

The Balkans Scientific Center  
of the Russian Academy of Natural Sciences

**4**<sup>th</sup>  
International  
Symposium

**MODERN  
TRENDS IN AGRICULTURAL  
PRODUCTION,  
RURAL DEVELOPMENT  
AGRO-ECONOMY  
COOPERATIVES  
AND ENVIRONMENTAL  
PROTECTION**

P R O C E E D I N G S



29 - 30 June 2022  
Vrnjacka Banja

# **The Balkans Scientific Center of the Russian Academy of Natural Sciences**



**4<sup>th</sup> International Symposium:**

**Modern Trends in Agricultural Production, Rural Development,  
Agro-economy, Cooperatives and Environmental Protection**

**Vrnjačka Banja, Serbia**

**29 – 30. Jun, 2022.**

# **Modern Trends in Agricultural Production, Rural Development, Agro-economy, Cooperatives and Environmental Protection**

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## **INVITED PAPERS**



**FIRST DISCOVERY OF SUBTERRANEAN SPECIES  
NIPHARGUS PECARENSIS S. KAR. & G. KAR. 1959  
(FAM. NIPHARGIDAE) IN ROMANIA  
(CONTRIBUTION TO THE KNOWLEDGE OF THE  
AMPHIPODA 327)**

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**ABSTRACT**

*The subterranean freshwater member of the family Niphargidae, Niphargus pecarensis pecarensis S. Karaman & G. Karaman, 1959 is discovered at the first time in Romania (subterranean waters near Mehadia, N. of Orsava, at the foot of Southern Carpathian Mts. Species is described and figured, and its morphological characters are compared with these of N. pecarensis from type-locality (Bulgaria, Pečara Dupka Cave near Belogradtschik) and with these from eastern Serbia (cave near Gabrovnica village, Kalna region).*

*The specimens of spring near Mratinje (NW Montenegro) described and figured as N. pecarensis occultus, ssp. nov. by G. Karaman (1998), morphologically allied to N. pecarensis but rather different, are not treated here in detail, because further studies and new samples of this subspecies are needed to resolve taxonomical status and relations of this taxon within N. pecarensis group.*

**Keywords:** taxonomy, Amphipoda, subterranean, Niphargus pecarensis, occultus, Romania, Bulgaria, Serbia, Montenegro.

**INTRODUCTION**

The subterranean fauna of Amphipoda in Romania, especially taxa of family Niphargidae, is very rich, thanks to presence of numerous caves and karstic phenomena, geographical position, geological history, hydrography, etc. By this way, elements of various types of fauna: pontocaspian, medioeuropean, glacial, Tertiary, postglacial, etc., are present here. All these conditions caused the presence of numerous members of the family Niphargidae (over 50 known species) in Romania, discovered and described by numerous Romanian and foreign researchers (Carausu, Dobreanu, Manolache, Dancau, Schellenberg, Petrescu, G. Karaman, Dudich, etc.).



As many species were only partially described, further studies on morphological and molecular / genetic level will confirm the value of all these taxa and reveal existence of various other new or known species in subterranean waters of Romania, what confirm present discovery of *Niphargus pecarensis* S. Kar. & G. Kar. 1959 in Romania.

## MATERIAL AND METHODS

The studied material was preserved in 70% ethanol. Studied specimens were dissected using a WILD M20 microscope and drawn using a camera lucida. All appendages were temporarily submersed in a mixture of glycerin and water for study and drawing. All illustrations were manually drawn. Dissected body-parts were submerged in Liquid of Faure and covered by thin cover glass forming permanent slides.

Some morphological terminology and seta formulae follow Karaman's terminology (Karaman G, 1969, 2012): for the last mandibular palpus article [A= A-setae on outer face; B= B-setae on inner face; D= lateral marginal D-setae; E= distal long E-setae], and for propodus of gnathopods 1 and 2 [S= corner S-spine; L= lateral serrate L-spines; M= corner facial M-setae; R= subcorner spine on inner face].

Terms "setae" and "spines" are used based on shape, not origin. This study was made based on morphological, ecological and zoogeographical data.

## TAXONOMICAL PART

Family NIPHARGIDAE

***NIPHARGUS PECARENSIS PECARENSIS* S. Karaman & G. Karaman, 1959**

Figures 1-7

*Niphargus tauri pecarensis* S. Karaman & G. Karaman, 1959: 143, figs. 1-8; Andreev, 1972: 65; Barnard, J.L. & Barnard, C.M., 1983: 696; G. Karaman & Ruffo, 1986: 533.

*Niphargus pecarensis pecarensis* G. Karaman, 1998: 116, figs. 1-4; G. Karaman, 1999: 168.

*Niphargus pecarensis* G. Karaman, 2011: 151.

### MATERIAL EXAMINED:

ROMANIA: S-5025= Mehadia (N. of Orsava), 3 exp. (196?) (leg. ?Dancu);

SERBIA: S-4775= cave in village Gabrovnica near Kalna (SE. of Knjaževac), water temperature 13°C, 29.7.1973, 8 exp. (leg. Milika Pljakić).

BULGARIA: type-material.

### **DESCRIPTION: Male 6.8 mm (Mehadia, Romania):**

Body moderately slender, metasomal segments 1-3 with 2 dorsoposterior marginal setae (fig. 1 I); urosomal segment 1 with one dorsolateral seta on each side; urosomal segment 2 on each dorsolateral side with one seta, urosomal segment 3 glabrous (fig. 4G). Urosomal segment 1 at each ventroposterior corner with one stronger spine-like seta near basis of uropod 1 peduncle (fig. 4G).

Epimeral plates 1-3 with completely subrounded ventroposterior corner and convex posterior margin bearing rather stronger dorsoposterior seta and 2-3 posterior marginal setae (fig. 1 I), plates 2 and 3 with 3 subventral stronger setae.

Head with short rostrum and short subrounded lateral cephalic lobes, ventroanterior excavation developed (fig. 1A), eyes absent.

Antenna 1 reaching nearly half of body; peduncular articles not elongated, progressively shorter towards article 3 (ratio: 51:35:20), scarcely covered with several setae (fig. 3A), article 3 twice longer than broad; main flagellum consisting of 21 scarcely setose articles; the most of articles with one short aesthetasc (fig. 3B); accessory flagellum 2-articulated, shorter than article 3 (ratio: 38:62), with long distal seta, (fig. 3C) accompanied by 2 short setae (fig. ).

Antenna 2 moderately slender, peduncular article 3 short, with distoventral bunch of setae (the longest setae as long as article itself); peduncular article 4 longer than article 5 (ratio: 66:53), ventral margin with several distal and lateral setae (the longest setae exceeding diameter of article itself), dorsal margin with several shorter setae; article 5 with several ventral setae (the longest setae exceeding diameter of article), and several dorsal shorter setae. Flagellum moderately slender, consisting of 8 articles bearing short setae (fig. 3D). Antennal gland cone short (fig. 3D).

Mouthparts well developed. Labrum with convex distal margin (fig. 1B). Labium with small well developed inner lobes and larger entire outer lobes (fig. 1C).

Mandible molar triturative. Left mandible: incisor with 5 teeth, lacinia mobilis 4 teeth and 8 rakers (fig. 1D); right mandible: incisor with 4 teeth, lacinia mobilis serrate, accompanied by 7 rakers. Mandibular palpus 3-articulate: first article glabrous, second article with 4 setae; article 3 subfalciform, hardly longer than article 2 (ratio: 63:57), with only 2-3 marginal D-setae and 4-5 distal E-setae (fig. 1F), on outer face with 2 A-setae (fig. 1E), on inner face 2 B-setae (fig. 1F).

Maxilla 1: inner plate with 1 seta, outer plate with 7 spines: 6 spines with one lateral tooth, one (inner) spine with 2-3 teeth (fig. 2A); palpus 2-articulated, reaching tip of outer plate-spines and provided with 4 distal short setae.

Maxilla 2: inner plate slightly smaller than outer one, both with distomarginal setae only (fig. 2B).

Maxilliped: inner plate short, with 2 distal spines mixed with several setae; outer plate hardly exceeding half of palpus article 2, bearing at mesial margin 8 spines and 4-5 distal setae (fig. 1G); palpus article 3 with distal groups of long setae; article 4 with 1-2 unequal setae at inner margin near basis of the nail (fig. 1H).

Coxae relatively short. Coxa 1 broader than long (ratio: 44:27), with subrounded ventroanterior part and provided with 6-7 setae (fig. 2C). Coxa 2 broader than long (ratio: 43:35), with 6 marginal setae (fig. 2D). Coxa 3 slightly broader than long (ratio: 43:37), with 6 marginal setae (fig. 3E). Coxa 4 distinctly broader than long (ratio: 47:38), without ventroposterior lobe and bearing 4-5 marginal setae (fig. 3G).

Coxae 5-7 shallow. Coxa 5 much broader than long (ratio: 60:30), bilobed, with 3-4 marginal setae (fig. 4A). Coxa 6 bilobed, smaller than coxa 5, broader than long (ratio: 50:25), with 5-6 marginal setae (fig. 4C). Coxa 7 shorter than coxa 6, entire, broader than long (ratio: 45:21), with 1-2 marginal setae (fig. 4E).

Gnathopods 1-2 relatively small, with propodus nearly as large as corresponding coxa. Gnathopod 1: article 2 with long anterior and posterior marginal setae; article 3 with distoposterior bunch of setae (fig. 2C). Article 5 rather shorter than propodus (ratio: 30:36), with distoanterior bunch of setae and posterior row of setae. Propodus trapezoid, slightly longer than broad (ratio: 80:70), at posterior margin with 3 transverse rows of setae (fig. 2D); Palm slightly convex inclined nearly half of propodus-length, defined on outer face by corner S-spine accompanied laterally by 3 serrate L-spines and 2 facial corner M-setae, on inner face by one subcorner R-spine (fig. 2E). Dactylus reaching posterior margin of propodus, with one median seta at outer margin and 3-4 short setae at inner margin (fig. 2D).

Gnathopod 2 slightly larger than gnathopod 1, article 2 with long setae at anterior and posterior margin; article 3 with distoposterior bunch of setae. Article 5 nearly as long as propodus, with distoanterior bunch of setae (fig. 2F). Propodus trapezoid, hardly broader than long (ratio: 82:76), with 6 transverse rows of setae at posterior margin (fig. 2G). Palm inclined hardly over half of propodus-length, convex, defined on outer face by corner S-spine accompanied laterally by 2-3 L-spines and 3 corner facial M-setae, on inner face by subcorner spine (fig. 2H). Dactylus reaching posterior margin of propodus, with one median seta at outer margin and 4 short setae at inner margin (fig. 2G).

Pereopods 3 and 4 rather similar. Pereopod 3 article 2 with anterior short setae and posterior long setae. Articles 4-6 of different length (ratio: 45:29:34); articles 4-5 with several longer setae at posterior margin and short setae at anterior margin (the longest setae exceeding diameter of articles themselves; article 6 with 3-4 groups of short spines and single setae at posterior margin (fig. 3E). Dactylus much

shorter than article 6 (ratio: 15:33), strong, with one stronger seta at inner margin near basis of the nail, and one median plumose seta at outer margin; nail slightly shorter than pedestal (ratio: 25:28) (fig. 3F).

Pereopod 4 pilosity like that of pereopod 3. Articles 4-6 of different length (ratio: 40:30:37) (fig. 3G), dactylus much shorter than article 6 (ratio: 14:37), with one stronger seta at inner margin and one median seta at outer margin (fig. 3H), nail nearly as long as pedestal.

Pereopod 5 remarkably shorter than pereopods 6 and 7 (fig. 4A), with article 2 longer than broad (ratio: 53:35), ventroposteriorly rather dilated but not lobed, at posterior margin with 5-6 setae, anterior margin with 4 groups of setae (distal seta much longer than article 3). Articles 4-6 of different length (ratio: 30:34:27), articles scarcely setose, article 5 with 2 groups of short spines. Article 2 longer than article 6 (ratio: 53:27). Dactylus shorter than article 6 (ratio: 12:27), with spine-like seta at inner margin and median plumose seta at outer margin (fig. 4B); nail shorter than pedestal (ratio: 25:35).

Pereopod 6: article 2 longer than broad (ratio: 66:38), unlobed, with 7 posterior marginal setae and 4-5 longer setae at anterior margin, article 3 with 2 long distoanterior setae. Articles 4-6 of different length (ratio: 40:50:47), with several short marginal spines and setae (fig. 4C). Article 2 longer than article 6 (ratio: 66:47). Dactylus shorter than article 6 (ratio: 25:44), with spine-like seta at inner margin and plumose median seta at outer margin (fig. 4D); nail shorter than pedestal (ratio: 25:50).

Pereopod 7 longer than pereopod 6, article 2 longer than broad (ratio: 73:42), unlobed, posterior poorly convex margin with 9 setae, anterior poorly convex margin with 5 stronger setae (fig. 4E); article 3 with short distoanterior seta. Articles 4-6 of different length (ratio: 40:57:60), articles along both margins with bunches of short spines and single short setae. Article 2 longer than article 6 (ratio: 73: 60). Dactylus shorter than article 6 (ratio: 28:60), with one spine-like seta at inner margin and one median plumose seta at outer margin (fig. 4F), nail shorter than pedestal (ratio: 28:45).

Pleopods with elevated number of retinacula. Pleopod 1 peduncle with 3-4 retinacula and 4 long lateral plumose setae (fig. 1J); pleopod 2 peduncle with 4 retinacula and 3 lateral plumose setae (fig. 1K); pleopod 3 peduncle with 4 retinacula and without plumose setae (fig. 1L).

Uropod 1 peduncle longer than rami, with dorsoexternal and dorsointernal setae (except distal spine). Outer and inner ramus inflated (dilated), with 5 distal spines each (fig. 4G); outer ramus rather longer than inner one.

Uropod 2 much shorter, rami not dilated, with 5 short unequal distal spines each

(fig. 4G), outer ramus hardly longer than inner one.

Uropod 3 very long; peduncle elongated, much longer than broad (ratio: 54:18), with 2 distal spines and several short lateral setae; inner ramus scale-like, with 2 distal spines and one lateral simple seta (fig. 4H); outer ramus very long, narrowed, 2-articulated; first article at outer margin with 2 lateral and one distal group of short simple setae, at inner (mesial) margin with 2 groups of simple setae; second article as long as first one, with 3 marginal short simple lateral and 6 short distal simple setae.

Telson only slightly longer than broad, deeply incised over  $\frac{3}{4}$  of telson length; each lobe with 6 long unequal slender distal spines (the longest spine exceeding  $\frac{2}{3}$  of telson length; a pair of short plumose setae attached rather over half of external margin of each lobe (fig. 3 I).

Coxal gills not reaching ventral margin of corresponding article 2 of extremities, curved on gnathopod 2 (fig. 2F), elongated on pereopod 4 (fig. 3G), ovoid on pereopod 3, 5 and 6 (figs. 3E, 4A, C).

### **Female ovig. 5.8 mm (Mehadia, Romania):**

Head like that in male. Metasomal segments 1-3 with 2-4 dorsoposterior marginal setae (fig. 5F); urosomal segments 1-2 with one seta on each dorsolateral side, urosomal segment 3 glabrous. Urosomal segment 1 on each ventroposterior corner with one slender spine (fig. 5J).

Epimeral plates 1-3 like these in male, broadly subrounded, with corner and 1-3 posterior marginal setae (fig. 5F), epimeral plates 2-3 with 3 ventral stronger setae each.

Antenna 1 like that in male, main flagellum with 16 articles (most of them with one aesthetasc); accessory flagellum 2-articulated, shorter than last peduncular article.

Antenna 2 like that in male, peduncular articles 4-5 with several groups of setae (the longest setae exceeding diameter of articles themselves); flagellum longer than last peduncular article, consisting of 7 articles.

Mouthparts like these in male. Palpus mandibulae article 1 glabrous, article 2 with 6 setae; article 3 nearly as long as article 2, with 4 unequally long D-setae and 3-4 distal long E-setae, on inner face by 2 facial B-setae, on outer face by 2 facial A-setae (fig. 5E).

Maxilla 1 inner plate with one seta, outer plate like that in male, palpus reaching distal tip of outer plate spines and provided with 4 setae. Maxilla 2 and maxilliped like these in male.

Coxae short, but mainly slightly longer than these in male. Coxa 1 broader than long (ratio: 50:32), ventroanterior corner subrounded, with 5-6 marginal setae (fig. 5A). Coxa 2 hardly broader than long (ratio: 45:43), with 5-6 marginal setae (fig. 5B). Coxa 3 hardly longer than broad (ratio: 51:48), with 5-6 marginal setae (fig. 5C). Coxa 4 unlobed, hardly broader than long (ratio: 53:48), with 4-5 marginal setae (fig. 4D).

Coxae 5-7 shallow. Coxa 5 bilobed (ratio: 57:33), with single marginal setae (fig. 6C). Coxa 6 broader than long (ratio: 43:27), bilobed, with single marginal setae (fig. 6D). Coxa 7 entire, broader than long (ratio: 42:21) (fig. 6E).

Gnathopods 1-2 relatively small. Gnathopod 1 rather smaller than gnathopod 2, with articles 2-4 like these in male. Article 5 hardly shorter than propodus (ratio: 61:68), with distoanterior bunch of setae (fig. 6A). Propodus trapezoid, nearly as long as broad, with 4 posterior marginal transverse row of setae (fig. 6A). Palm slightly convex, inclined nearly half of propodus-length, defined on outer face by corner S-spine accompanied laterally by 3 L-spines and corner facial 2 M-setae, on inner face by subcorner R-spine. Dactylus with one median seta at outer margin, and 4-5 short setae at inner margin (fig. 6A).

Gnathopod 2: articles 2-4 like these in male. Article 5 nearly as long as propodus, with distoanterior bunch of setae (fig. 6B). Propodus trapezoid, slightly broader than long (ratio: 78:73), with 5 posterior marginal transverse rows of setae (fig. 6B). Palm slightly convex, inclined rather over half of propodus-length, defined on outer face by corner S-spine accompanied laterally by 3 L-spines and 2 corner facial M-setae, on inner face by one subcorner R-spine. Dactylus with one median seta at outer margin and 4-5 short setae along inner margin (fig. 6B).

Pereopods 3-4 rather similar to these of male. Pereopod 3: article 2 with row of long setae at posterior margin and short distoanterior marginal setae. Article 4-6 of different length (ratio: 42:32:40), scarcely setose (fig. 5C); article 6 with 3 groups of short setae at posterior margin. Dactylus remarkably shorter than article 6 (ratio: 19:40), with spine-like seta at inner margin near basis of the nail, and short median seta at outer margin; nail slightly shorter than pedestal.

Pereopod 4 like pereopod 3.

Pereopod 5 remarkably shorter than pereopods 6-7: article 2 longer than broad (ratio: 51:30), unlobed, with 3 anterior marginal spine-like setae and 3 distal setae (the longest seta exceeding length of article 3). Articles 4-6 of different length (ratio: 27:34:33); articles 3 and 4 with long distoanterior long seta mixed with single shorter setae (fig. 6C). Article 2 longer than article 6 (ratio: 51:33). Dactylus shorter than article 6 (ratio: 16:33), at inner margin with spine-like seta near basis of the nail.

Pereopod 6: article 2 longer than broad (ratio: 62:35), unlobed, anterior margin with 3 longer spine-like setae and distal group of setae (the longest seta exceeding tip of article 3), posterior margin with 8 setae. Articles 4-6 of different length (ratio: 33:47:52), articles scarcely setose, mixed with single short spines (fig. 6D). Article 2 longer than article 6 (ratio: 62:52). Dactylus shorter than article 6 (ratio: 21:52), with short spine-like seta at inner margin near basis of the nail; and short median seta at outer margin; nail shorter than pedestal.

Pereopod 7 rather longer than pereopod 6, unlobed. Article 2 longer than broad (ratio: 68:40), anterior margin with 4 longer spine-like setae, posterior, medially almost straight margin, with 8 setae. Articles 4-6 of different length (ratio: 33:48:60), articles with single or groups of spines mixed often with single short setae (fig. 6E). Article 2 longer than article 6 (ratio: 68:60). Dactylus shorter than article 6 (ratio: 29:60), with one slender spine at inner margin near basis of the nail, and one median plumose seta at outer margin; nail shorter than pedestal.

Pleopods 1-3 with 4 retinacula each. Pleopod 1 peduncle with 6 lateral external plumose setae (fig. 5G); pleopod 2 peduncle with 5 lateral external plumose setae (fig. 5H); pleopod 3 peduncle naked (fig. 5I), outer ramus shorter but consisting of 5 articles, inner ramus longer but consisting of 4 articles.

Uropods 1-2 with normal, not inflated rami. Uropod 1 peduncle with dorsoexternal row of 3 spines, dorsointernal margin with distal spine only (fig. 5J); rami shorter than peduncle. Outer ramus slightly longer than inner one, both rami with 4-5 distal spines (the longest spine of inner ramus reaching half of ramus-length; lateral spines absent).

Uropod 2 peduncle with 3-4 distal spines; rami nearly of the same length (fig. 5K), with 4-5 distal spines each (the longest spines remarkably exceeding half of ramus-length (fig. 5K).

Uropod 3 missing.

Telson nearly as long as broad, deeply incised over 2/3 of telson-length, tapering distally. Each lobe with 4-5 distal long spines (the longest spine exceeding 2/3 of telson-length (fig. 6F); lateral and facial spines absent. A pair of unequally short plumose setae attached medially at outer margin of each lobe.

Coxal gills curved on gnathopod 2 (fig. 5B), ovoid on pereopods 3, 5, and 6 (fig. 5C, 6C, D) and rather elongated ovoid on pereopod 4 (fig. 5D).

Oostegites very broad, with single marginal setae (fig. 5C).

### **VARIABILITY:**

The morphological variability of some taxonomical characters within populations of Bulgaria, Serbia and Romania is observed by number and length of spines of telson, number of lateral teeth of maxilla 1 outer place, number of retinacula,

number of lateral plumose setae on pleopod-peduncles, by L-spines and M-corner setae on gnathopods 1-2-propodus, number of subventral strong setae on epimeral plates 2-3, shape and number of distal spines on telson, etc.

Observed stable morphological characters: maxilla 1 inner plate is always with one seta, and palpus reaching distal tip of outer plate-spines; mandibular palpus article 3 with low number of A, B and D setae and very long E-setae, absence of dorsolateral spines on urosomal segments 1-2 (replaced by setae), short accessory flagellum, unlobed article 2 of pereopods 5-7, strong dactylus of pereopods 3-7; gnathopod 1-2 propodus with lateral position of L-spines and one outer marginal median seta on its dactylus.

By this way, *N. pecarensis pecarensis* is morphologically well recognized by specific pilosity of mandibular palpus with scarce number of D-setae and more lateral position and reduced number of B-setae, very long E-setae; maxilla 1 inner plate with one seta, palpus reaching tip of outer plate spines; dactylus of gnathopods 1-2 with one median seta at outer margin, L-spines sitting laterally regarding S-spine. Dactylus of pereopods 3-7 strong but with weak spine or spine-like seta on inner margin near basis of the nail; pleopods with elevated number of retinacula and presence of long lateral plumose setae mainly on peduncle of pleopods 1-2; telson deeply incised with distal long more or less slender spines. Gills on gnathopod 2 curved, gills of pereopod 4 elongated, other coxae ovoid, short. Dorsolateral margins of urosomal segments 1-2 with single setae, spines absent in males and females; uropod 3 in female with elongated distal article of outer ramus.

#### **LOCUS TYPICUS:**

Pečara dupka" near Belogradčik in NW-Bulgaria. Beron (2015) mentioned that the real name of this cave is Bashovishki pech, Vidin reg.

#### **LOCALITIES CITED:**

**Karaman S. & Karaman, G. 1959:** Pečara dupka" near town Belogradčik, NW Bulgaria.

**Karaman, G. 1998:** locus typicus.

**Karaman, G. 1999:** cave near Grabovnica Village by Kalna reg., NE Serbia.

**Karaman, G. 2011:** NE Serbia.

**Beron 2015:** NW Bulgaria, region Vidin (original spelling by Beron):

Vd 5 Haydushkata propast (Haydushkata propast 1) cave pot hole near Belogradchik.

Vd 9. Bashovishki pech (= Vodni pech, Pečara dupka or Pečova dupka) – cave near Oreshets Railway Station (loc. typ.);



Vd 11. Golemi Pech – cave near Varbovo Village;

Vd 13. Desni Suhi pech – cave near Dolni Lom Village, Alt. 500 m;

Vd 21. Rushkovitsa (Prelaz) – cave near Salash Village;

Vd 28. Yame 2 – pot hole near Targovishte Village;

**Present data:** ROMANIA: Mehadia (N. of Orsava).

## REMARKS AND AFFINITIES

The present discovery of this species in Romania (Mehadia, N. of Orsava, Southern Carpathian Mts.) showed similarity of taxonomic characters of these specimens with specimens of *N. pecarensis pecarensis* from Bulgaria, and no significant morphological difference were observed. except less number of setae on maxilla 1 palpus.

The specimens from eastern Serbia (cave near Gabrovnica by Kalna, Suva planina Mts.) (Karaman, G., 1999) showed its morphological similarity to specimens of *N. pecarensis* from Bulgaria:

**Male 7.2 mm** pleopods 1-3 with 4-3-3 retinacula, pleopod 1 peduncle with 4 lateral plumose setae and one proximoanterior short simple seta; pleopod 2 peduncle with 3 lateral plumose setae and one proximoanterior short simple seta; pleopod 3 peduncle naked. Uropods 1-3 and telson like these in type. Maxilliped palpus with one seta at inner margin near basis of the nail. Maxilla 1 like that in type.

**Female ovig. 6.5 mm:** pleopods 1-3 with 3-4 retinacula; pleopod 1 peduncle with 9 lateral plumose setae and 2 proximoanterior simple short setae; pleopod 2 peduncle with 5 lateral plumose setae and one proximoanterior simple seta; pleopod 3 peduncle with 2 lateral plumose setae. Uropods 1-2 not inflated, with rami of equal length. Uropod 3 outer ramus with second article slightly exceeding half of first article, like that in paratype. Telson like that in type.

Evidently females have more setose peduncle of pleopods 1-3 than male.

Beron (2015) cited *N. pecarensis* from several caves in region of Vidin (NW Bulgaria, northern tip of Stara planina Mts) without any morphological data (see above).

## ***NIPHARGUS PECARENSIS OCCULTUS* G. Karaman, 1998**

*Niphargus pecarensis occultus* G. Karaman, 1998: 123, figs.5-8; G. Karaman, 1999: 168.

## REMARKS

G. Karaman (1998) described *Niphargus pecarensis occultus*, ssp. nov. from

springs in Mratinje, NW Montenegro (Maglič Mts., Dinarid Mts), nearly 350 km W of type-locality of *N. pecarensis pecarensis*, based on morphological characters regarding specimens from type-locality in NW Bulgaria (Vidin reg., Stara planina Mts.) (elevated number of retinacula, less number of plumose setae on pleopods, presence of more setae on pleopod 3 peduncle, etc.). Population from Montenegro show morphological affinity to *N. pecarensis pecarensis*, but differs distinctly by long second article of uropod 3 in female. Understanding its taxonomical and zoogeographical position regarding *N. pecarensis* request further study and finding of new localities.

**LOCUS TYPICUS:** spring on the old road near Mratinje, N. part of Montenegro (Crna Gora).

**DISTRIBUTION:** Montenegro (Crna Gora):

G. Karaman 1998 and 1999: loc. typ.; second spring near Mratinje.

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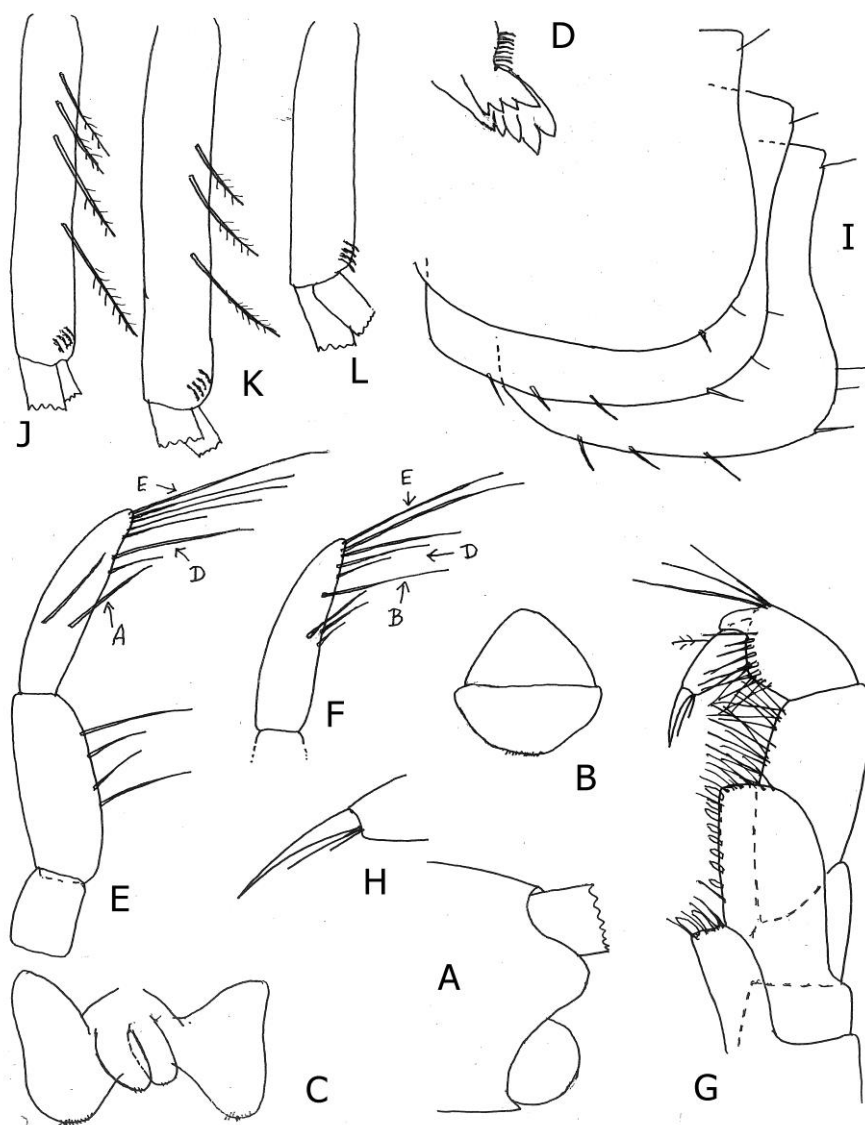


Fig. 1. *Niphargus pecarensis pecarensis* S. Kar. & G. Kar. 1959, Mehadia, Romania, male 6.8 mm: A= head; B= labrum; C= labium; D= left molar with lacinia mobilis and rakers; E= left mandibular palpus, outer face [D= marginal D-setae, E= distal E-setae; A= facial A-setae]; F= right mandibular palpus distal article, inner face [D= marginal D-setae; E= distal E-setae; B= facial B-setae]; G= maxilliped; H= distal tip of maxilliped palp article 4; I= epimeral plates 1-3; J= pleopod 1 peduncle; K= pleopod 2 peduncle; L= pleopod 3 peduncle.

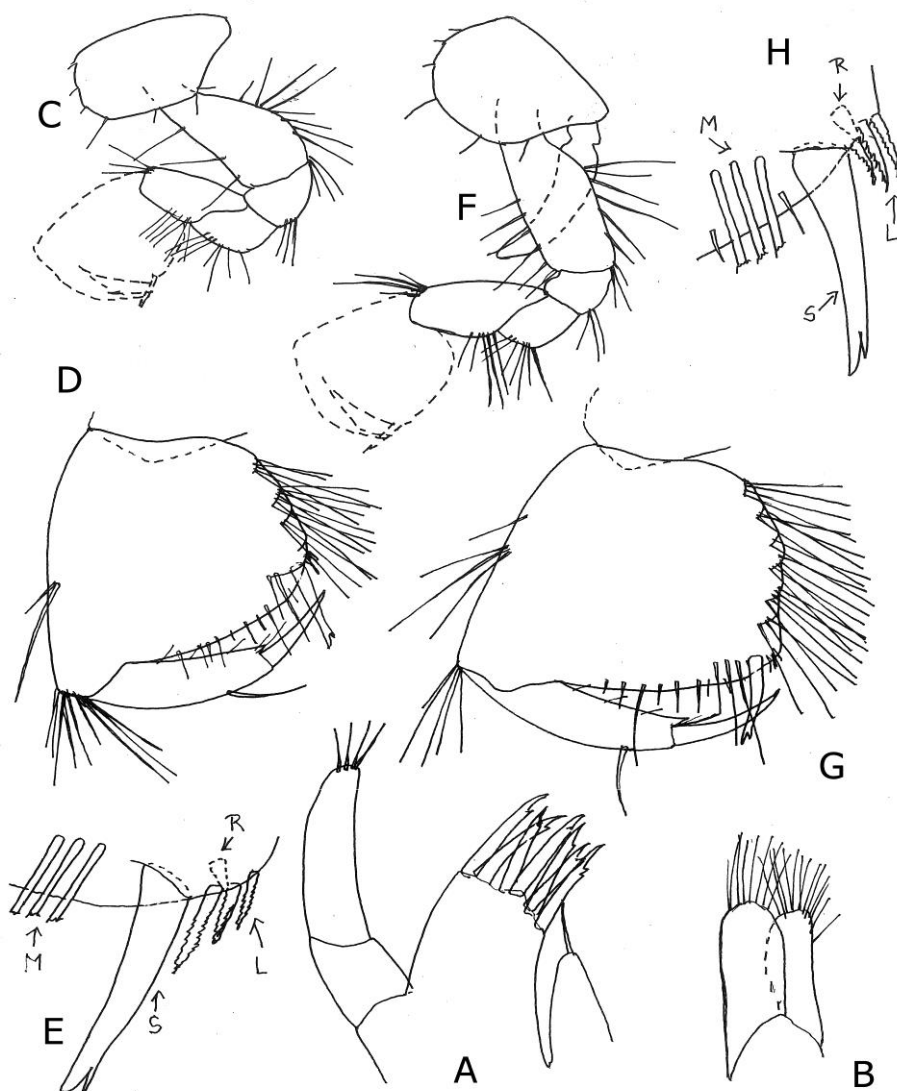


Fig. 2. *Niphargus pecarensis pecarensis* S. Kar. & G. Kar. 1959, Mehadia, Romania, male 6.8 mm: A= maxilla 1; B= maxilla 2; C-D= gnathopod 1, outer face; E= distal corner of gnathopod 1-propodus, outer face [S= corner S-spine; L= lateral L-spines; M= corner facial M-setae; R= subcorner R-spine, inner face]; F-G= gnathopod 2, outer face; H= distal corner of gnathopod 2-propodus, outer face [S= corner S-spine; L= lateral L-spines; M= corner facial M-setae; R= subcorner R-spine, inner face].

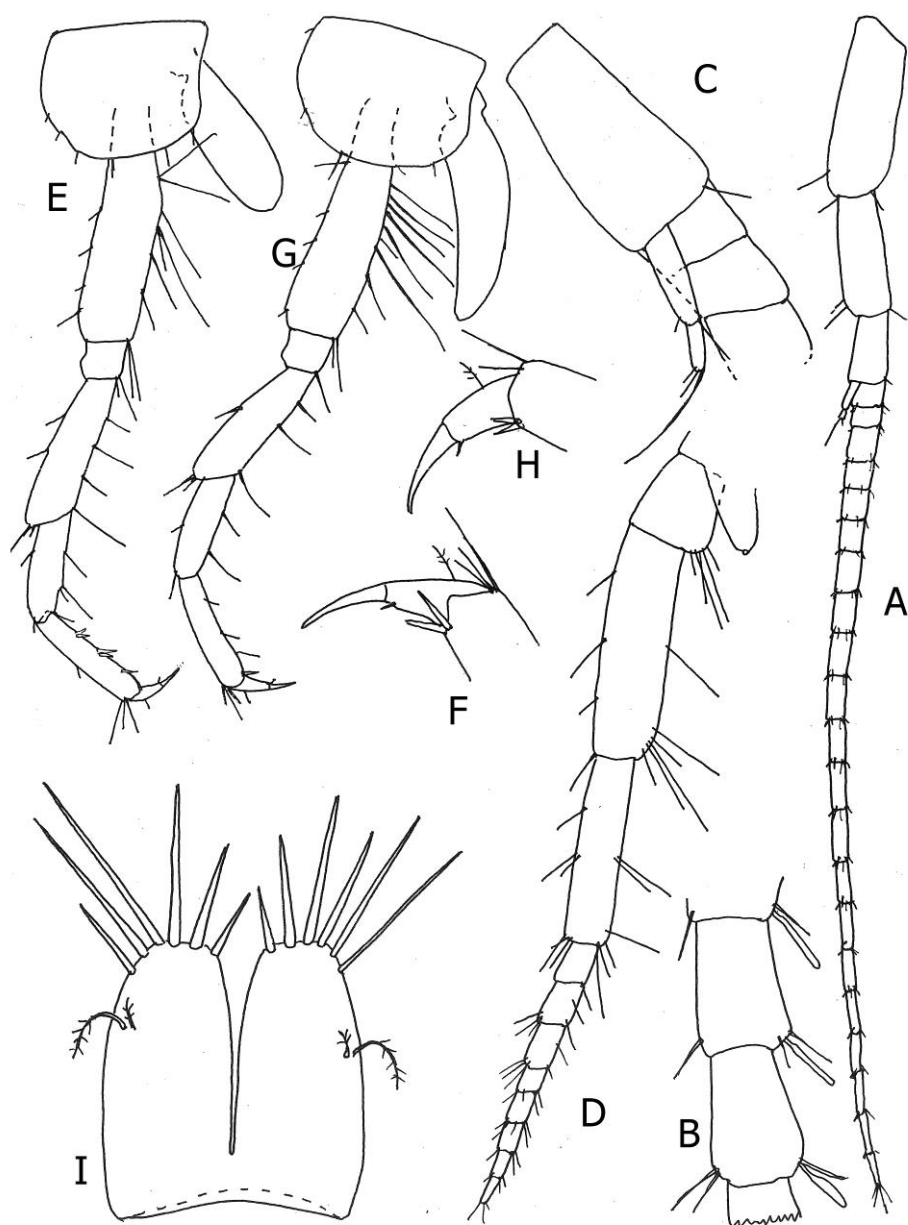


Fig. 3. *Niphargus pecarensis pecarensis* S. Kar. & G. Kar. 1959, Mehadia, Romania, male 6.8 mm: A= antenna 1; B= aesthetascs on antenna 1 flagellum; C= accessory flagellum; D= antenna 2; E-F= pereopod 3; G-H= pereopod 4; I= telson.

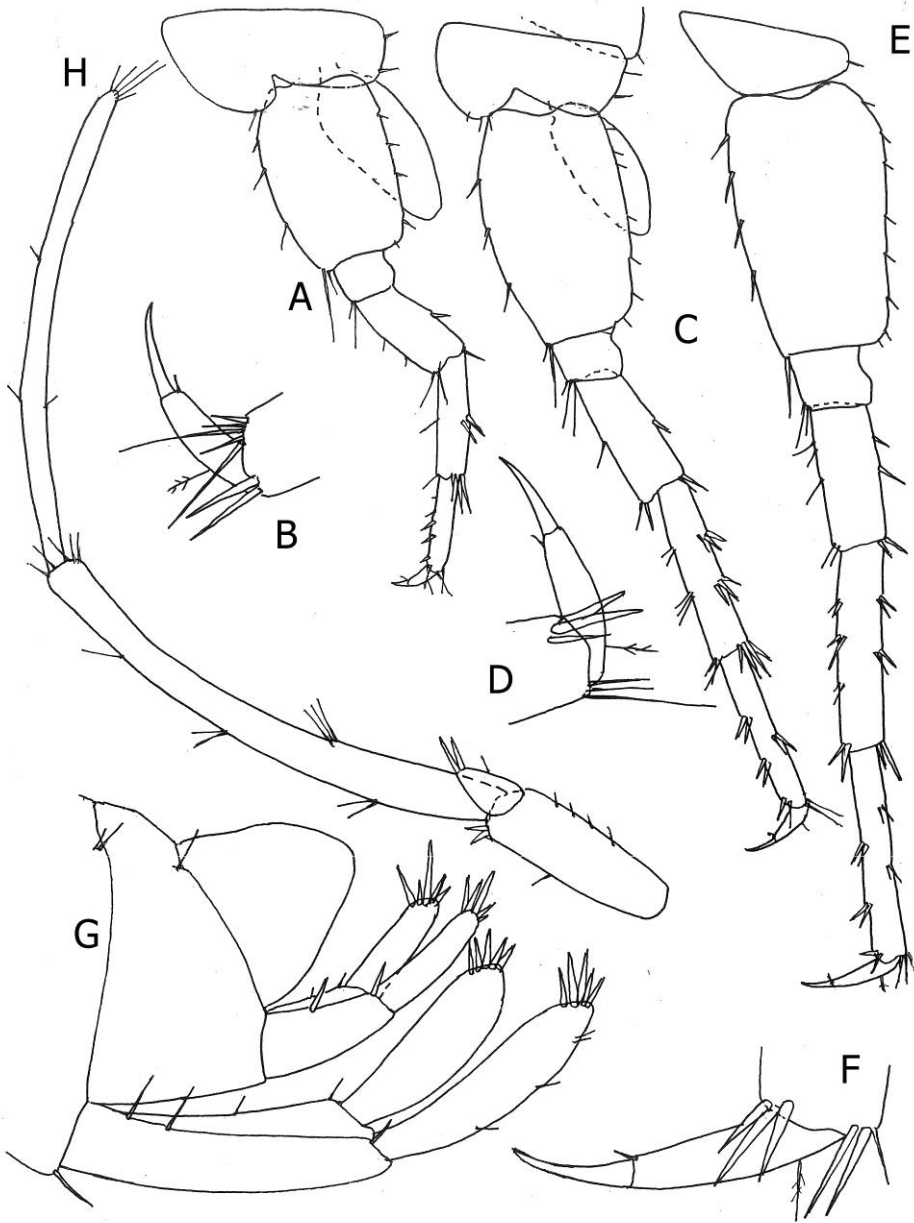


Fig. 4. *Niphargus pecarensis pecarensis* S. Kar. & G. Kar. 1959, Mehadia, Romania, male 6.8 mm: A-B= pereopod 5; C-D= pereopod 6; E-F= pereopod 7; G= urosome with uropods 1-2; H= uropod 3.

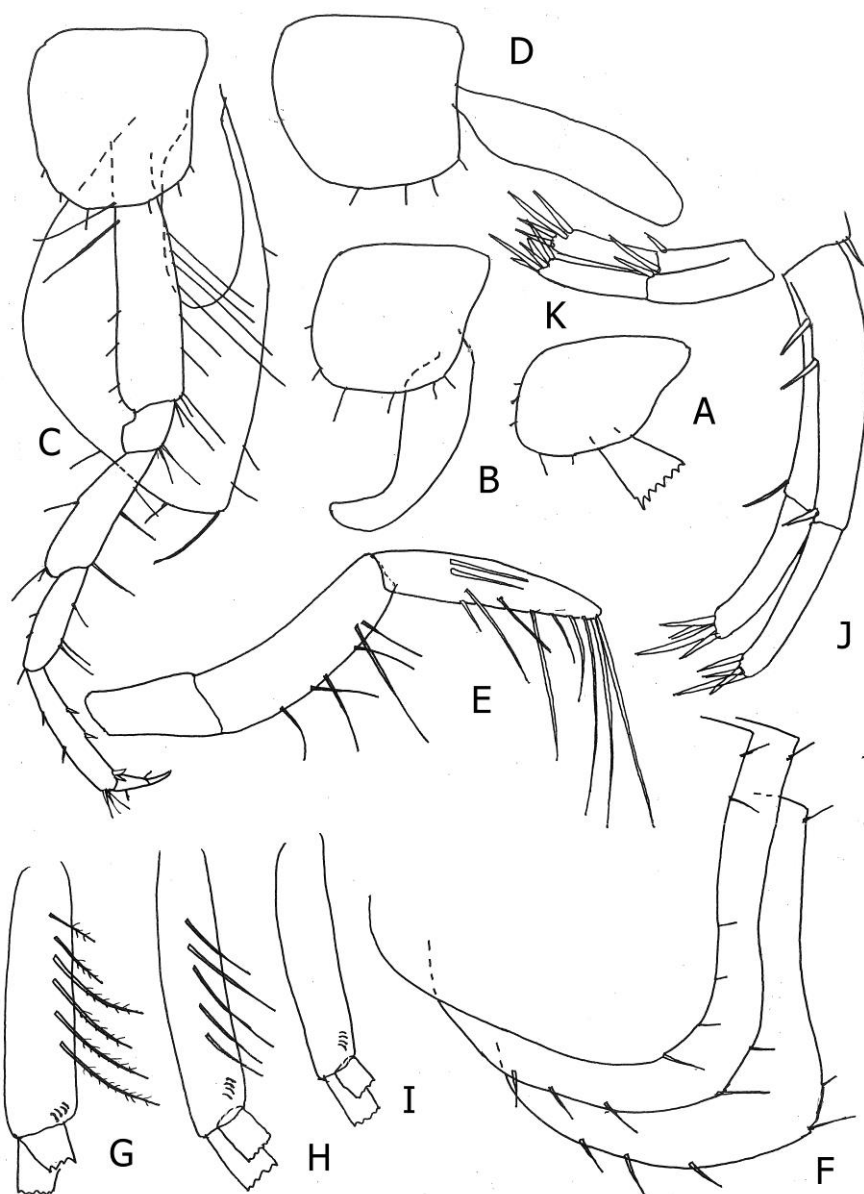


Fig. 5. *Niphargus pecarensis pecarensis* S. Kar. & G. Kar. 1959, Mehadia, Romania, female 5.6 mm: A= coxa 1; B= coxa 2; C= pereopod 3; D= coxa 4; E= mandibular palpus, outer face; F= epimeral plates 1-3; G= pleopod 1 peduncle; H= pleopod 2 peduncle; I= pleopod 3 peduncle; J= uropod 1; K= uropod 2.



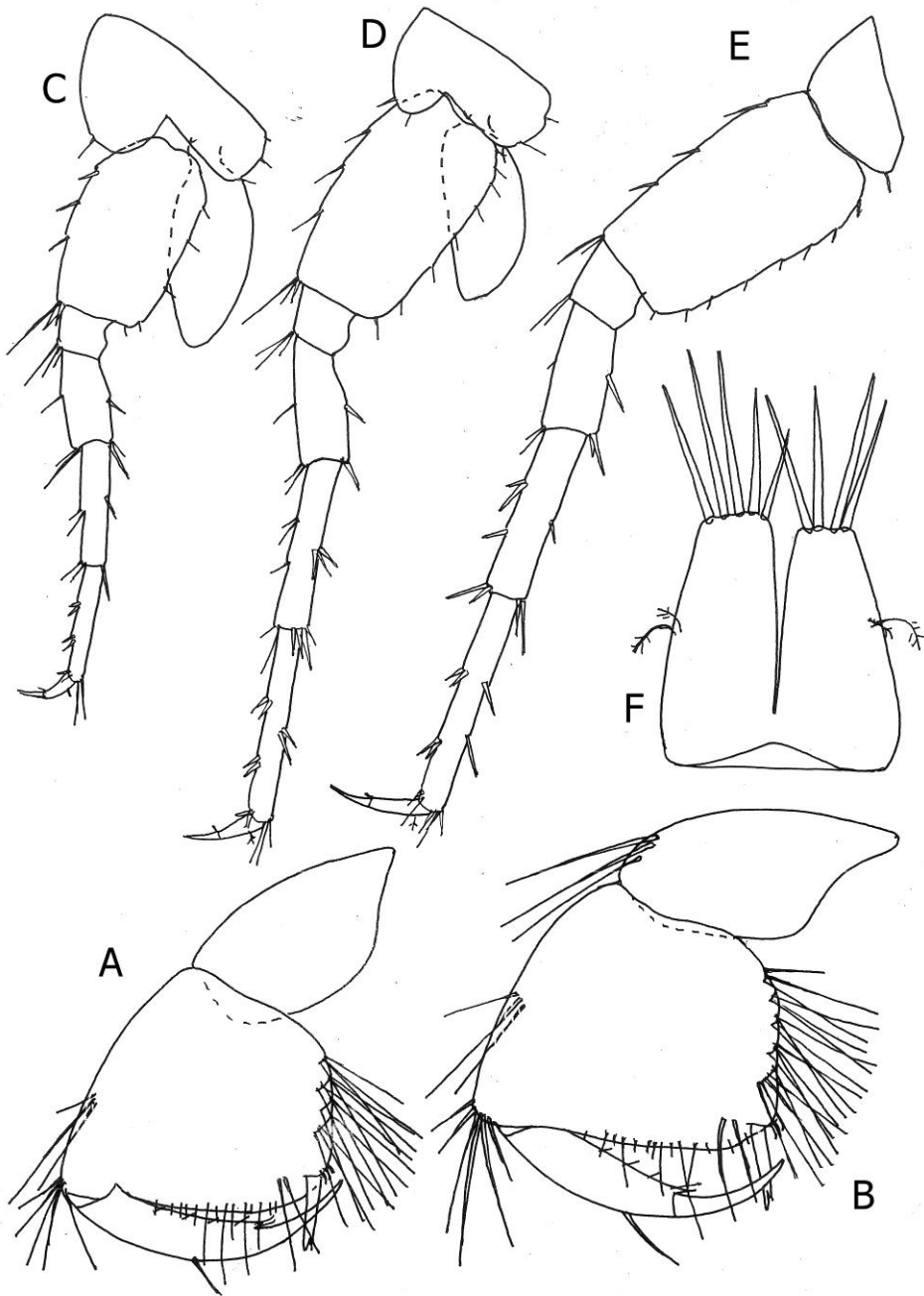


Fig. 6. *Niphargus pecarensis pecarensis* S. Kar. & G. Kar. 1959, Mehadia, Romania, female 5.6 mm: A= gnathopod 1-propodus, outer face; B= gnathopod 2, propodus, outer face; C= pereopod 5; D= pereopod 6; E= pereopod 7; F= telson.



Fig. 7. Distribution of *Niphargus pecarensis*:

O = *Niphargus pecarensis pecarensis* S. Kar. & G. Kar. 1859

U= *Niphargus pecarensis occultus* G. Kar.1998

Insert from map: internet: <https://www.freeworldmaps.net/europe/balkan-peninsula/>

## **ACHIEVEMENTS IN UNDERSTANDING THE HEALTH OF SOIL ECOSYSTEMS IN THE 21ST CENTURY AND CHALLENGES FOR THE FUTURE**

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### **Introduction**

A good theory means correct, bringing practical results, fetus crops. The theory of soil fertility - "the birth of a fetus" as well as concept soil quality is still a defining characteristic for the soil, although the knowledge of nature has advanced far. Modern requirements in food production oblige to preserve the environment. The environment is an ecosystem in which soil is the basis of everything that makes up an ecosystem. Modern scientific definition of the notion a soil it is soil ecosystem (SE) representing an organic–mineral natural product formed under certain natural and climatic conditions; it is maintained by continuous microbial–plant interactions in the initially quantitatively predominant inorganic substance. This product includes the biota, mortmass, and metabolites affected by continuous enzymatic and chemical transformations; it also accumulates biophilic elements. Important biological and physicochemical processes are ongoing in it, including biogeochemical elemental cycles and microbial cycles. The product features a significant buffer capacity against various stress factors, provides nutritional substances for plants and heterotrophic biota, and acts as a source and discharge of biophile elements and biodiversity (Kupriyanov et al., 2010; Semenov, Sokolov, 2016).

Comprehension and understanding that the soil is not a "bioinert body", but a biological, ecological system and, therefore, like all biological objects, has health, can be ill and needs treatment, arose at the very end of the twentieth century (Doran et al., 1996).

There was a fairly quick understanding of the importance and depth of the content of this concept. (Van Bruggen, Semenov, 2000). There was a need to develop methods for the quantitative determination of the essentially immense concept - soil health. "Soil health" (SH) is a biological category that reflects the state of activity dynamics of the biotic component in the organic-mineral complex of the soil; this biological category is characterized by adequate activity of biotic processes (synthesis and hydrolysis) that corresponds to the natural climatic zone, their resistance to disturbing influences (biotic and abiotic stressors), and the "closedness (self-sufficiency)" of biophilic element and microorganism cycles. The healthy soil of agrocenoses is also characterized by the conformity of its quality to standard indicators and adequate fertility (for a specific climatic zone).

This definition of soil health is applicable to any soil (with the exception of anomalous soil) and does not contradict the substantive essence of traditional characteristics, but rather integrates their content, since the indicators of the dynamics of the biotic component's activity are interrelated with both the physicochemical parameters of the soil and the actual soil fertility. (Semenov et al., 2011; Semenov and Semenova, 2018).

The purpose of the message to list the main successes in the knowledge of soil health - soil ecosystem achieved in the 21st century and to identify some significant tasks for a speedy solution.

### **Soil health, soil ecosystem health and some quantitative parameters of this characteristic**

At the very beginning of the XXI, the method quantitative the determination has been developed and patented in Russia, named as a soil health parameter. The method have been based on the idea of quantitative comparison of substrate-induced respiration (SIR) of two or more soil samples after initiation of respiratory activity of the soil microbial community (MS) heterotrophic substrate (glucose). The method is published in Russian and English publications (Semenov 2011a; Semenov, Sokolov, 2016; Semenov, Djukich, 2017; Semenov, Semenova, 2018; Spiridonov et al., 2019).

It should be noted that the development of a method for quantitative determination of SH is based on the fundamental ecological concept "disturbing effects and wave-like development of microbial populations and microbial communities (Semenov, 2001; Semenov, 2011; Van Bruggen et al., 2006). Thus, the method for the quantitative determination of the heterotrophic (since glucose initiates the heterotrophic component of the soil MS) SH parameter has been developed and is available for use.

Like everything dialectical, the idea and method for determining of the soil health ecosystem needs implemented and developed. It is well known that

providing the soil ecosystem with N and P compounds is key for the functioning of any pedocenosis, and for organic farming, it is critical. The need arose for a method for determining the SH, taking into account the influence of key biophilic elements (N, P) in the soil ecosystem. In other words, the method for judgments the ability of soil ecosystem to self-sufficiency by the biophilic elements - N, P were needed.

### **Method for determining the ability of a soil ecosystem to self-sufficiency in biophilic elements - N, P.=**

It is generally known that the deficiency of N and P compounds in PE is a key limiting factor in the functioning of any natural pedocenosis, this is especially significant for consumers of agrophytocenosis, and becomes critical for organic farming (Semenov et al., 2016). For assess the health of the soil knowledge is required not only about the activity of the general biotic processes in the soil, which reflects the soil SIR indicator, but also knowledge about the “closedness” of the cycles of biophilic elements in SE, or the ability of SE to self-sufficiency in biophilic elements. By “closedness” is meant the ability of a healthy soil ecosystem to local, cyclical self-provision with biophilic elements, primarily nitrogen (N) and phosphorus (P) compounds (Semenov, Sokolov, 2016; Semenov, Semenova, 2018). We experimentally studied the processes of the dynamics of nitrogen fixation and ammonification (as the input of N into SE) and nitrification and denitrification (removal of N from SE) (Emer et al., 2014; Semenov et al., 2019). The dynamics of these experiments demonstrated that the processes, especially nitrogen fixation, but also ammonification in SE, have serious limitations, which do not allow us to consider them as indicators of the “closedness” or “openness” of the processes of N entry into SE.

It was proposed to "judge" the state of the cycles of "biophilic elements" by the activity of the microbial community (MC) after the disturbing effect - the enrichment of the soil with mineral compounds of these elements (N, P). To do this, it was proposed to enrich soil samples by analogy, to identify the heterotrophic parameter of SH, simultaneously with carbon and nitrogen substrates, and, if necessary, with phosphorus. To measure the soil SIR, the rate (V) of CO<sub>2</sub> emission induced by these biophilic elements, in dynamics (by analogy with the determination of the heterotrophic parameter of the SH). Samples of the compared soils (investigated and “conditionally healthy” - reference) are incubated under optimal conditions of humidity and temperature. The SIR inductors are aqueous solutions of glucose, ammonium nitrate, or (if necessary!) disubstituted sodium phosphate. Determination of SIRs is carried out daily for at least 5 days. The SIR indicator in this case acts as an induced parameter of the health of the soil, soil ecosystem, characterizing its carbon,

nitrogen, and even phosphorus exchange of this SE. The calculation of the "nitrogen" and "phosphorus" parameters of the SH is performed in the same way as when calculating the heterotrophic parameter of the SH (Semenov et al., 2011; Semenov, Semenova, 2018).

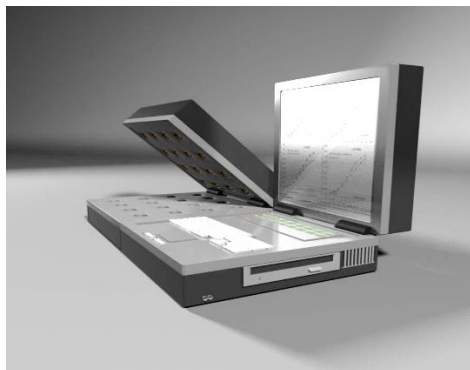
These methods, already widely known, but of course, need to be developed and supplemented. The methods urgently need to be supplemented with several more methods. There is an urgent need for express methods for testing the chemical and biological toxicity of a healthy soil ecosystem. We need not only general methods for determining the SH, we also need specific methods. Methods for determining the specific "bacterial" and "fungal" SH are needed to differentiate the state of the source of health and the sources of the SE disease, for the differential diagnosis of SE. Methods are needed that would reflect the role and contribution of soil mesofauna to the health of the soil ecosystem. It was noticed long ago that in the soil, in common parlance, called rich, fertile, many different soil biota live, in particular, for example, earthworms. In this regard, new, different indicators are needed that would reflect the role and treasure of a very significant component of the soil mesobiota. Soil mesobiota is an incubator and distributor of various microorganisms. To do this, it is necessary to study the influence of the population of, for example, earthworms on the microbial activity of the soil, to determine the contribution of the population of earthworms to the health of the soil ecosystem.

Thus, methods of quantitative determination of SH, general and one specific parameter - nitrogen and phosphorus are considered. The problems of further development of general, specific and special methods for determining the SH are discussed. All such methods require widespread application and accumulation of databases on SH in different soils and in different conditions.

**The tool for the determination the health of soil through quantitative determination of greenhouse gas emissions: CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O from soil sample; automation and exclusion of subjectivity**

It is obvious that the more data is obtained and, therefore, accumulated on the health parameters of different soils; different parameters and different characteristics that contribute to the knowledge of the soil ecosystem, the more adequately it will be possible to manage both the fertility and quality of soils and the preservation of ecosystems. The proposed methods for determining soil health parameters are relatively easy to use. However, any scientific methods and approaches require knowledge, skills and time, which is most expensive. For the widespread use of the proposed methods for determining the SH, devices are needed that would facilitate, help and save the user's time and results. For this purpose, it was proposed to create an automated, robotic, computerized tool. The

design and software of such a device would make it possible to determine the current (actual) state of the SH, compare and save the data, of course, contribute to the protection of the environment and climate, since parting is programmed to determine three greenhouse gases  $\text{CO}_2$ ,  $\text{CH}_4$ ,  $\text{N}_2\text{O}$ .



A description of the proposed device is in the patent, which is publicly available (in web) and of course the author has (Semenov et al., 2009; [https://yandex.ru/patents/doc/RU90212U1\\_20091227](https://yandex.ru/patents/doc/RU90212U1_20091227)). The introduction of such a tool into practice will undoubtedly contribute to the production of products and the protection of the environment.

**To the method for determining the SH for cultivated areas of agro-soil adjacent or distant from each other, of the same genesis and landscape, but in the absence of "fallow", i.e. (control) as known to be healthy soil**

The soil health can be quantified using substrate-induced respiration using the samples from two or more the soils. According to the developed method, as a comparative, i.e. control ("healthy") soil sample, it is recommended to use a known healthy soil, for example, "fallow" soil, of the same genesis and landscape. However, to find (select) such sample of "fallow", i.e. knowingly healthy soil is not always possible. The problem arises not only for non-specialists in soil science, but also even for specialists in soil sciences. The problem becomes more complicated when it becomes necessary to determine the SH of two plots of field soil of the same genesis and landscape, but distant from each other, but "fallow", i.e. there is no known healthy soil of the same genesis and landscape. If this problem is solved subjectively, then there will be unreliable results in the SH and wrong decisions will be made.

We have proposed to solve this problem by determining the amount, i.e., the concentration of the substance - geosmin (geosmin) in the compared soil samples. Geosmin is a product of the vital activity of soil microorganisms: bacteria of the actinomycete line, the genus streptomycetes, blue-green bacteria (cyanobacteria) and possibly microomycetes. This compound is a gaseous volatile organic substance, i.e., in general, is a product of the soil microbial community. The concentration of this substance is also associated with the "smell" of freshly cultivated soil (plowed, moistened, that is, exposed to the disturbing effect [URL: [http://aromatic.ucoz.ru/publ/chem\\_pakhnet\\_zemlja/1-1-0-20](http://aromatic.ucoz.ru/publ/chem_pakhnet_zemlja/1-1-0-20)].

The concentration of this substance, geosmin, can be determined chromatographically by comparing two or more soil samples. Assuming, for example, that "healthier soil has a higher concentration of geosmin." The proposed method, along with the proposed device for measuring greenhouse gas emissions from SE, will allow solving many everyday difficulties in determining the SH.

### **Molecular biological methods (MBM) in the determination of SH**

Before discussing the application of molecular biological methods (MBM) and their capabilities in determining the SH, let us name some of the advantages of MBM for the ecology of soil ecosystems. The advantage of MBM is modernity, attractiveness. Molecular biological methods make it possible to determine the list of bacteria in a soil sample. MBM essentially gives the results of the microbial biodiversity of the soil sample, suggesting the potential activities of the uncultivated microbiome.

The known method for the determination of SH, which in essence can be called traditional, is based on the measurement of substrate-induced respiration (SIR) of soil samples after the initiation of respiration of microorganisms by the substrate (glucose). This approach is based on one of the important population laws of biology (ecology): "only that process is significant and noticeable if this process is carried out by microorganisms, of which there are many and they are active" (Semenov et al., 2019a).

The SIR method for determining health also has several advantages. The main advantage is that the method provides relevant results. The disadvantages of the method are its limitations: only aerobic microorganisms (MO) are "taken into account", only glucose-active microorganisms, the method does not include MOs that are in an inactive state (although their contribution and is not important for actual health). The explanations for the listed restrictions are as follows: most agricultural crops, with the exception of the rice rhizosphere, grow under aerobic conditions; the beginning of the metabolism of aerobic microorganisms - glycolysis, where the initial metabolite is glucose. The cycle of tricarboxylic acids and as the beginning of the respiratory chain - everywhere begins with glucose; inactive cells - do not affect health either positively or negatively.

MBM also has some limitations. MBM studies of SE provide only potential, predictive results. They have many objective difficulties, performance difficulties. MBMs are very expensive, time-consuming, complex, requiring a highly qualified technician and laboratory.

To use the MBM to determine the SH, it is necessary, of course, to compare again, the performance indicators of the test subject and obviously healthy soils, i.e., the principle of comparing dynamics is inevitable. The results



of which MBMs used for the analysis of the two compared soils can be applied in determining the SH? For example, the results of the DGGE method or metabarcoding in the form of operational taxonomic units - OTU, i.e., amplicons, (sequences), of the test soil and selected as healthy soil. For example, to compare the dynamics of the number of OTUs for several days after the disturbing impact, as it is in the publication (Semenov et al., 2013).

It is obvious that in order to transform the results of "comparative bacterial biodiversity" into the results of the SH assessment, either fundamentally new approaches or adaptation of known ones are needed. Of the known, it is possible to try to adapt the approach and calculation of the SH index by analogy with the already approved approach in the method for determining salary based on the dynamics of SIR. So, the use of MBM to determine the SH needs serious development and testing.

**Determination and use of the suppressive activity properties in soil ecosystems; methods of activation (induction) of such activity and methods of healing and therapy of soil ecosystems.**

The suppressive activity of soil ecosystems in relation to some microorganisms is a well-known property and is already being used in practice. This property requires further development of assessment the methods, its stimulation and management methods. It should be noted that the phenomenon of the suppressive activity of the soil ecosystem is more appropriate to consider and discuss for organic farming soils. In agro ecosystems that use even "low-input" agriculture and furthermore conventional agriculture the suppressive properties of soils are little or no significance. Still for today some methods for determining the suppression activity of SE are already known. (Alabouvette et al., 2004; Toropova, Kirichenko. 2015; He et al., 2012). Methods of activation (induction) of suppressive activity of SE are also known (Toropova et al., 2016; Toropova et al., 2017). However, the above-mentioned methods of detecting, evaluating and using knowledge about soil suppressiveness, with their undoubted necessity and significance, have a complex of drawbacks. This is the tangible complexity and duration of the analysis (time and laboriousness), the need for a microbiological laboratory and a qualified analyst - performer and other. This limits their availability for the average land user. Therefore, it is necessary to use and improve other fundamental approaches that are proposed for the "treatment" of soils (Semenov, Semenova, 2018). Therefore, to solve the problems of determining and using the properties of the suppressive activity of PE in everyday practice, for the widespread use of methods of activation (induction) of suppressive activity of PE, further serious developments are needed. New important realizations need to be used. Soil is a biosystem, an ecosystem. For a living system, the category of

health is appropriate. The category of health undoubtedly includes many components. Soil suppressive activity is diverse. There are proposals to consider the suppressive activity of the soil, by analogy with the immunity of animals, as a manifestation of the immunity of the soil ecosystem. To do this, it is necessary to involve knowledge about the soil microbial community, to consider the soil microbiome as a source and generator of the soil immune response (Raaijmakers, Mazzola, 2016).

### **Oligotrophication of terrestrial ecosystems as a methodology for chronic health improvement of soil ecosystems**

It is quite obvious that all or almost all of the proposed methods, when applied, can show their significance, efficiency, first of all, in organic agriculture (organic agriculture, organic farming system). Slightly less - with highly intensive agriculture (conventional agriculture). However, the technology of oligotrophication of agroecosystems, as a method of conscious management of agrosystems, is applicable not only to organic farming technologies. This method can (and should!) be used to maintain and health any SE. There is a discussion of approaches to ligotrophication of agroecosystems as a way to improve and maintain soil health (Semenov et al., 2011; Semenov at al., 2017).

It remains only to understand that oligotrophic agroecosystems are not only more environmentally friendly, but also more economically profitable due to the reduction of irreplaceable energy costs for "eutrophication.

### **Conclusion**

The proposed methods are undoubtedly significant and their demand is increasing. These methods are not equivalent in their elaboration. Some of the methods need to be improved and calibrated. A broad comparison database is also needed. However, the intensity of the development of the SH problem, progress in the development of methods and their application, depends largely on the reaction of the world, first scientific, and then consumer society. The discussed methods are mainly developed by the authors of this communication. The answer to a possible question about the contribution of the international scientific community in the development of the SH topic, in the development of methods for determining the SH, given that the SH problem was posed by American scientists (USA), is rather difficult to find. An analysis of the literature leads to an interesting conclusion. In the USA and Western European countries, the scientific community and practitioners focused on the "depths" of the use of organic farming, and the studies of the theory and practice of soil health, the health of the soil ecosystem were postponed for "later" (\* [www.greencoverseed.com](http://www.greencoverseed.com)).

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## **RURAL TOURISM IN THE COVID-19 PERIOD IN SERBIA WITH PREDICTIONS OF DEVELOPMENT IN THE POST COVID PERIOD**

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### **Abstract**

*Serbia has numerous resources for the development of tourism, with the inclusion of economic and non-economic activities. The spread of the corona virus for almost more than two years has left severe consequences on the travel and tourism sector in the world and in Serbia. The aim of the research was to determine the extent to which the quality of rural service in the villages of Serbia was achieved during the pandemic, because it is known that during this bad period all movements were focused on domestic tourism. The authors of the paper conducted a field survey in 30 rural households, which provide services to visitors. The research was conducted in the period of 2021 on a total sample of 329 respondents. The obtained data were analyzed in the software SPSS, version 26.00, and descriptive analysis determined the average scores for all factors of rural service quality. Then, the goal was also to determine whether a given group of factors can predict the future development of rural tourism, but through the mediator COVID-19. Multiple regression analysis with mediation was performed. The importance of the research is reflected in determining the current quality of service in Serbian villages during the pandemic, and based on corrective measures to achieve better business after the pandemic and ensure long-term business with minimal costs in times of crisis.*

**Key words:** rural development, tourism, COVID-19, Serbia

## **Introduction**

Rural tourism is related to the rural environment and its surroundings, as well as all activities that take place in it: gastronomy, agriculture, ethnology, rural events and other economic and entrepreneurial activities. Today, there are more than 900 rural households registered in Serbia to work with tourists. Among them, there are almost a hundred who host up to 1.300 people a year. Many people recognize how important rural tourism is for our country. Since 2004, the Ministry of Agriculture, with its funds, ie competitions for incentive funds, has turned to households engaged in rural tourism. Later, the Ministry of Trade, Tourism and Telecommunications joined the credit funds, and there are various foreign associations that deal with it, and even some embassies helped the development of rural tourism in Serbia. The corona virus pandemic has threatened the tourism industry around the world, which has guided tourists to visit domestic destinations. This automatically meant an increase in visitors to Serbian villages, over 90%. Unlike "urban" tourism, resorts and mountains, where the consequences are particularly serious, rural tourism has experienced a real renaissance. The authors of the paper aimed to determine the current state of quality of rural tourist offer in 30 rural households in Serbia, during 2021, during the COVID-19 pandemic (Cvijanović et al., 2021). After determining the average score for all quality factors, the authors commented that determining the impact of these factors on predicting the future development of rural tourism in the postcovid period. Exploratory factual analysis and hierarchical factor analysis were used to obtain the desired number of factors, according to the authors, the required number of factors. Then, multiple linear regression to determine the influence of mediators on the predictive power of factors. The obtained data indicate partial mediation or direct influence of each factor on the score on the criterion variable.

The importance of research is reflected in the current state of quality of rural tourist offer in a time when the crisis situation for all forms of tourism, including rural tourism. With the help of the obtained data, service providers will be able to contribute to better strategic development in the future, which means reduced costs and long-term business. However, the importance of exploration serves broader economic and social purposes, especially when it comes to looking at the consequences of a pandemic and preparing for perhaps similar crises in the future.

## **Literature Review**

Serbia is a country where rural tourism is yet to see the light of day and get a well-deserved place in the domestic and world tourism market. However, in recent years, due to the COVID-19 pandemic, the development of many economic activities has been halted, but tourism has had the greatest consequences (Gajić et al., 2021; Milovanović et al., 2021). The tourism sector in Serbia is an industry that has suffered huge losses due to the corona virus pandemic. It is not easy to anticipate how many losses there will be and when the recovery of the tourism sector will begin, because it is still unknown how long Serbia and the world will fight COVID-19, but they are estimated at millions of euros. In the first five months of 2020, the total number of tourist arrivals in Serbia dropped by 52.8 percent compared to the same period in 2019, according to a report by the Ministry of Trade, Tourism and Telecommunications. In May 2020, the total number of tourist arrivals decreased by 87.6 percent compared to May 2019, and when it comes to foreign tourists, this decline is 97.8 percent (Cvijanović et al., 2020). The pandemic and the fear of the virus are changing the rules in tourism, and that is felt everywhere (Bourar et al., 2020). There is a need for a better understanding of travelers and employees in the crisis period (Assaf et al., 2021). Touat and Tabani (2021), found in their research that the pandemic made irreparable losses in air traffic and tourism in Algeria. Tourism is a key sector in the sustainable development of rural areas. Its ability to create stable employment and acceptable levels of profit is conditioned by the stability of tourism activity throughout the year (Martinez, 2019). Fears of consumer market disruption in crisis situations affect human behavior in this unique situation (Laato et al., 2020). One of the most important issues of the current topic is the efficient preservation and rational use of existing ecological, climatic, health and recreational resources of summer resorts and rural areas (Ivolga & Erokhin, 2013). Tourism is particularly vulnerable to pandemic measures due to limited mobility and social distancing. The paper compares the impact of COVID-19 on previous epidemics / pandemics and other types of global crises and explores how a pandemic can change society, the economy and tourism (Gosling et al., 2021). Rural destinations became the most attractive choice after the outbreak of the pandemic. Rural areas have been a great alternative during the summer for those tourists who want to travel while still maintaining social distance Petrović et al., 2018; (Rosalina et al., 2021). Based on the latest preventive measures taken by countries, recent developments in Asia and the pattern of previous crises (SARS 2003, economic and financial crises 2008-2009), the UN World Tourism Organization (UNWTO) estimates a reduction of 20% to 30% in international arrivals, tourists and corresponding economic earnings in 2020 compared to 2019 (Romagosa, 2020).



The crisis particularly emphasizes the need for local communities that rely on rural tourism to improve their resilience to the risks posed by both pandemics and the accelerated impacts of climate change (Gabriel-Campos et al., 2021). In the case of Portugal, the propensity for tourism in rural areas has increased significantly and the regions best positioned to offer rural accommodation have experienced a stronger and more lasting recovery in domestic demand (Carlos et al., 2021). Polukhina et al. (2021), discusses issues of sustainability in rural areas and claims that tourism is one of the most promising sectors for the development of domestic tourism on the example of the Russian tourism industry in the period COVID-19. According to empirical results, a large number of tourists have recently preferred rural tourism. Potential participants in rural tourism paid the most attention to the realization of performances and the time cost of picturesque places, while the psychosocial risk posed by COVID-19 had little impact. The inherent risk nature of risk aversion attitudes made pneumonia risk knowledge less effective in reducing tourists' intentions, while pneumonia risk knowledge was more effective in alleviating the perception of potential tourists' risk perception of rural tourism (Zhu & Deng, 2020). As consumers return to old habits, they are likely to be modified by new regulations and procedures in the way consumers buy and purchase products and services. New habits will also emerge with advances in technology, changing demographics, and innovative ways consumers have learned to cope with blurring boundaries at work, leisure, and education (Sheth, 2020).

Based on the issues of study and the given literature, the following hypotheses have been set:

*H1: Good quality of rural service has been achieved in rural households in Serbia.*

*H2: Based on grouped factors, it is possible to predict the Development of rural tourism in the post COVID period.*

*H3: Complete mediation or indirect effect of factors on the criterion variable was achieved.*

## **Methodology**

### **Sample and Measurement procedure**

In 2021, a field survey was conducted in 30 rural households in Serbia (Vojvodina - 8 rural households, Eastern Serbia - 12 rural households and Western Serbia - 10 rural households), which provide services to tourists.

The period marked by the outbreak of the COVID-19 pandemic certainly contributed to slower research, as many facilities were closed during the year. The total sample consisted of 329 respondents, of which 62.1% were women and 37.9% were men. Regarding the age structure, it is as follows: a total of 18.3% aged 18 to 25, then 24.6% aged 26 to 36, about 20% aged 37 to 50 and 37.1% of respondents over 50. The largest number of research participants was 63.6% with a university degree, 25.2% with a high school diploma and 12.2% with a PhD or MSc degree. When looking at earnings, a total of 19.7% have a monthly salary below 200 euros, then 35.9% between 200 and 500 euros, a total of 33.4% between 500 and 1.000 euros, and 11% over 1.000 euros. Descriptive statistical analysis was used to determine average scores, while exploratory factor analysis was used to group items. Factor analysis is a technique of interdependence because it looks for a group of variables that are similar in the sense that they "move together" and therefore have great interdependence. When one variable has a large value, then the other variables in the group have a large value. one of the main goals of factor analysis is to look for a group of similar statements by respondents because they express the same basic idea in ways that differ in nuances. To obtain a smaller number of factors, the authors used higher order factor analysis or hierarchical factor analysis. Then, in order to determine the predictor influence of factors on the future development of rural tourism through mediators, multiple linear regression was used. The mediator effect is the association of one variable with another, in such a way that this correlation is, in fact, determined by the third variable. In other words, the interconnection of two variables of main interest is realized in part or in full thanks to the third, ie. mediator variable. Categorical variables (features) are shown by relative (%) frequency. The central tendency of numerical features is shown by the arithmetic mean (m) and the scatter by the standard deviation (sd). The frequency distribution of numerical features was examined with indicators of Skewness and Kurtosis. Since all variables are normally distributed, parametric statistics methods were used. The value of Cronbach's alpha is 0.927, which makes the questionnaire very reliable.

## Results and Discussion

Table 1 gives average estimates or arithmetic values, as well as standard deviations for all 29 items, which will later be grouped by exploratory factor analysis as well as hierarchical factor analysis.

Table 1. Descriptive values of given items

| <b>Descriptive Statistics</b>                 |          |           |
|-----------------------------------------------|----------|-----------|
|                                               | <b>m</b> | <b>sd</b> |
| All tourist requirements are met              | 1.45     | .715      |
| Meeting the wishes and needs of tourists      | 1.79     | .841      |
| Providing assistance                          | 1.89     | .860      |
| Timely response to problems                   | 1.74     | .857      |
| Care for a pleasant atmosphere                | 1.89     | .806      |
| Each guest is treated as an individual        | 2.34     | .819      |
| The hosts know a foreign language             | 2.11     | .806      |
| The hosts do the job properly                 | 1.98     | .900      |
| The hosts provide all the information         | 1.99     | .839      |
| The hosts are always present in the household | 1.93     | .884      |
| The hosts are always available to guests      | 1.69     | .892      |
| The hosts provide guide roles                 | 1.81     | .758      |
| The hosts know all the ingredients            | 1.78     | .886      |
| Provided quality food                         | 2.34     | .614      |
| Hygiene at a satisfactory level               | 2.64     | .876      |
| Homemade food and products                    | 1.90     | .923      |
| Souvenir availability                         | 2.19     | .725      |
| Preserved furniture                           | 1.81     | .951      |
| Interior authentic                            | 1.61     | .762      |
| Exterior rural                                | 1.12     | .410      |
| Provided security to guests                   | 1.45     | .740      |
| All rooms are marked                          | 2.08     | .927      |
| Credible marketing                            | 2.60     | .622      |
| Price is a reflection of quality              | 1.95     | .843      |
| Costume as a form of marketing                | 1.11     | .772      |
| Tourist animation                             | 1.91     | .889      |
| Tasting of domestic products                  | 1.61     | .907      |
| Sports and recreational activities            | 2.06     | .594      |
| Field trips                                   | 2.32     | .859      |

Source: authors reserach, \*m=arithmetic mean;sd= standard deviation

It can be noticed that the highest average rating of the service is credible marketing  $m = 2.60$ , then item each guest is treated as an individual  $m = 2.34$  and the inclusion of excursions as activities for tourists  $m = 2.32$ . commercialization, and that tourists do not see the authentic rural interior, which is not good for the future development of rural tourism. Tasting of domestic products was also present ( $m = 1.61$ ;  $sd = 0.907$ ). Quality food is provided to tourists, which is the average arithmetic value for this item  $m = 2.34$ . Hygiene is also at a satisfactory level ( $m = 2.64$ ;  $sd = 0.876$ ). The attitude of the hosts towards the guests and towards the provision of services in general, in general, all items have good marks. Hypothesis H1 was confirmed that a good quality of the complete rural offer was achieved in rural households.

An exploratory factor analysis was performed to determine the number of factors that grouped all questions. In this study, 29 basic variables were reduced by exploratory factor analysis to eight latent factors with properties greater than one, the values of which are shown in the table. So this model explains a total of 85% of the variance, which is very good.

**Table 2. Exploratory factor analysis - Total Variance Explained**

| Component | Initial Eigenvalues |               |              | Extraction Sums of Squared Loadings |               |              | Rotation Sums of Squared Loadings <sup>a</sup> |
|-----------|---------------------|---------------|--------------|-------------------------------------|---------------|--------------|------------------------------------------------|
|           | Total               | % of Variance | Cumulative % | Total                               | % of Variance | Cumulative % | Total                                          |
| 1         | 7.915               | 27.292        | 27.292       | 7.915                               | 27.292        | 27.292       | 6.180                                          |
| 2         | 5.115               | 17.638        | 44.929       | 5.115                               | 17.638        | 44.929       | 6.323                                          |
| 3         | 3.474               | 11.978        | 56.907       | 3.474                               | 11.978        | 56.907       | 2.922                                          |
| 4         | 2.713               | 9.355         | 66.262       | 2.713                               | 9.355         | 66.262       | 3.434                                          |
| 5         | 1.824               | 6.289         | 72.552       | 1.824                               | 6.289         | 72.552       | 3.355                                          |

|    |           |       |         |           |       |        |       |
|----|-----------|-------|---------|-----------|-------|--------|-------|
| 6  | 1.38<br>2 | 4.766 | 77.317  | 1.38<br>2 | 4.766 | 77.317 | 2.933 |
| 7  | 1.31<br>1 | 4.520 | 81.838  | 1.31<br>1 | 4.520 | 81.838 | 4.615 |
| 8  | 1.00<br>5 | 3.464 | 85.302  | 1.00<br>5 | 3.464 | 85.302 | 3.064 |
| 9  | .823      | 2.837 | 88.138  |           |       |        |       |
| 10 | .497      | 1.713 | 89.851  |           |       |        |       |
| 11 | .457      | 1.576 | 91.427  |           |       |        |       |
| 12 | .328      | 1.132 | 92.559  |           |       |        |       |
| 13 | .312      | 1.075 | 93.635  |           |       |        |       |
| 14 | .277      | .955  | 94.590  |           |       |        |       |
| 15 | .222      | .766  | 95.356  |           |       |        |       |
| 16 | .212      | .730  | 96.086  |           |       |        |       |
| 17 | .180      | .619  | 96.705  |           |       |        |       |
| 18 | .157      | .542  | 97.247  |           |       |        |       |
| 19 | .134      | .460  | 97.708  |           |       |        |       |
| 20 | .115      | .398  | 98.105  |           |       |        |       |
| 21 | .104      | .358  | 98.463  |           |       |        |       |
| 22 | .089      | .307  | 98.770  |           |       |        |       |
| 23 | .079      | .272  | 99.042  |           |       |        |       |
| 24 | .069      | .237  | 99.280  |           |       |        |       |
| 25 | .055      | .188  | 99.468  |           |       |        |       |
| 26 | .049      | .169  | 99.637  |           |       |        |       |
| 27 | .040      | .138  | 99.775  |           |       |        |       |
| 28 | .034      | .117  | 99.892  |           |       |        |       |
| 29 | .031      | .108  | 100.000 |           |       |        |       |

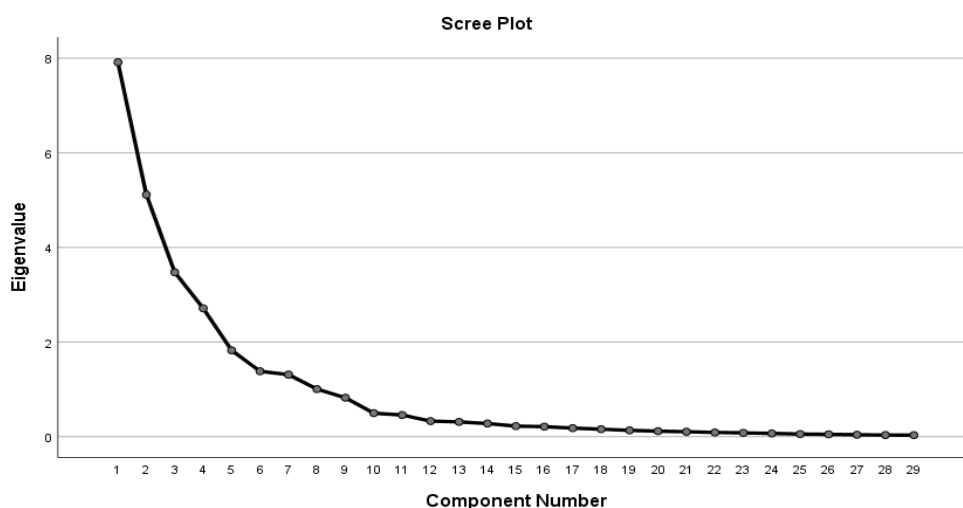
Source: authors reserach ; Extraction Method: Principal Component Analysis.

a. When components are correlated, sums of squared loadings cannot be added to obtain a total variance.

Variables that have little in common with other variables and have a small factor load (less than 0.30) according to all common factors are often called "independent variables". The factors that have the greatest impact (the greatest factor load) and cover most of the variability of the data are factors for further study. The most common method used is the latent root criterion. According to this criterion, only those factors that have eigenvalues greater than 1 are taken into account. Factors that have less eigenvalues greater than 1 are considered irrelevant because they explain less variability than the variables themselves. The highest saturation has the first factor of 27.2%, while the last factor has the lowest saturation, at the very limit of acceptability of 3.46%. Anything below the value of one is automatically discarded.

The Scree Plot chart is used to determine how many factors we want to keep and in principle the rule is that the "cut" should be made in the fold. In this case, the existence of eight factors is confirmed, which can be seen at the intersection.

Graph 1. Graphic representation of the obtained factors



Source: authors reserach

Before starting a higher order factor analysis procedure, one should check whether the variables are correlated and use the Bartlett sphericity test (tests the null hypothesis that the matrix of intercorrelation of variables inserted in the identity matrix procedure is zero). Bartlett's test for sphericity compares your correlation matrix (Pearson's correlation matrix) with the identity matrix. In other words, it checks for redundancy between variables that can be summed up with some factors. If the Bartlett test is not statistically significant, the application of factor analysis does not make sense (without a strong theoretical justification). The value of the Bartlett test for the statistical significance of the correlation

matrix is  $\chi^2 = 11042.995$ , with degrees of freedom  $df = 306$  and  $p = 0.00$ , which confirms the suitability of statistical processing of the collected data by factor analysis. According to the Kaiser-Guttman criterion, only those factors that best explain variability greater than one are considered. The Kaiser-Meier-Olkin (KMO) test is a measure of how suitable your data is for factor analysis. The test measures the adequacy of sampling for each variable in the model and for the complete model. Statistics is a measure of the proportion of variance among variables that could be common variance. The lower the proportion, the more relevant your data is to factor analysis. KMO values between 0.8 and 1 indicate the sampling is adequate (Dodge, 2008). In this case, the value of KMO is 0.912. From the previous table it can be seen that this criterion in this case satisfies the first eight components, which cumulatively explain 85% of the variance. In the case of a higher order hierarchical factorial analysis, the percentage of explained variance is lower, but still acceptable. We found that the percentage of explained variance was 57%, but we obtained the desired number of factors, based on which we will more easily perform multiple regression analysis with mediation. We named the factors: General quality, Attitude towards the guest, Host as a service provider.

**Table 3. Hierarchical factor analysis - Total Variance Explained**

| Component | Initial Eigenvalues |               |              | Extraction Sums of Squared Loadings |               |              | Rotation Sums of Squared Loadings <sup>a</sup> |
|-----------|---------------------|---------------|--------------|-------------------------------------|---------------|--------------|------------------------------------------------|
|           | Total               | % of Variance | Cumulative % | Total                               | % of Variance | Cumulative % | Total                                          |
| 1         | 7.915               | 27.292        | 27.292       | 7.915                               | 27.292        | 27.292       | 6.974                                          |
| 2         | 5.115               | 17.638        | 44.929       | 5.115                               | 17.638        | 44.929       | 6.540                                          |
| 3         | 3.474               | 11.978        | 56.907       | 3.474                               | 11.978        | 56.907       | 3.782                                          |
| 4         | 2.713               | 9.355         | 66.262       |                                     |               |              |                                                |
| 5         | 1.824               | 6.289         | 72.552       |                                     |               |              |                                                |

|    |           |       |         |  |  |  |  |
|----|-----------|-------|---------|--|--|--|--|
| 6  | 1.38<br>2 | 4.766 | 77.317  |  |  |  |  |
| 7  | 1.31<br>1 | 4.520 | 81.838  |  |  |  |  |
| 8  | 1.00<br>5 | 3.464 | 85.302  |  |  |  |  |
| 9  | .823      | 2.837 | 88.138  |  |  |  |  |
| 10 | .497      | 1.713 | 89.851  |  |  |  |  |
| 11 | .457      | 1.576 | 91.427  |  |  |  |  |
| 12 | .328      | 1.132 | 92.559  |  |  |  |  |
| 13 | .312      | 1.075 | 93.635  |  |  |  |  |
| 14 | .277      | .955  | 94.590  |  |  |  |  |
| 15 | .222      | .766  | 95.356  |  |  |  |  |
| 16 | .212      | .730  | 96.086  |  |  |  |  |
| 17 | .180      | .619  | 96.705  |  |  |  |  |
| 18 | .157      | .542  | 97.247  |  |  |  |  |
| 19 | .134      | .460  | 97.708  |  |  |  |  |
| 20 | .115      | .398  | 98.105  |  |  |  |  |
| 21 | .104      | .358  | 98.463  |  |  |  |  |
| 22 | .089      | .307  | 98.770  |  |  |  |  |
| 23 | .079      | .272  | 99.042  |  |  |  |  |
| 24 | .069      | .237  | 99.280  |  |  |  |  |
| 25 | .055      | .188  | 99.468  |  |  |  |  |
| 26 | .049      | .169  | 99.637  |  |  |  |  |
| 27 | .040      | .138  | 99.775  |  |  |  |  |
| 28 | .034      | .117  | 99.892  |  |  |  |  |
| 29 | .031      | .108  | 100.000 |  |  |  |  |

Source: authors research; Extraction Method: Principal Component Analysis.

a. When components are correlated, sums of squared loadings cannot be added to obtain a total variance.



After determining exactly three factors with the help of hierarchical factor analysis, multiple linear regression was approached, in order to determine the predictor influence of factors on the criterion variable, with the help of a mediator effect. In this case, the mediator is COVID-19, and thus it was determined whether partial or indirect full mediation is present in the forecast soon on the criterion variable development of rural tourism in the post COVID period.

**Table 4. Model Summary- Percentual explanation of variance**

| Model | R                 | R Square | Adjusted R Square | Std. Error of the Estimate | Change Statistics |          |     |     |               |
|-------|-------------------|----------|-------------------|----------------------------|-------------------|----------|-----|-----|---------------|
|       |                   |          |                   |                            | R Square Change   | F Change | df1 | df2 | Sig. F Change |
| 1     | .996 <sup>a</sup> | .992     | .992              | .652                       | .992              | 9225.924 | 3   | 210 | .000          |
| 2     | .996 <sup>b</sup> | .993     | .992              | .646                       | .000              | 4.603    | 1   | 209 | .033          |

a. Predictors: (Constant), General quality, Attitude towards the guest, Host as a service provider.

b. Predictors: (Constant), Mediator COVID-19

Table 4 provides an insight into the percentage of saturation or explanation of variance. It can be noticed that the model fits or explains a total of 99.2% of the variation, which is considered to be an excellent percentage of the explained sample. It can also be noticed that even in the phase of introducing mediators, the state of explanation of variances is unchanged.

**Table 5. ANOVA<sup>a</sup>**

| Model |            | Sum of Squares | df  | Mean Square | F        | Sig.              |
|-------|------------|----------------|-----|-------------|----------|-------------------|
| 1     | Regression | 11760.011      | 3   | 3920.004    | 9225.924 | .000 <sup>b</sup> |
|       | Residual   | 89.227         | 210 | .425        |          |                   |
|       | Total      | 11849.238      | 213 |             |          |                   |
| 2     | Regression | 11761.934      | 4   | 2940.484    | 7039.301 | .000 <sup>c</sup> |
|       | Residual   | 87.304         | 209 | .418        |          |                   |
|       | Total      | 11849.238      | 213 |             |          |                   |

a. Dependent Variable: Development of rural tourism in the post COVID period

b. Predictors: (Constant), General quality, Attitude towards the guest, Host as a service provider.

c. Predictors: (Constant), Mediator COVID-19

Table 5 shows that in the first phase of multiple linear regression there is statistical significance in the power of factors to predict the criterion variable, which is the case even after the introduction of the VOCID-19 mediator, the statistical significance remains unchanged.

Table 6 shows the partial contribution of each of the given factors in two phases, before and after the introduction of the mediator. All three factors have a statistically significant contribution in predicting the Development of rural tourism in the post COVID period. B represents a standardized partial contribution to the prediction, more precisely how strong it is in itself to predict the score on the criterion variable. Beta is interpreted as a correlation, as far as each predictor is related to the criterion.

**Table 6. Coefficients<sup>a</sup> - Partial contribution factors**

| Model |                            | Unstandardized Coefficients |            | Standardize<br>d<br>Coefficients | t      | Sig. |
|-------|----------------------------|-----------------------------|------------|----------------------------------|--------|------|
|       |                            | B                           | Std. Error | Beta                             |        |      |
| 1     | (Constant)                 | -2.823                      | .342       |                                  | -8.248 | .000 |
|       | Attitude towards the guest | 4.744                       | .134       | .228                             | 35.412 | .000 |
|       | Host as a service provider | 6.900                       | .128       | .388                             | 53.971 | .000 |
|       | General quality            | 18.095                      | .218       | .635                             | 83.163 | .000 |
| 2     | (Constant)                 | -2.707                      | .344       |                                  | -7.879 | .000 |
|       | Attitude towards the guest | 4.797                       | .135       | .230                             | 35.515 | .000 |
|       | Host as a service provider | 7.087                       | .154       | .398                             | 46.073 | .000 |
|       | General quality            | 18.058                      | .216       | .633                             | 83.447 | .000 |
|       | Mediator COVID-19          | -.276                       | .129       | -.016                            | -2.145 | .033 |

a. Dependent Variable: Development of rural tourism in the post COVID period

The Attitude towards the guest factor statistically significantly predicts the criterion variable ( $B = 4.744$ ;  $\beta = .228$ ;  $p = 0.00$ ). Then, the factor Host as a service provider also significantly predicts the criterion variable with the following partial values:  $B = 6,900$ ;  $\beta = .388$ ;  $p = 0.00$ . The General quality factor gives the following values with statistical significance:  $B = 18,095$ ;  $\beta = .635$ ;  $p = 0.00$ . It can be seen from the same table that after the introduction of the mediator variable, the statistical significance of each predictor remains unchanged. The introduction of the COVID -19 mediator was also statistically significant from  $p = 0.03$ . This is about direct influence or partial mediation. Hypothesis H2 was confirmed, that it is possible to predict the Development of rural tourism in the post COVID period, based on the obtained three factors. However, hypothesis H3 is denied, because it is a direct influence of predictors or partial mediation.

## Conclusion

The concept of rural tourism is the broadest and refers to all activities in rural areas. Rural tourism is a type of tourism in which tourists go to villages or farms to experience rural life. The term rural tourism is used in many cases when rural culture is a key component of the offered tourist product. A rural tourist household is a facility or group of facilities in which accommodation, food and beverage services are provided. In order for the farm to generate more income, there is a possibility to achieve that by providing services to tourists - domestic and foreign. Maybe it's a chance for more young people to stay in the countryside. There are households that have turned to families with children, so their contents are the same. There are also those that are a bit cheaper, for mass visits of hikers and organized groups, and there are also very exclusive households, which have accommodation from 1,200 RSD for full board, up to 5,000 RSD for some who have practically a hotel offer. And as the number of countries in which Serbian citizens are currently allowed to enter is decreasing every day, one of the solutions is to spend the summer at one of the domestic destinations. The authors of the paper dealt with the issue of the achieved quality of rural service in rural households in Serbia, during 2021, when there was a pandemic that is still going on. In that period, tourist movements were focused mainly on domestic tourism, and rural areas. The aim of the research was to determine the quality of supply in rural households, and whether some of the given factors can predict the state of rural tourism and its supply in the post COVID period. Factor analysis was performed to determine the number of factors, but after that a hierarchical factor analysis of a higher order was performed, in order to obtain the desired number or number of factors. Of course, hierarchical analysis found that the level of saturation or the percentage of explanation of variance is still high. Three factors

have been identified as predictors. By multiple linear regression, it was found that factors can significantly predict the future development of rural tourism, but that the mediator variable does not play a significant role in changing the partial contribution of three factors to the criterion variable. Two hypotheses were confirmed, while the third hypothesis was denied, because it is a direct or partial mediation, and not indirect or full mediation.

The importance of research can certainly contribute to the improvement of scientific, economic and social spheres, in discovering the quality and current state of rural supply in Serbia, in this way it will be possible to predict consumer behavior or not. This will avoid unnecessary business problems, as well as unnecessary costs.

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## AGRICULTURE AND THE ECONOMIC SIGNIFICANCE OF LIVESTOCK PRODUCTION FOR THE REPUBLIC OF SERBIA

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**Abstract:** *The aim of this paper is to point out the state and possibilities of improving Serbian livestock production, based on the analysis of important available data. Namely, it is known that animal husbandry is a strategic branch of the economy and that the degree of its development significantly affects the intensity of agricultural development, and thus the overall economy of a country. The Republic of Serbia has extremely favourable natural conditions for breeding all species of domestic animals and a long tradition in livestock breeding. Also, our country has excellent crop production, ie. has an adequate fodder base for feeding far more animals than it now has. Also, it is known that in the Republic of Serbia there has been a trend of reducing livestock for years and that the warnings of experts about the need to stop this trend are becoming more dramatic. Based on the above, this paper presents the results of the analysis of the number by calculating average values for five-year periods, starting in 2000. In addition, the average production of important animal products such as meat, milk, eggs and wool by analyzed periods was calculated. Furthermore, when it comes to the number of livestock and their production, indexes were calculated and based on them, trends in the past two decades were analyzed. Based on the established trends in the above indicators, conclusions were drawn on the state and possibilities of further directions in the development of Serbian livestock production.*

**Key words:** *agriculture; livestock; the number of domestic animals in Serbia; production of milk, meat, eggs and wool; improvement of livestock production.*

### **Introduction**

Agriculture is synonymous with food production and food security in the country, but it is also important for the production of raw materials for other industries and export. It has multiple meanings and roles in the socio-economic development of the Republic of Serbia. When we add to this the availability of significant natural and human resources, as well as the achieved level of production and processing, social, demographic and other aspects, it is clear that agricultural production is determined as an extremely important economic activity in our country. In support of this are data on the share of GDP of agriculture in the total GDP of Serbia, and that share ranged from 17% in 2002, ie. 9.0% in 2012, to 7.8% in 2020. Although the above data show a declining trend, analyzes have shown that agricultural production itself is not the cause, but the fact that some other activities, primarily services, have significantly improved their position (Novaković, 2019).

Livestock production is a particularly important branch of agriculture. It provides the necessary products (milk, meat, eggs) for the nutrition of the domestic population. In addition, livestock production provides raw materials for the food industry, dairies, slaughterhouses, confectionery industry and leather processing industry. Livestock production in Serbia is facing serious challenges as a result of the transition and development of new applications in food production (Petrović et al., 2012). This branch of agriculture is expected to provide quality products for export, primarily beef and lamb. Also, there is an opportunity for the export of cheese (especially sheep and goat milk cheese) with a defined origin and standard of quality (Aleksić et al., 2009). In addition, livestock production as a strategic branch of an economy affects the intensity of agricultural development, ie. the intensity of agriculture is measured by the degree of development of livestock production and its participation in the structure of the total agricultural production. Hence, the share of livestock production in highly developed countries is more than 66% of the total value of agriculture. Also, 85% of crop production is used as animal feed. In this way, crop products are further refined and converted into high-quality protein products of animal origin.

Based on the above, statistical analyzes of data on the value of agricultural and livestock production in Serbia in the past two decades have been performed. These analyzes showed that the share of plant production in the total value of agricultural production in our country was on average about 69%, and the share of the value of livestock production was 31% (Novaković, 2009). Further analysis showed that the total value of crop production statistically significantly contributes to the formation of GDP agriculture, which leads to the conclusion that extensive production is still dominant in our country. On the other hand, the

total value of livestock production does not significantly contribute to the realization of the GDP of agriculture, so the search for answers and solutions to the long-term unfavourable state of livestock production in Serbia is still current and extremely important.

### **State and future trends for the development of livestock production in Serbia**

The number and quality of livestock are indispensable indicators for drawing relevant conclusions in assessing the state of livestock production. Also, it is known that the generation interval and the reproductive cycle in animal husbandry are quite long and they last several years for some species of domestic animals. In addition, the oscillations from year to year in numbers and production are large, and cyclical fluctuations are also characteristic. Given these specifics that significantly complicate the analysis of livestock production and achieved results, the analysis should include a longer period and multi-year results.

Based on the above, and to understand the trends in the number of livestock and poultry, as well as the production of the most important animal products, five-year averages for the period from 2000 to 2020, was calculated and presented in Tables 1 to 4.

Thus, Table 1 shows data on the number of cattle, as well as milk and beef meat production in the past two decades.

**Table 1. Number of cattle and production of cow's milk and beef meat in Serbia in period 2000-2020**

| Year      | Number of cattle (1000) | Index | Cow's milk production (million litres) | Index | Beef meat production (1000 t) | Index |
|-----------|-------------------------|-------|----------------------------------------|-------|-------------------------------|-------|
| 2000      | 1246                    | 100   | 1566                                   | 100   | 104                           | 100   |
| 2001-2005 | 1117                    | 89.6  | 1589                                   | 101.5 | 92.5                          | 88.9  |
| 2006-2010 | 1038                    | 83.3  | 1522                                   | 97.2  | 94.6                          | 91.0  |



|           |     |      |      |      |      |      |
|-----------|-----|------|------|------|------|------|
| 2011-2015 | 921 | 73.9 | 1472 | 94.0 | 76.6 | 73.7 |
| 2016-2020 | 891 | 71.5 | 1501 | 95.8 | 74.0 | 71.2 |

Source: Statistical Office of Serbia, Ministry of agriculture, forestry and water management

The data in Table 1 clearly show the decreasing trend in the number of cattle in our country. Thus, the average number of cattle in the previous five-year period was 891 000, which is 355 000 or almost a third less than in 2000, which can be concluded from the movement of the index, which in the five years 2016-2020 amounted to 71.5 compared to the index of 100 from 2000. The decrease in the number of cattle was especially reflected in the production of beef meat, which reduced from 104 000 tons in 2000 to 74 000 tons in the period 2016-2020. It can be concluded that the trend of decreasing beef meat production has continuously followed the decreasing trend of the number of cattle.

**Production of milk** in Serbia is one of the major livestock production regarding many agricultural households engaged in this production as well as in the value of this production which is additionally increased (added value) by the processing of milk into dairy products (Aleksić et al., 2009). It is important to note that the decrease in cow's milk production in the period 2000-2020 is much smaller. This can be concluded from the absolute numbers on the average milk production by the analyzed five-year periods, but also the calculated index. There is no doubt that such results in milk production are a consequence of the genetic improvement, but also the improvement of breeding technology. All this affected the increase in milk yield of cows and higher overall milk production in Serbia. It should be borne in mind that the current average milk yield per cow is 3 500 litres of milk in lactation, which is higher than the average production than it was in the first three analyzed five-year periods. However, we cannot be satisfied with the average milk yield per dairy cow, especially if know that it is lower by about 20% compared to the world average and by as much as 40% compared to the European average. With the production of about 1.5 billion litres of milk and about 74 000 tons of meat annually, cattle production in Serbia participates with about 41% in the value of our livestock production, while in the total meat production it has a share of 14%. In addition to the above data on the number of cattle and their production, should certainly be borne in mind that the Simmental breed makes up about 75%, a group of black and white Holstein-Frisian cattle about 15%, while beef, indigenous breeds and crossbreeds make up about 10% of the total number of cattle in Serbia (Institut for Animal Husbandry Belgrade-Zemun, 2021).

**Pig breeding** is another important branch of animal husbandry that participates significantly in the total meat production in Serbia and significantly contributes to the gross value of our livestock production. Table 2 shows data on the number of pigs and the production of pork meat.

**Table 2. Number of pigs and production of pork meat in Serbia in the period 2000-2020**

| Year      | Number of pigs<br>(1000) | Index | Pork meat<br>production<br>(1000 t) | Index |
|-----------|--------------------------|-------|-------------------------------------|-------|
| 2000      | 4066                     | 100   | 283                                 | 100   |
| 2001-2005 | 3488                     | 85.8  | 255.5                               | 90.3  |
| 2006-2010 | 3709                     | 91.2  | 266.2                               | 94.1  |
| 2011-2015 | 3218                     | 79.1  | 261.6                               | 82.4  |
| 2016-2020 | 2920                     | 71.8  | 301.5                               | 106.6 |

Source: Statistical Office of Serbia, Ministry of agriculture, forestry and water management

From the data in Table 2, we can see the decreasing trend in the number of pigs since 2000, analyzed based on five-year average values. Today, Serbia has about 2 900 000 pigs, which is about 28% less than in 2000. Despite the reduction in the number of pigs, pork meat production is stable with slight oscillations. The average annual production in the previous five-year period amounted to 301 000 tons, which is slightly more than in 2000 (by about 6.6%).

Pork meat production ranks second in Serbian livestock production, with a share of 42% in gross value. On the other hand, in the total meat production in Serbia, pork meat participates with more than 55%. Interestingly, family farms are engaged in pig farming in our country, with about 80% of the total number of heads. Namely, small farms and medium-sized farms are the most numerous and 2/3 of all pigs are reared on them. This is especially important because, in the description of the situation in this branch of animal husbandry, it is important to point out the dominant traditional breeding on family farms. On these farms, pig production has a highly expressed natural character, ie. the average marketability is low and amounts to only 20% in some years.

**Sheep breeding** is also an important branch of animal husbandry, which in our country significantly contributes to the overall results of livestock production. Table 3 shows the data on the number of sheep and the production of sheep meat, milk and wool in the past two decades.

**Table 3. Number of sheep and production of sheep's milk, meat and wool in Serbia in period 2000-2020**

| Year      | Number of sheep (1000) | Index | Sheep's milk production (million litres) | Index | Sheep meat production (1000 t) | Index | Wool production (t) | Index |
|-----------|------------------------|-------|------------------------------------------|-------|--------------------------------|-------|---------------------|-------|
| 2000      | 1611                   | 100   | 19                                       | 100   | 19                             | 100   | 2264                | 100   |
| 2001-2005 | 1523.2                 | 94.5  | 13.5                                     | 71.1  | 19.5                           | 102.6 | 2424.5              | 107.1 |
| 2006-2010 | 1549.2                 | 96.2  | 12.6                                     | 66.3  | 21.8                           | 114.7 | 2490.4              | 110   |
| 2011-2015 | 1649.6                 | 102.4 | 16                                       | 84.2  | 26.6                           | 140   | 2700                | 119.3 |
| 2016-2020 | 1681.6                 | 104.4 | 13.8                                     | 72.6  | 33                             | 173.7 | 2826                | 124.8 |

Source: Statistical Office of Serbia, Ministry of agriculture, forestry and water management

The data in Table 3 point to the conclusion that sheep breeding is the only branch of animal husbandry in Serbia where there has been a slight increase in the number of animals. Thus, in the last analyzed five-year period (2016-2020), about 1 681 000 sheep were reared in Serbia, which is about 70 000 more than in 2000. Also, the data in this table point to the conclusion that in the analyzed period, the production of sheep's milk was significantly reduced, but also the production of sheep's meat and wool was significantly increased. These results of sheep production point to the conclusion that in the past two decades, rearing

sheep for meat production has been an absolute priority concerning milk production, which is understandable because market demands and marketing opportunities were far more favourable for meat. On the other hand, it should be borne in mind that sheep's milk was only significantly realized through meat production because the milk was sucked by lambs in the period of their intensive growth and development.

Sheep breeding in Serbia is realized with about 98% in small and medium-sized (in terms of economic strength) agricultural households. This is especially important and should be kept in mind when creating future development programs that would encourage the intensification of sheep production in Serbia. By the way, the analysis showed that in the past few years, sheep production was in the fourth place in the gross value of Serbian animal husbandry with a share of about 8.7% and the fourth place in terms of meat production. According to Institut for Animal Husbandry Belgrade - Zemun (2019, 2021) about 80% of animals belong to Pramenka (the Sjenica strains makes about 80% and Svrljig about 16% of the controlled Pramenka population). Tzigai sheep participate with about 5% of the total sheep population, and 15% are crossbreds of Pramenka and imported foreign breeds. Merinolandschaf (72,5%) and Ile de France (21.5%) dominate the total number of imported breeds.

**Poultry breeding** in Serbia is also an extremely important branch of animal husbandry, which is best illustrated by the fact that, in the past decade, meat production is in second place in the total meat production in Serbia. Data on this, as well as on the movement of poultry and egg production are shown in Table 4.

**Table 4. Number of poultry and production of eggs and meat in Serbia in the period 2000-2020**

| Year      | Number of poultry (1000) | Index | Egg production (millions) | Index | Poultry meat production (1000 t) | Index |
|-----------|--------------------------|-------|---------------------------|-------|----------------------------------|-------|
| 2000      | 20360                    | 100   | 1374                      | 100   | 67.0                             | 100   |
| 2001-2005 | 17736                    | 87.1  | 1449                      | 105.5 | 63.0                             | 94    |
| 2006-2010 | 18635                    | 91.5  | 1293.8                    | 94.2  | 77.0                             | 114.9 |

|           |       |      |        |       |       |       |
|-----------|-------|------|--------|-------|-------|-------|
| 2011-2015 | 17963 | 88.2 | 1952.4 | 142.1 | 93.8  | 140   |
| 2016-2020 | 15968 | 78.4 | 1777.8 | 129.4 | 103.6 | 154.6 |

Source: Statistical Office of Serbia, Ministry of agriculture, forestry and water management

From the data in Table 4, the number of poultry has decreased in the period from 2000 until today. Thus, the average number of poultry in the last five-year period (2016-2020) was lower by 21.5% than in 2000 and about 10% lower than the average for the period 2001-2005. On the other hand, the production of poultry meat and eggs had a predominantly growing trend in the analyzed period. Thus, the average annual production of poultry meat in the period 2016-2020 was as much as 55% higher than in 2000. The growing trend was in egg production, which is clear from the absolute values, but also the calculated index is given in Table 4. Reduction in the number of poultry, and at the same time growth in egg and meat production point to the conclusion that in the past two decades, Serbian poultry marked has a qualitative improvement in terms of using more productive hybrids and significantly improved breeding technology. Thus, the production results per housed bird have been significantly improved, as well as the overall results of Serbian poultry production. Also, it is interesting that the carriers of poultry production in our country are large agricultural farms where more than 60% of the total number of poultry is reared (Popović, 2015). These are farms with a high degree of production specialization, which also contributes to the improvement of the overall production results of this branch of animal husbandry in our country.

From the data shown in the previous tables, it is clear that livestock production in the past two decades marked a significant decrease in the number of cattle, pigs and poultry, but also the production of the most animal products had a positive growth trend. However, the positive trends did not contribute to a higher share of livestock production in the total gross value of agriculture, as it remained at around 30%. Thus, the analysis of the contribution of certain branches of livestock production to agriculture GDP in the past two decades amounts to an average of 13.2% for cattle, 1.8% for sheep, 10.6% for pigs and 5% for poultry, ie. the overall contribution of livestock was on average 30.6% (Novaković, 2019). The presented analysis of important indicators of the state and trends in animal husbandry in our country indicates the importance of stopping the decreasing trend in the number of livestock and further and even more intensive work on

modernizing livestock production following the latest scientific knowledge and achievements in this field. Only in this way is it possible to significantly improve the share of the value of livestock production in the agriculture GDP in our country in the next years.

## **Conclusions**

Livestock production plays an important role in achieving better results in Serbian agriculture and its greater contribution to total GDP. The previous analysis showed numerous weaknesses of our livestock production. Hence, its share in the GDP of agriculture is small (about 31%) and far below compared to agriculturally developed countries. In addition, this situation in animal husbandry determines our overall agriculture as underdeveloped, because it is dominated by plant production with a share of over 50% of crop production. Namely, a small percentage of crops are used as animal feed, while most are exported as a primary product. In economic terms, the export of value-added products such as meat, milk and eggs, would be far more profitable. Therefore, our livestock production needs fast and big steps towards positive changes and new, much more pronounced positive trends.

It is extremely important to stop the further decrease in the number of livestock and poultry, especially breeding categories, to create conditions for gradually more significant renewal and strengthening of the base of this branch of our agriculture. Increasing its competitiveness is also important for the revitalization and intensification of our livestock production. In the long term, the strategy of livestock development is important, which should be based on efficiency in the production of the most important animal products. In that sense, the good connection and organization of our farmers are also important. Stable and well-known prices for livestock products, as well as well-set price parities, are a guarantee for stable livestock production (Petrović et al., 2013).

Also, previous experiences in the improvement of animal husbandry and the achieved results in the world, as well as experiences in technology transfer, point to the conclusion that better organized and strengthened professional and scientific infrastructure is necessary for faster overall improvement of animal husbandry. Only in this way, through the solid synergy of society, agriculture, science and profession, changes can be initiated and significantly better results in livestock production can be achieved.

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## PYRAMIDING STRATEGIES FOR DURABLE RESISTANCE TO LEAF RUST OF WHEAT

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**ABSTRACT:** *The individual use of single race-specific resistance genes with major phenotypic effects has rarely provided lasting resistance. However, breeding and combining or pyramiding of resistance genes into individual cultivars has had considerable success, particularly in situations in which the pathogen does not reproduce sexually, as in the case of wheat leaf rust pathogen. In European-Mediterranean region performed international investigations of wheat leaf rust proved that breeding of new lines of wheat resistant to Puccinia triticina Eriks. for differentiation of pathogen population, as well as for sources of durable resistance is necessary. Breeding of such resistant lines has proved necessary due to the unsatisfactory survey results of these regions on standard isogenic Lr lines. It has become clear that these regions needed new, more efficient differential resistance genes, as well as sources of resistance. In the beginning, after extensive screening tests of several International Rust Nurseries, 18 donors of resistance had been selected as crosses with recurrent parents' varieties Princ and Starke. These hybrid lines had been comparatively tested with twenty six Lr single gene lines using twenty especially virulent cultures of P. triticina in order to check the presence of these known Lr genes in our hybrid lines. Considerable influence of recurrent parent to the number of resistant genes in used donors was demonstrated. On the other hand, considerable influence of the pathogen culture was established to the number of resistance genes in used donors. In order to enhance resistance and pyramiding genes in these hybrids, the most interesting selected eight lines have been crossed with only effective isogenic ones, containing the strong genes Lr9, Lr19 and Lr24. On the basis of different segregation ratios of all crossing combinations it was proved that no one of resistant donors contained the applied strong resistant genes. It means that our hybrid lines contained resistant genes from the donors, as well as three strong resistant genes Lr9, Lr19 and Lr24.*

**Key words:** *Puccinia triticina, hybrid lines, accumulation of resistance genes, resistant hybrid wheat lines.*



## INTRODUCTION

Leaf rust, caused by *Puccinia triticina* Eriks., is an important foliar disease of common wheat (*Triticum aestivum* L.) worldwide [1]. Variability of wheat rust pathogen has always represented the greatest problem of successful selection of resistant cultivars. Sixty years ago international studies of wheat rust pathogens and greater testing of resistant genotypes have started. In 1966 [2] in our country the centre for national and international studies of leaf rust pathogens was founded. In the paper are presented results of our long term studies on durable resistance to wheat leaf rust pathogene *Puccinia triticina* Eriks.

### TYPES OF RESISTANCE TOWARD WHEAT RUST PATHOGENS

In 1905. more intensive studies and use of resistance toward wheat rust begun when [3] proved that resistance toward yellow rust pathogen has inheritable character. Van der Plank [4] was the first one who differentiated two resistance types; specific and horizontal, common or non-specific. Specific type is the most frequently occurring in plants as hyper sensible reactions and it is efficient only toward some variants of virulent parasites. Hypersensible reactions *Gramineae* are causers of cell death after infection. Common or non-specific resistance is preconditioned by more minor genes that take effect to whole pathogen population in longer time interval. In VAN DER PLANK publication [5] this resistance type was discussed in more details in epidemiological sense, but inadequate genetic categorization aroused high controversy in scientific circles. It has been proved that genetic categorization sometimes does not correspond to reality. It has been established that resistance is not of polygenetic character but it depends upon one or several specific genes. Good example for this is resistance of all maize lines and hybrids toward *Cochliobolis carbonum* U11. Combinations of more specific major resistance genes can provide effects of durable horizontal resistance. In India in such a manner wheat cultivars with combinations Lr1, Lr10, Lr13 and Lr14 preserved resistance in longer time period, while those with single genes showed higher susceptibility [6]. Last years similar resistance effects toward leaf rust pathogen are obtained also by accumulation, i.e. ‘pyramiding genes’ in selection to resistance. Cytoplasm can also have impact to efficiency and longevity of single major genes transferred into different parents. WASHINGTON and MAN [7] have proved that the same mayor resistance gene in wheat cultivar Chris, under influence of cytoplasm of relatives *Triticum timopheevi* and *Aegilops speltoides* has different longevity and efficiency. NELSON [8] named synthesis of specific resistance genes

“pyramiding genes”. KOLMER *et al.* [9] emphasized that the most significant strategy has been selection to durable resistance of pyramiding genes. Certain programs that have been directed to combination of genes proved very successful in increase of durable resistance [10]. Durable resistance that lasted in the period 1966 – 1980 [9] toward leaf rust has been stipulated for cultivars Chris, Era (Lr13+Lr34) and Columbus (Lr13+Lr16). In our breeding program we have been oriented toward accumulation of the most efficient strong genes and their wild relatives Lr9, Lr19 and Lr24. The last approach comprises in creation of spatial genetic diversity that can successfully counteract diversified virulence inside pathogen population. This is maintained by growing of multi-line, compound cultivars or by coordinated use of different resistance genes in certain geographic areas. Many works in selection were directed to creation and growing of multi-line cultivars and their mixtures with the aim of obligatory parasite control. Multi-line cultivars have not been made especially for *P. triticina*. Tolerance is the special type of resistance. Tolerant genotype is able to sustain corresponding infection level with significantly lower yield losses in contrast to other genotypes under identical conditions. For tolerance and horizontal resistance mutual feature is absence of specific selective pressure to the parasite population. Difference make occurrence of horizontal resistance from special natural obstacles of parasite development, while tolerance refers to adaptation of metabolism and energetic production of the host. Selection to tolerance in some fungi and bacteria has been methodologically successful. SOTO *et al.* emphasizes that selection to tolerance can be successful in virus diseases that are transmitted by insects-vectors. Tolerance toward *P. triticina* has been evaluated as in cases of other parasites by comparison of infection intensity and yield.

## INHERITANCE MODES OF WHEAT RESISTANCE TO PUCCINIA TRITICINA

Since the very beginning [16], studies of *P. triticina* resistance inheritance have provided basic scientific information for selection to resistance. MARTINEZ *et al.* [17] performed comprehensive inheritance studies of resistance toward some parasite races in stadium of seedlings and adult plants, in combination of resistant wheat genotype NS No. II-39-2 (Premier x Bobin-Gaza-Bobin) with susceptible cultivar Thatcher. Seedlings reactions depended upon two pair of resistance genes toward only some races, whereas field reactions were preconditioned with three pairs of genes. For inheritance of resistance of the mature plant stadium for cultivars Rio Negro and Frondozo two complementary genes were established [18]. Studies of UPADHYAYA *et al.* [19] showed that in some crossings of three dominant resistance genes two proved to be

complementary. Two complementary dominant and one recessive resistance gene were established in other crossing combinations. It was emphasized that modification genes are important for reaction toward *P. triticina* in the stadium of adult plants, but very susceptible to the influence of environmental factors [20]. Inheritance of resistance in adult plants to cultivars, *P. triticina* was studied. Combinations of resistance genes of seedlings with genes resistance of adult plants are known. Lr1 and Lr10 stipulate resistance of seedlings, and Lr 22a is especially efficient in the stadium of third and fourth pair of leaves [22]. The same, LrT2 with reaction of medium resistance of seedlings gives high resistance in the stadium of adult plants in the field. RUSSELL *et al.* [23] proved differences in expression of resistance of the adult plant stadium in wheat in interaction of host and pathogen. In characterization of wheat resistance to leaf rust parasite MILUS and LINE [24] identified seven resistance classes in the study of four components in the stadium of seedlings and adult plants. MODAWI *et al.* [25] established that in susceptible cultivars Belinda and Thatcher in adult stadium of development significant reduction in number of uredopustals per cm<sup>2</sup> of the wheat flag leaf area.

## **MODELS OF GENE-FOR-GENE RELATIONSHIP HOST: PATHOGEN**

Based upon FLOR's [26] hypothesis, PERSON [27] was the first one to develop theoretical model in the system gene-for-gene including five corresponding gene pairs (CGP) and by this he provided new directions in data analysis of infection types (IT) of rust parasites. In the model of ROBINSON [28] was applied the identical Person's rule, by which resistance genes of hosts and genes of high pathogenicity of parasites result in susceptible infection types, but with differently classified genotypes. However, his arrangements of interaction genotypes are not based upon bionomical widening proposed by PERSON [27]. ROBINSON [28] suggested that there exist other mathematical relationships that can be applied during classification of genotypes based upon nature of alleles at each locus.

MCINTOSH and WATSON [59] broadened gene-for-gene relationship model in another manner. In their models, only two corresponding gene pairs are treated, but genes of low pathogenicity and genes that lead to reaction characteristic for resistant plants that in interaction provide low infection type (IT) are assorted. Boolean's model is based upon concept of agricornus. This model applies Boolean's algebra symbolization that is very useful, as there are no traditional mathematic or biological marks. It has its special genotype that is

integration of parasite and host interaction, and agricornus phenotype qualifies its genotype. LOEGERING *et al.* [30] developed computerized method of grouping host lines toward their reaction to the corresponding parasite culture out of which conclusions upon host and parasite genotypes can be made. Definitive phenotype in wheat rust is usually connected with resistance, while in interaction of some other parasites and host exist exceptions. Wheat allele Lr13 for resistance to *Puccinia triticina* in adult plant stadium, can be found in stadium of seedlings when plants are inoculated with the corresponding culture of parasites at 25.5°C. Also, resistance gene of adult plant stadium of wheat cultivar Atlas 66 was found with some cultures of *Puccinia triticina* at 5°C, at it was not discovered with identical cultures at 19°C. Delayed parasite development of wheat rust parasites in cultivar Suwaon 85 can be even visually observed when infected seedlings are grown at 5 and 12°C, but not when grown at 19 and 26°C [31].

### **SOURCES OF RESISTANCE AND PUCCINIA TRITICINA PATHOTYPES**

In 1966. in Novi Sad the International European Centre for Wheat Rust was founded [2]. After many years of testing in the central nursery in such a manner two groups of genetically different sources of resistance have been selected to wheat rust pathogen. The first group of eight sources of resistance exhibited greater genetic differentiations and it has been used for complex breeding. In the second group eight other sources of resistance have been included that participated only in the first phase of breeding. At the time wheat cultivar Sava from Novi Sad was resistant, and was used as a control genotype. Majority Lr isogenic lines selected up to 1980 showed susceptibility toward European pathotypes, i.e. races of *Puccinia triticina* [34; 35; 33; 36].

### **TRANSFER OF RESISTANCE GENES TOWARD P. TRITICINA INTO IDENTICAL RECURRENT PARENTS**

1980. in cooperation with Prof. Mac Kay from Sweden begun breeding program of creation of European resistant lines toward *Puccinia triticina*, by transfer of new, more efficient genes into identical recurrent parents. Hybrid combinations of the first back-crossing ( $F_1b_1$ ) of the first group of eight resistance sources with cultivars Prince and Starke were tested in stadium of seedlings with three international cultures *P. triticina* [37; 14]). Cultures of the resistant type of reaction “1” were taken from strong genes of wide spectrum Lr9 and Lr19, as

these are more virulent. Hybrid material from these crossings is grown and tested in the Central Nursery in Rimski Sancevi with use of the pedigree method of breeding of resistant offspring in conditions of artificial nursery inoculations.

Relationship of segregation with the culture Yu-13-19-1 with each of eight resistance sources (66,77,26,32,37,49, 95,146) have shown that line 66/2 with recurrent parent Princ segregated to two resistance genes, and hybrid 66/4 with the cultivar Starke showed activity of three resistance genes. This relation of segregation suggests that there exist complementary action of genes. Significance of recurrent parent has been noticed, as the identical source of resistance with the cultivar Princ showed two resistance genes, and with the cultivar Starke three genes. In contrast to these, two hybrids of the following hybrid combinations: 77/1 x Princ and 77/3 x Starke, as well as 32/2 x Princ and 32/3 x Starke have shown presence of only one resistance gene in both recurrent parents. The identical combinations of resistance genes occurred in lines 26/1 x Princ and 26/2 x Princ x Starke in regard to hybrids 94/1 x Princ and 94/2 x Starke. Both sources (26 and 94) with Princ had one resistance gene, and identical sources with Starke had two resistance genes. Three resistance genes have been determined in relationships of segregation lines 37/2 x Princ and 37/3 x Starke, and two resistance genes in hybrids 146/3 x Princ and 146/1 x Starke. The influence of the recurrent parent in line 46/2 x Princ with two resistance genes toward line 46/1 x Starke with one gene has shown again.

The relationship of segregation with another culture H-13-9-1 revealed no differences between the parents in the recurrent parents in crossing combinations 77/1 x Princ and 77/3 x Starke, followed by 37/2 x Princ and 37/3 x Starke, as well as 146/3 x Princ and 146/1 x Starke. For these hybrid combinations the identical results were achieved with both of the used pathotypes. Namely, both recurrent parents with a source of resistance 77 showed the presence of one gene, and with the source 37 three resistance genes and the source 146 two resistance genes. The identical results for both cultures were obtained by remaining hybrids, except lines 32/2 x Princ and 32/2 x Starke. The first one segregated in the presence of one, and the second in the presence of three resistance genes with complementary effect. Analysis of segregation toward the third culture CZ-13-Ar-3 to the number of present genes in some breeding combinations with one and other recurrent parent revealed the identical results as well as for the previous cultures. It is interesting to emphasize that difference again occurred only in hybrid 32/2 x Princ and 32/3 x Starke. With this culture both named lines had only one resistance gene, i.e., identically as with the first culture. From this it can be concluded that genetic of host and parasite interaction for these two hybrids in certain measure is more complex in regard to the remaining hybrid combinations.

In the presented results of segregation relationship of  $F_1B_1$  with three used cultures it is obvious that presence of one, two or three resistance genes was shown. Inheritance of resistance was dominant as it was established in many papers. Even the first works of resistance inheritance toward leaf rust pathogen suggested that there exists possibility of a dominant, recessive and intermediary inheritance, depending on the combination of parents [14; 38; 39; 40]). The influence of recurrent parent has also appeared to be significant, such as for instance sources of resistance 26 and 94 with Prince that had one resistance gene, and the identical sources with the cultivar Starke had two genes. Number of resistance genes in sources of resistance, i.e. hybrids was established by analysis of  $F_1B_1$  generation from crossings of sources of resistance with susceptible cultivars Princ and Starke, based upon relationship of segregation. Of eight sources of resistance, three genes were established only in:

37- Bowie/Quaderna, S 66 R 5803

66- Nadadores 63, Traper (2), Lancer, Ks 62 136, Co 701354

In conventional genetic analysis of  $F_1B_1$  generation of used resistance sources based upon relationship of segregation it is possible to determine only number of present resistance genes. Application of Boolean's algebraic gene-for-gene approach relationship illustrates significant advantages in genetic analysis in regard to conventional methods. Boolean's algebraic approach to the models gene-for-gene relationship with three pairs of corresponding resistance genes for two sources, 37 and 66 in recurrent parents Princ and Starke, and in their variants and different environmental conditions illustrated several different features of this model. Extensions of Model I, applied in this paper differ from PERSON's [27]. in the following. He did not use the identical concept for definitive variants, but he used terms "genes for resistance", and "genes for virulence", that produce susceptible genotype (S). This phenotype occurs where there are neither resistance genes nor virulence genes. In applied model in this paper,  $1p \times 1h \times 1e$  always provide definitive agricornus 1 ap. Parasite and host genotypes that he classified according to increasing number of genes for resistance and genes for virulence. In this paper the factor of multiplication system was used in arrangement of genotypes. Person used the binomial methods of the model expansion in calculation of the expected number of phenotypes in serials, and in this paper binary formulas were applied for these calculations.

Literature data in the field of resistance toward parasites is in great part based upon VAN DER PLANK's [24] classification of vertical and horizontal resistance. According to this classification, vertical resistance is characterised simply by inherited differences in phenotype. It is considered that vertical resistance in majority cases is controlled by gene-for-gene relationships.

Horizontal resistance, according to the same author, is characterized by small, but very important differences in development of parasites in regard to different host genotypes. Various studies and publications support explanation of horizontal resistance as specific character of relationship parasite: host: environmental conditions, controlled by relationships gene-for-gene in different environmental conditions.

## ACCUMULATION OF GENES TOWARD PUCCINIA TRITICINA

Results of long lasting studies in the frame of international and national projects were outlined under scheme in which international analysis of *P. triticina* population and testing of new lines of accumulation of efficient resistant genes toward leaf rust pathogen in nurseries on wide epidemiological territory were included. According to this approach classifications and nomenclatures of pathogens were excluded, and everything is directed to better and more comprehensive practical solution of selection to resistance toward leaf rust pathogen [14; 40]).

During two years lasting testing of International nurseries for wheat rusts, in the Central field nursery some nurseries were included [37; 40] in a manner given. Based upon reaction of seedlings of 1118 numbers of the Central nursery, twenty cultures of *P. triticina* from various epidemiological area, as well as reactions of testing of adult stadium in the field, 410 numbers were previously chosen from International leaf rust nursery. Remaining 708 were tested for the first time in CIMMYT's nurseries of summer wheat lines. In reactions of winter wheat lines seedlings, of the total of 410 numbers, 48% was resistant to all cultures, 42% differentially, and only 10% was susceptible to all cultures. This is completely understandable as tested materials originated from International nursery were chosen as resistant in previous years. Very close proportion per reactions were obtained from total number of 708 summer genotypes of CIMMYT's nurseries. In total, there were 53% of resistant lines per cultures, differential or in segregation 38%, and susceptible to all cultures only 9%. It revealed that CIMMYT's summer nurseries have numerous resistant wheat genotypes toward *P. triticina*.

Reactions of seedlings from 1412 tested lines from the Central nursery is performed in regard to twenty other new cultures of *P. triticina* from European collection, as well as reaction of adult stadium in the field suggest that maintained infection was sufficient. Of the total of 704 lines of the first group (of winter lines) in the field there were 57% of susceptible ones. Similar occurred with the other group (of spring lines) in which of the total of 708, susceptible reactions occurred

in 51% of lines. Susceptibility was also more expressed in reactions of seedlings. Of the total number of spring lines (CIMMYT's nurseries), 57% were susceptible, and of the total number of the first group (of winter lines), lower number, i.e. 33% were susceptible lines. In category of resistant seedlings toward all cultures in the first group there was 24%, in the second 17%; in the category of differential resistance or segregation in the first group 43%, and in the second 26%. These differences showed that in regard to resistance toward *Puccinia triticina* lines from the first group are genetically more unstable in regard to the second group lines.

In the first year, winter selection materials were tested toward twenty pathotypes of *P. triticina*. As these were hybrid offspring obtained by complex breeding for accumulation of resistant genes, more detailed analysis of reaction toward used cultures of *P. triticina* was required. Hybrid lines represented offspring of breeding of eight donors of resistance (66, 77, 26, 32, 37, 46, 94 and 146) with recurrent parents Prince and Starke; after this the best lines of these offspring were crossed with genes that were only efficient, Lr9, Lr19 and Lr24. Lacking are only combinations of crossings 37 x Lr19 and 146 x Lr24, as all offspring from these crossings were excluded due to inconvenient recombination features [39]. The most interesting are crossing combinations with the highest number of lines and spectrums of reaction, VR – very resistant and R – resistant, and these are: 66 x Lr19; 66 x Lr24; 77 x Lr24; 32 x Lr24; 94 x Lr9 and 94 x Lr19. From all of this it can be concluded that in good combining capabilities Lr24 with donors 66, 77 and 32, as well as Lr19 with 66 and 94, and Lr 9 only with 94. The other categories of weaker combining capabilities is observed from numeric relationships, and negative evaluation had also combinations of crossings in which somewhat more lines with spectrum reactions in segregation (LD,S; MD,S; HD,S; R,S; and S,R) were found. This segregation confirms genetic heterogeneity of corresponding hybrid lines toward used cultures of *P. triticina*. In selection to resistance, the aim is certainly choice of lines that show genetic uniformity of resistance toward reactions. As inside each of crossing combination there are 33-39 lines, further testing and choice of genetically uniform lines are possible. Higher numerical participation of lines with spectrum of reactions in segregation was seen for crossing combinations 55 x Lr19; 26 x Lr24; 32 x Lr9 and 37 x Lr24. In such a manner combination 77 x Lr19 had 18 lines with spectrum LD,S, three lines MD,S and 6 lines R,S, which makes 27 of the total of 39 lines of this crossing combination. Only 12 lines with reaction spectrum VR and R could be evaluated with genetically homozygote resistance. Numerical relationships in other numbered crossing combinations were similar, and in the following period, due to this for these offspring significantly more detailed testing and choice of corresponding homozygote resistant line is needed.



The most interesting were combinations of crossing with greater number of lines in reaction spectrum VR – very resistant and R – resistant. This indicates good combining capabilities of donor 66 with Lr19 and Lr24; and with Lr9 and Lr19. From this it is obvious that Lr24 combines better with corresponding donors than Lr9 and Lr19. Next year selection material, i.e. numerous lines of certain crossing combinations of eight resistance sources (66, 77, 26, 32, 37, 46, 94 and 146), with recurrent parents Prince and Starke, and each of these combinations crossed with Lr9, Lr19 and Lr24 were tested with twenty new cultures of *Puccinia triticina*. Significant participation of segregation confirms that young hybrid material is heterogeneous in greater measure to the feature of resistance toward parasite [39; 40]). It should be noted that lines 37 x Lr19 and 146 x Lr24 were excluded due to bad recombination features.

### **SELECTION OF HYBRIDS WITH ACCUMULATION OF RESISTANCE GENES TOWARD *P. TRITICINA***

Detailed crossings to gene resistance accumulation to *P. triticina* were performed. Lines from previous crossings with the highest degree of resistance in stadium of seedlings with different *P. triticina* cultures, as well as in field trial and European nurseries for leaf rust were approved. Those were wheat lines with resistance sources under numbers 66, 77, 26, 32, 37, 46, 94 and 146.

Each of these lines was crossed with efficient genes in isogenic lines Lr9, Lr19 and Lr24. In our country and in the world for these lines were known for they were the only one of the created Lr lines that possessed wider spectrum of resistance. Similarly as in previous crossings, hybrid material was grown in field trials, with use of artificial inoculation and pedigree method of selection in choice of resistant plants. With the aim of resistance monitoring, offspring from this crossings were tested in F<sub>2</sub> generation with three *P. triticina* cultures parallel with parental components and susceptible control. These cultures were chosen from previously named twenty cultures from European nurseries, and all three were taken from resistant wheat genotypes of infection type ‘1’ and minimal fructification of parasites ([14]. Comparison of the results with resistance gene accumulation, possessed by both parents, and in reactions with different cultures, i.e. virulence of *P. triticina*, lead to complex genetic interactions with different relationship of segregation. However, novel approaches in analysis of genetic of host: parasite and environmental conditions relationship, by use of mathematic models gene-for-gene relationship provide possibility for better choice of the most comprehensive resistance sources. In such a manner, presence of specific or horizontal resistance is possible to determine as slow rusting and partial resistance, and etc.

## IDENTIFICATION AND MARKING OF LR RESISTANCE GENE USING OF DNA MARKERS AND THEIR MARKING

Last years, based on DNK markers great advantage has been made in facilitation and shorting time and financials in identification of resistance genes. Leaf rust resistance genes: Lr1, Lr9, Lr10, Lr13, Lr19, Lr23, Lr24, Lr25, Lr27, Lr28, Lr29, Lr31, Lr34, Lr35, Lr37 and Lr47 have been mapped on chromosomes, usually by RFLP molecular markers. Improved breeding programs demand a series of molecular markers that are connected to resistance. Up to now, 26 different molecular markers have been reported that had been tightly connected to 14 different Lr resistance genes. The widest applied marker system is based on RFLPs. However, RFLP markers have been very difficult for use in the practical program of breeding.

In comprehension study of the same authors named markers were chosen from the following isogenic lines of wheat rust: in TcLr3, 3bg with ISSR prajmer (ACTG94) and TcLr20 with RAPD prajmer (OP/J-17), and for Lr29 gene with OP/Y-10. It is clear that each of polymorph PCR fragments was detected, but it had been developed as the result by exchange in genomes with back crossing for incorporation time Lr resistance gene by back crossing. All of this suggested to research of new binding target sites for prajmers RAPD, SSR and ISSR. The first molecular STS marker was discovered by SCHACHERMAYER *et al.* [42] for Lr9 gene derived from *Aegilops umbellulata*. Resistance genes toward leaf rust resistance Lr1, Lr9, Lr24, Lr28, Lr29 and Lr37 were mapped on hromozomes with RAPD markers; Lr19, Lr26 and Lr46 were mapped on hromozomes with AFLP markers. However, resistance genes toward leaf rust Lr1, Lr9, Lr10, Lr24, Lr28, Lr35, Lr37 and Lr40 were mapped with hromozomes with STS markers and leaf rust resistance genes Lr13, Lr16, Lr22a, Lr22b, Lr39 and Lr50 were mapped with hromozomes with SSR markers. Leaf rust resistance gene Lr11 was mapped on hromozomes with CAPS marker, Lr37 with SCAR marker. RFLLP markers are reliable but costly, demand intensive work and need high purity DNA, so they were not used for marker-assisted breeding. From the reasons of practical use, RFLP markers that are completely linked with given resistance genes transferred into specific PCR markers, STS or CAPS markers and RADP markers that can be converted into SCAR markers [42]. Presence of Lr9, Lr19 and Lr24 genes in off spring was confirmed through identification of seedling tests. The most efficient were lines with resistance genes Lr9, Lr19 and Lr24. Lr9 is introgressed from *Aegilops umbellutata*. Presence of Lr19 was confirmed by specific strengthening of single fragment 130bp in Thatcher NIL (Tc\*Lr19) and INIA66/CMH81A575. Leaf resistance gene Lr24 was introgressed from *Agropyron elongatum*. Of 41 lines of differently crossed combinations tested to

leaf rust pathogen, 38 lines showed presence of Lr19. Lr24 gene is known to bind to Sr24 gene for wheat stem rust that is combination efficient against all kinds of rusts [43].

These studies with molecular markers connected with Lr isogenic lines (NILs) are very significant also for future studies in our country, for better and more successful work on breeding to resistance toward leaf rust pathogen.

## **MAPPING OF LEAF RUST RESISTANCE GENES IN WHEAT**

Until recently, chromosome locations of many mapped leaf rust resistance genes were determined by use of monosomic analysis and telocentric mapping. SEARS [44] has developed set of 21 monosomics for each chromosome by use of bread wheat cultivar Chinese spring. Monosomic analysis includes crossing of 21 monosomics as female parent with resistant cultivar that possesses unknown resistance genes. F1 plants with 41 chromosome allowed self-pollination and F2 plants have evolved rust resistance. Monosomic that coincide with location of gene chromosome provides reverse segregation into F2 and the following segregation of generations [43]. SEARS [44] has also produced addition chromosome lines and other kinds of aneuploidy (multisomic, trisomic, tetrasomic) that have also been used in mapping. Monosomic analysis often have often been combined with telocentric mapping in which ditelosomics developed by SEARS [45] used for discovering of chromosomes of location position (locus) and distance gene recombination from centromere. These cytogenetical stocks have also recently been used for determination of molecular marker's positions. *P. tritici* is wheat parasite that often and in very short period can cause serious yield losses up to 40%. In order to reduce such losses, with their researches, breeders reached ecological and the most efficient method and it is use of molecular markers. Molecular markers have found wide application in breeding of plants. Breeders have isolated 80 resistance gene toward wheat leaf rust pathogen by molecular markers. Of all resistance genes, Lr9, Lr19 and Lr24 are considered as the strong ones. And long-lasting resistance is achieved with combination of two or more Lr genes. It is considered that improved strategy of breeding relates to integration of molecular markers into conventional breeding programs, for this leads to breeding efficiency that could not be reached earlier; this shortened the process, and financial costs have been reduced. Finally, it would be a good time to update the advice provided by Nelson in [27] as follows: Go back once more young man and gather up not only your most efficient genes, but also molecules, natural enemies and practices, and build a highly durable strategy ... and be wise enough to make this strategy economically and ecologically sustainable.

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## **CURRENT KNOWLEDGE ON BOVINE CORONAVIRUSES AS A CAUSATIVE AGENTS OF RESPIRATORY AND ENTERIC DISEASES**

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**ABSTRACT:** Bovine coronaviruses (BoCoVs) are very prevalent worldwide. Two forms of the disease are recognizable, as a consequence of BoCoVs infections - enteric and respiratory, with three clinical conditions: neonatal calf diarrhea (NCD), winter dysentery of adult cattle (WD) and respiratory BoCoV infections which covers all age categories of cattle (most of all, bovine respiratory disease complex - BRDC). Veterinary virologists pays special attention to viral interspecies transmission. High morbidity and low mortality are characteristic of BoCoV infections in cattle, which can be revealed by detection of the virus (virus isolation), viral antigen, viral genomic RNA or specific antibodies in samples from infected individuals. Opportunities for the curing of diseased cattle are more limited than the potential for prevention, although for successful recovery they must be implemented in a planned and synergistic manner. This review paper deals with the issue of infection of cattle and other animal species with bovine coronaviruses in a holistic manner, with special reference to their zoonotic potential.

**Key words** - *bovine coronaviruses (BoCoV), BRDC, enteric disease, preventive, therapy*

## **INTRODUCTION**

Coronavirus (CoV) infections lead to disorders of the respiratory and intestinal tract, neurological, liver and kidney disorders in a wide array of different species: humans, cows, small ruminants, pigs, dogs, cats, horses, mice, poultry and wild-ruminants (Han et al., 2006; Alekseev et al., 2008; De Groot et



al., 2012; Burimuah et al., 2020). In the last twenty years, the ability of the CoV to adapt quickly to new hosts and ecological niches has been confirmed in scientific circles, as evidenced by the spread of a severe pandemic of acute respiratory coronavirus (SARS-CoV), the respiratory coronavirus epidemic (MERS-CoV) in the Middle East, SARS-CoV-2 pandemic, the outbreak of the porcine epidemic diarrhea virus in North and South America, and the outbreak and spread of the swine deltacoronavirus in Asia, followed by its spread to the United States (Ksiazek et al., 2003; Rota et al., 2003; Graham and Baric, 2010; Borucki et al., 2013; Drosten et al., 2015; MacLachlan and Dubovi, 2017; Dawson et al., 2019; Wang et al., 2019; Ralph et al., 2020). Veterinary virology pays special attention to interspecies transmission. SARS-CoV and MERS-CoV originate from animals, and their reservoirs are in close contact with the human population (Dawson et al., 2019). Isolation of SARS-related CoVs in palm civets and raccoon dogs originating from live animal markets in China was the first indication of interspecies transmission, but it was soon proven that these animals were random hosts, and suspicions are currently being raised that different species of horseshoe bats represent the ultimate reservoir of SARS-associated CoVs (Guan et al., 2003; Dawson et al., 2019).

Results of Wardeh et al. (2021) demonstrate the large underappreciation of the potential scale of novel CoV generation in wild and domesticated animals and identified high-risk species for CoV surveillance. They state that there is a consensus that SARS-CoV-2 was generated by homologous recombination; originally derived from CoV in bats (Lau et al., 2005; Zhou et al., 2020) and then shifted to humans *via* an intermediate reservoir host - a species of pangolin (Lam et al., 2020). As a warning for the future, the origin of SARS-CoV-2 was deduced only after the outbreak in humans. Greater understanding of the extent of mammalian host reservoirs and the potential recombination hosts could help in prevention and mitigation strategies and provide a vital early warning system for the emergence of new strains of CoV. Weiss (2020) has a completely justified position expressed as a viewpoint after forty years of research on coronaviruses, and in her chronological review she states that the latest outbreak of SARS-CoV-2 has paralyzed the world, and the scientific public is mobilized. This fact results in incomparably greater availability of contemporary literature to researchers around the world, which motivated us to inform the scientific and professional public about CoV of animal origin as potential zoonotic pathogens. Researchers are also considering options for cross-protection of human population against Covid-19 used BoCoV immune milk against COVID-19 and benefits for the human respiratory system and potential contributions for clinical management of COVID-19 by taking bovine colostrum (BC) and observation of biological effects of supplementation for improving the respiratory health in humans and the

potential role of bioactive molecules from BC as adjunctive therapy for SARSCoV-2 infection (Coronavirus Disease 2019 - COVID-19) (Arenas et al., 2021; da Silva Galdino et al., 2021).

Quick adaptation to new hosts and ecological niches originated from mutation rates, relatively low fidelity of viral RNA polymerase, large CoV genomes, and high frequency of homologous recombination events during RNA replication. CoVs are the only described RNA viruses with a developed mechanism for correction of their genomes (through the activity of non-structural proteins 10–14), which enables them to avoid fatal errors and to generate and maintain very diverse and sustainable groups of quasi-species (Vlasova and Saif, 2021).

Bovine coronavirus (BoCoV) primarily affects the respiratory and intestinal tracts. BoCoV is classified into a species *Betacoronavirus 1* (subgenus *Embecovirus*) of the *Betacoronavirus* genus along with wild ruminant CoVs, porcine hemagglutinating encephalomyelitis virus, equine coronavirus, HCoV-OC43, HECoV-44, and canine respiratory coronavirus (de Groot et al., 2012; MacLachlan and Dubovi, 2017). Bovine coronavirus (BoCoV) is related to SARS-CoV-2 and has caused disease in humans on at least one occasion (Han et al., 2006). BoCoV virions are enveloped and pleomorphic, 65-210 nm in diameter, contained 5 major structural proteins: the nucleocapsid (N) 50 kDa protein, the integral membrane (M) 25 kDa protein, the small membrane/envelope (E) 8 kDa protein, the haemagglutininesterase (HE) 120-140 kDa protein and the spike (S) 190 kDa protein (Clark, 1993). Spike protein (S) consists of an S1 subunit that contains the dominant neutralizing epitopes and an S2 subunit that mediates virus entry into host cells. Thus, they are responsible for the range of viral hosts and tissue tropism and initiating the generation of virus-neutralizing (Clark, 1993; Bidokhti et al., 2013, Li, 2016; Brownlie, 2017).

In addition to diseased cattle, BoCoV can be identified in the respiratory and intestinal tracts of healthy individuals (Clark, 1993; Saif, 1990; Saif et al., 1991; Saif, 2010; Saif and Jung, 2020), where it is excreted in nasal secretions and faeces and causes three different clinical syndromes in cattle (Saif and Jung, 2020): neonatal calf diarrhea - CD (Clark, 1993; Tsunemitsu and Saif, 1995), winter dysentery (WD) - hemorrhagic diarrhea of adult cattle (Saif, 1990; Tsunemitsu and Saif, 1995; Yavru et al., 2016) and respiratory infections in cattle of different ages, usually as part of a bovine respiratory disease complex (BRDC) or transport fever in cattle in the feeder (Clark, 1993; Storz et al., 2000a, 2000b; Saif, 2010; Fulton et al., 2013; Yavru et al., 2016; Franzo et al., 2020; Hodnik et al., 2020; Orozco-Cabrera et al., 2020). WD must be differentiated from dietary diarrhea, coccidiosis, Bovine Viral Diarrhoea Virus (BVDV) infection, and salmonellosis (Peek et al., 2018).

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## ***Epidemiology of BoCoV***

Researchers around the world have confirmed that BoCoV is present on all continents and seroprevalence studies revealed that over 90% of cattle have been exposed to BoCoV during its longevity, identified in the intestinal and respiratory tracts whether healthy or diseased cattle (Fulton et al., 2015). Franzo et al. (2020) has been demonstrated a remarkable role of recombination and high mutation rate to generate the genetic variability in response to selective immune pressure which have a major role, favoring the diversification of the spike protein in regions exposed on the viral surface. The occurrence of co-evolution linking different sites is likely the result of the compensatory mutation necessary to preserve protein stability and functionality. On the contrary, while some weak evidence of host/tissue tropism-induced selection could be detected, overall, BoCoV tropism appears to be more closely linked to innate flexibility rather than to specific adaptations. Perhaps surprisingly, studies reviewed in Weiss and Leibowitz (2011) showed that it was not only the spike protein that impacted tissue tropism; other „background genes”, including nucleocapsid and replicase, as well as accessory genes, were also important determinants of tropism.

Oma et al. (2018) reported about possibilities for prevention spread of the endemic pathogens BoCoV and bovine respiratory syncytial virus (BRSV) between herds. Successful prevention requires knowledge about indirect transmission by personnel and fomites. The authors tried to determine the duration of viral RNA carriage and the infectivity of virions on fomites and human nasal mucosa after exposure to BoCoV and BRSV.

Winter dysentery (WD) was typically reported in colder regions. Recently reports revealed occurrence in warmer seasons (summer in Korea) or in tropical countries like Thailand, Brazil and Cuba (Singasa et al. 2017).

One of the most serious syndromes of fattening cattle and dairy cows is bovine respiratory disease complex (BRDC), which arises as a result of complicate interactions between cattle of all age and sexes, environment, and pathogens (Setiyaningsih et al., 2018), manifested with significantly high morbidity and mortality. BRDC leads to extremely high costs in livestock, due to falling production, increased labor costs and reduced carcass value, as well as costs of prevention and treatment - modern vaccines, antibiotics and supportive therapy (Irsik et al., 2006; Gershwin et al., 2015). BRDC is caused primarily by several viruses (alone or in combination): bovine respiratory syncytial virus (BRSV), bovine herpes type 1 (BoHV-1) and bovine viral diarrhea virus (BVDV). BoCoV has been isolated and detected in many outbreaks of respiratory disease

in cattle of all ages (Decaro et al. 2008a; Saif, 2010). The controversy about BoCoV role in BRDC onset is based on the fact that many studies have isolated aforementioned virus from feedlot cattle with clinical signs of BRDC and without or with limited co-infection with other respiratory pathogens (Storz et al. 2000a, 2000b; Paller et al. 2017), while others have achieved the same in clinically healthy cattle (Workman et al., 2019). However, BoCoV should not be dismissed as a respiratory pathogen but should rather still be considered as a participant in the multifactorial etiology of BRDC. Above mentioned viruses predispose animals to coinfection with one or more bacteria: *Pasteurella multocida*, *Mannheimia haemolytica*, *Mycoplasma bovis* and *Histophilus somni*, indicating a multifactorial process (Taylor et al., 2010a; 2010b; Gershwin et al., 2015). Viral agents such as BRSV and bovine parainfluenza virus 3 (BPIV-3) are designated as the most common challengers of BRDC. The most important predisposing factors which enable the development of BRDC are: stress, weaning, too high temperature, overcrowding of cattle in stalls in the facility, dust, humidity, transportation and frequent changes in diet composition (Taylor et al., 2010a). One part of the scientific community has concluded that the viral pathogens are the main pathogens of BRDC that causing primarily respiratory lesions (Kirchhoff et al., 2014). Unique viral agents is BVDV, which cause intrauterine infection in the first trimester of pregnancy and lead in persistently infection (PI) of cattle, chronically ill or dying in feedlots (Kurćubić et al., 2011). Immunosuppressive effect of the BVDV infections contributes to the onset of BRDC, primarily due to the creation of favorable conditions for the development of numerous bacterial agents: sixteen serotypes of *M. haemolytica*, *P. multocida*, *Histophilus somni*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Corynebacterium bovis*, *Streptococcus* spp. and *Micrococcus* spp. (Taylor et al., 2010a). Also, Mycoplasmas caused serious cattle disease, which leads to negative economic and welfare impacts (Parker et al., 2018). *M. bovis* is recognized as one of the most important Mycoplasma species associated like cattle disease (Bednarek et al., 2012; Fox, 2012) and they are also often isolated *Mycoplasma bovirhinis*, *Mycoplasma dispar*, *Ureaplasma diversum* and *Mycoplasma canis* (Szymańska et al., 2010).

Calves with BRDC are detected in 91% of cases in the first 27 days after arrival at the in fattening units (Buhman et al., 2000). Morbidity risks of BRDC cases in feedlot cattle are highest within the first 45 days after arrival to the feedlot. Morbidity was highest in weeks 1 to 3, and decreased through the 12-week period (Edwards, 1996). Unpredictable etiology of BRDC is almost always polymicrobial and associated with predisposing risk factors.

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***Transmission and pathogenesis of BoCoV***

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Oma et al. (2016) revealed that the infection of animals with BoCoV occurring through the faecal-oral route or inhalation of aerosol. BoCoVs enter the cell by binding to its membrane receptors with the S protein (S1 part). BoCoV penetrate into the cell by cleavage of the S protein by enzyme (trypsin-like proteases). The S2 part of the S protein interfere the fusion of the BoCoV with the host cell membrane (Popova and Zhang, 2002). Whether the initial replication takes place in the respiratory (nasal turbinates, trachea and lungs) or the gastrointestinal tract (enterocytes) is still a matter of debate. The clinical picture depends on various factors such as: secondary infections, stress, the animals overall immunologic status and environmental temperature (Saif, 2010). The widespread tropism of BoCoV is attributed to the fact that its host cell receptor is sialic acid, detected in enterocytes of the intestinal tract and in the epithelium of the upper and lower respiratory tract (Saif, 2010; Brownlie, 2017). With one side, claiming that the initial replication takes place in the respiratory tract and large quantities of virus protected by mucus are then swallowed to infect the intestine (Saif, 2010; Oma et al., 2016), while the other side claims that the gastrointestinal tract is infected first and the virus reaches the respiratory tract with viraemia (Park et al., 2007; Boileau and Kapil, 2010).

Wherever the primary site of replication is (respiratory tract - nasal turbinates, trachea and lungs; gastrointestinal tract - enterocytes), BoCoVs are shed both in faeces and in nasal secretions (Park et al., 2007). Kanno et al. (2018) determined by the nested PCR method that the duration of BoCoVs excretion can be very long, up to 932 days after infection detected in nasal secretions and 1058 days in feces. The most common way of transmitting the disease is from a cow to a calf or between calves. Infection occurs between herds via purchased cattle or contaminated fomites. The consequences of the pathogenic action of BoCoV are interstitial pneumonia or atelectasis with damage of the epithelium of nasal turbinates, trachea and lungs (Park et al., 2007; Oma et al., 2016; Vlasova and Saif, 2021). In gastrointestinal tract, the spread of BoCoV begins in the duodenum and continues to the large intestine, manifested with villous atrophy and other mucosal lesions (Park et al., 2007).

There are assumptions about the pathogenic role of respiratory BoCoV, which is still being investigated. The conclusion of Lathrop et al. (2000) study indicates that BoCoV was isolated from cattle in a fattening farm in a large number of different geographical locations. Most cattle develop an antibody response to BoCoV during the first 28 days after arriving at the fattening facility. Of the total number of examined cattle (n = 814), 473 (58%) seroconverted to

BoCoV during the first 28 days after arrival at the fattening farm, and the percentage of seroconversion ranged in certain groups ranging from 20.3 to 77.9%. However, further analysis is needed to determine the extent of the damage caused by BoCoV and to more clearly define its impact on the emergence of BRDC. The identification of BoCoV in fattening populations is incriminating, but does not in itself prove that it causes respiratory diseases. Hasoksuz et al. (2002) reported that seroconversion to BCV was inversely related to average daily weight gains ( $P < 0.06$ ). Twenty-eight respiratory and 7 enteric BoCoV strains were isolated from nasal and fetal samples of 32 cattle in HRT-18 cell cultures. These findings confirm the presence of enteric and respiratory BoCoV infections in feedlot calves. Further studies are needed to elucidate the differences between enteric and respiratory strains of BoCoV and their role in the BRDC of feedlot cattle. The pathogenesis of BRDC remains unclear, particularly in the advanced stage of chronic disease (Mehinagic et al., 2019). Storz et al. (2000b) have confirmed infections with BoCoV and *Pasteurella* using Evan's criteria for causation. Between 2011 and 2016, Klima et al. (2020) revealed a significant increase in the prevalence of Multi Drug Resistance (MDR) isolates of *M. haemolytica* and *P. multocida* associated with BRDC. The results of aforementioned investigations serve as a basis for the creation of innovative preventive and therapeutic strategies and for the selection of cattle with enhanced resistance to BRDC (Behura et al., 2017). Today's research results testify that it is possible to expect that selection may reduce susceptibility to BoCoV infection and BRDC in cattle (Kiser and Neibergs, 2021).

### ***Clinical conditions***

#### ***Neonatal calf diarrhoea (NCD)***

BoCoV is the causative agent of neonatal calf diarrhea (NCD), most often during the first three weeks of life, whether it is dairy or fattening cattle (Boileau and Kapil, 2010; Bok et al., 2018; Saif and Jung, 2020; Debelo et al., 2021). Due to the damage to the intestines by infection of calves with BoCoV, strong and bloody diarrhea sometimes occurs, followed by high mortality (Torres-Medina et al., 1985). BoCoV-infected intestinal epithelial cells die, exfoliate, and are replaced by immature cells. The compensatory crypt hyperplasia and increased fluid secretion leads to exacerbates of NCD. Lack of mature intestinal cells capable of absorption and digestion by enzyme secretion greatly reduces the absorption, metabolic and secretory power of the intestinal tract (Moon, 1978). Damaged small intestinal epithelium cannot absorb large amounts of food that is

continuously available to calves (Nappert et al., 1993), so animal feed remains undigested and ferments in the large intestine, leading to increased fluid accumulation, excessive bacterial growth, and organic acid production, which intensifies diarrhea (Moon, 1978). The consequences are dehydration, metabolic acidosis and electrolyte imbalance (due to losses of sodium, chloride, potassium, and bicarbonate). This condition is most difficult to tolerate in younger animals. NCD occurs in 24-hour-old calves that have not received colostrum or in those that are 5 months old.

### *Winter dysentery of adult cattle (WD)*

BoCoV infection allows the occurrence of winter dysentery (WD) in adult dairy cattle, which causes a dramatic reduction in milk production and huge economic losses (Symes et al., 2018; Saif and Jung, 2020). Moon (1978) report that BoCoV is detected in the feces of both healthy and calves with diarrhea, but the prevalence of diseased calves is significantly higher (8-69%) compared to healthy calves (0-24%). BoCoV proved to be more stable at lower ambient temperatures and reduced levels of ultraviolet rays. Excretion is 50-60% higher during the winter months (Collins et al., 1987; Clark, 1993), and is thought to favor WD in adult cattle (Cho et al., 2000). BoCoVs show interspecific transfer in which they cause diseases of susceptible species or transitional hosts, with the greatest importance as pathogens of all categories of cattle, of different production purposes. Anyway, the possibility of transmission to the human population is attracting a lot of attention from researchers: HECOV-4408 from a child suffering from acute diarrhea is genetically and antigenically closely related to BoCoV and is suggested to be a his variant (Zhang et al., 1994). There is experimental evidence that enteric BoCoV can infect dogs, as well as evidence for natural and experimental infection of different species of domestic and wild ruminants causing clinical (Tsunemitsu et al., 1995) or subclinical infections and seroconversion (Kaneshima et al., 2007; Amer, 2018).

### *Respiratory BoCoV infections*

Since the first scientific confirmation that BoCoV is one of the viruses that cause BRDC, over time (Thomas et al., 1982) to date, its role in the development of BRDC and reducing the results of fattening cattle is increasingly recognized (Lathorp et al., 2000a, 2000b; Storz et al. 2000a, 2000b; Haskuz et al., 2002; Hägglund et al., 2006; Saif, 2010; Hick et al., 2012; Ellis, 2019; Saif and

Jung, 2020; Duse et al., 2021; Blakebrough-Hall et al., 2022). Respiratory disease with mild symptoms (coughing, rhinitis) and pneumonia (various intensity) in two to six month old calves attributed to BoCoV infections (Saif, 2010; Saif and Jung, 2020). The worldwide high prevalence of BoCoV could be due to shedding of high titers of the BoCoV from bovine respiratory and intestinal tracts (Heckert et al., 1990), and existence of subclinically infected (carrier) animals (Ksiazek et al., 2003) within most herds. Calves seronegative for BoCoV and BRSV at arrival had an increased BRDC risk in the first 3 weeks of the production cycle and applications of screening veal calves determined Ig cut-off of 7.5 g/L like recommended. Seropositive status for these viruses either by vaccination on the herds of origin or by provision of colostrum from a vaccinated cow might be successful approach to minimize the BRDC risk and subsequently prudent antimicrobial use in the veal calf sector (Pardon et al., 2015). Extensive research (n = 1,074 cattle) revealed that feedlot calves shedding respiratory BoCoV nasally were more likely suffer from respiratory disease (1.6 times) and pulmonary lesions at slaughter (2.2 times) than animals that did not shed BoCoV respiratory virus (Lathorp et al., 2000b). Two trials in which similar results were obtained reported that calves shedding BoCoV respiratory virus nasally were 2.7 and 1.5 times more likely to have respiratory disease than those that did not shed BoCoV (Hasokuz et al., 2002; Thomas et al., 2006). Also, the results were noted that nasal BoCoV shedding or an Ab titer <20 correlated with increased risk of treatment for BRDC, while intranasal vaccination with BoCoV vaccine mitigated this risk (Plummer et al., 2004). Clinical signs are most often manifested by high fever (40-41.5 °C), depression, decreased appetite, nasal and ocular discharge, coughing and varying degrees of dyspnea (Saif, 2010). In 3 of 4 outbreaks in Italy, the classic signs of BRDC (dyspnea, fever, respiratory distress) were evident in 2–3-month-old calves positive for BoCoV and negative for other respiratory viral pathogens, while presence of common bacterial pathogens of cattle (*Staphylococcus* spp., *Proteus mirabilis* or *Mannheimia haemolytica*) was only confirmed in 2 of 4 outbreaks (Decaro et al., 2008a). A recent research of Workman et al. (2019) has identified BoCoV, *H. somni*, *M. haemolytica* and *P. multocida* (but not other pathogens involved in occurrence of BRDC) during a respiratory disease outbreak in pre-weaned beef calves marking these pathogens as the major pathogens. The multifactorial etiology of BRDC is extremely challenging: similar like in humans (Fajgenbaum and June, 2020), animal treatment with antibiotic may lead to massive release of bacterial lipopolysaccharides (LPS) inducing proinflammatory cytokine response and further enhancing lung damage (Van Reeth et al., 2000; Saif and Jung, 2020). Corticosteroid use can reduce the numbers of CD4 and CD8 T cells and certain cytokine levels compromising protective immunity and exacerbating disease



(Jung et al., 2007, Zhang et al., 2008). Thus, current evidence suggests that BoCoV alone may not be routinely associated with substantial infection/pathology of the lower respiratory tract, but likely is significant as a respiratory pathogen in the case of mixed infections and plays a major role in inciting the disease. However, experimental data on infection of SPF BoCoV seronegative cattle with BoCoV respiratory isolates is needed to better confirm the role of BoCoV in respiratory disease.

### ***Diagnosis and differential diagnosis of BoCoV infection***

After entering the body of cattle, pneumoenteric BoCoV multiplies (replicates) in the upper parts of the respiratory and intestinal tract, so nasal discharge or feces are taken as samples for diagnosis (Oma et al., 2016; Saif and Jung, 2020). BoCoV has also been detected or isolated from diseased animals with respiratory symptoms (nasal, pharyngeal, tracheal and lung tissues) or from distal small intestine and colon of those diseased animals that show enteric disorders (Reynolds et al., 1985; Storz, 2000a). Diagnostic tests of live animals include collecting nasal swabs or feces in appropriate sterile containers, which are transported chilled in hand-held refrigerators in the fastest manner to an accredited microbiological (virological) laboratory (Cho et al., 2001). It is also possible to aspirate fluid from live calves suffering from acute respiratory disease (tracheobronchial lavage), which was previously proven to be positive for BoCoV antigen by ELISA (Uttenthal et al., 1996). Stress caused by transport and arrival of animals to fattening facilities from different markets or farms as the main predisposing factors for BRDC lead to the highest level of nasal (or fecal) excretion of BoCoV during the first week after arrival to fattening pens (Storz, 2000a; Cho et al., 2001; Hasoksuz et al., 2002). Infections with BoCoVs (like most other viral infections) can be revealed by detection of the virus (virus isolation - VI), viral antigen (VA), viral genomic RNA or specific antibodies (Ab) in appropriate samples from infected bovine or aforementioned susceptible species (Nišavić and Milić, 2017). VI is most often performed on tissue culture (cells of bovine origin or human colorectal adenocarcinoma - HRT-18), but this method implies long-term work and certain strains of the virus do not find a suitable growth medium for them (Saif et al., 2010). Molecular methods are routinely used today such as RT-PCR, nested RT-PCR, real-time qRT-PCR (targeting most conserved genomic regions - ORF1ab or N gene) are the most sensitive, more reliable and faster methods currently available for BCoV detection (Cho et al, 2001; Decaro et al., 2008b; Saif, 2010; Gomez et al., 2017; Nišavić and Milić, 2017). BoCoV Ab are significantly represented in cattle

population, so it is necessary to collect paired acute and convalescent serum samples in order to achieve serologic diagnosis of BoCoV infections by various methods: virus neutralization (VN), immunofluorescence assay (IFA), haemagglutination-inhibition (HI) or enzyme-linked immunosorbent assay (ELISA) tests (Burimuah et al., 2020; Brownlie, 2017; Saif, 2010). Detection of BCoV-binding or VN Ab is performed in the convalescent phase of the disease (Hasoksuz et al., 2008; Storz et al., 2000a; Saif and Jung, 2020). The protective function of serum Ab against BoCoV infections has been proven on several occasions. Serological diagnosis of BCoV infections implies provision of paired acute and convalescent sera, due to the high prevalence of BoCoV Ab in cattle (Saif, 2010). The use of immunoelectron microscopy in the detection of BoCoV in nasal discharge or feces is limited by its relatively low sensitivity and expensive equipment, despite the advantage of detecting other viruses. Immunofluorescent/immunohistochemical staining with hyperimmune antiserum or monoclonal Abs on BoCoVs is a very useful and accurate method for detecting viral antigen in respiratory (trachea, lung) or intestinal (ileum, colon) tissues (frozen or embedded in paraffin) (Heckert et al., 1990; Tsunemitsu et al., 1995b).

### ***Therapy of cattle suffering of BoCoV infection***

There are no specific antiviral treatments for BoCoV infection in beef or dairy cattle. Boileau and Kapil (2010) reported that the often veterinarians-clinicians are limited to the use of supportive intravenous therapy (fluid, concentrated glucose, electrolyte supplementation,) which significantly increases the chances of diseased cattle to survive and recover. Vitamin cocktails also have roborant properties. When it comes to respiratory diseases in cattle (primarily BRDC), due to secondary bacterial infections that almost always develop after viruses cause the formation of primary lesions, the use of antimicrobial drugs (AMD), primarily and frequently antibacterial agents, and non-steroidal anti-inflammatory drugs (NSAIDs) is still recommended (Boileau and Kapil 2010). The results of a number of studies indicate that the widespread use of antibiotics (ABs) in the prevention and treatment of animals for slaughter inevitably leads to antibiotic resistance (U.S. Department of Health and Human Services, Food and Drug Administration, 2016). If certain ABs are used frequently and uncontrollably in the treatment of animals, there is a much greater possibility that bacterial resistance will develop through various mechanisms, so this drug will be less and less effective over time. BRDC is most often treated with the following ABs: tetracyclines, florfenicol and tulathromycin. (Kurćubić et al., 2021). If ABs applied for preventive purposes in low doses, we can expect the

development of resistance to the same AB when they are used in high (therapeutic) doses (WHO Report, 2016). Every experts program is multicomponent: vaccination/revaccination, use of AMD on arrival in feedlots, biosecurity measures, diagnostic procedures, treatment of ill animals, animal husbandry practices, refine quality of feeds and feeding programs, monitoring and animal health intervention programs (Brault et al., 2019; Lhermie et al., 2019).

### ***Economic impact of BoCoV infection***

Beaudeau et al. (2010) showed in his research that BoCoV infection herd status was not significantly associated with any production losses in cattle from infected herds compared with those in infection-free herds. A different opinion is published by Khamassi et al. (2021) that BoCoV infections are responsible of huge economic consequences associated or not to highly public health impact, in certain cases. Fewest investigators have shown that BoCoV-associated BRDC is correlated with decreased performance in feedlot cattle and shedding of respiratory BoCoV correlated with a reduction in weight gain (Martin et al., 1998; Cho et al., 2001; Hasoksuz et al., 2002). And in this case, there are divided views such as the one published by Lathorp et al. (2000b): 837 calves in 4 feedlots from 2 states (Ohio, Texas) involved in study and showed that the BoCoV shedding or seroconversion status did not affect the average daily gain - ADG. NCD remains an important cause of illness and death. Economic damage associated with the disease include decreased performance, mortality, and the expenses of medication and medication of sick animals., Beef cattle herds may experience death rates of 5%-10% or greater, and sometimes up to 100% morbidity, on the annual level. Accordingly, BoCoV infections may contribute directly to economic losses in feedlot cattle due to reduced daily gain or, similar to other respiratory viruses, facilitating the development of respiratory infections in cattle (Boileau and Kapil, 2010).

### ***Prevention of BoCoV infection***

Prevention of BoCoV infections is carried out through vaccination, good management practice (GMP) and a high level of hygiene. Vaccines prepared from BoCoV for the prevention of bovine respiratory diseases have not yet been developed. Vaccine for intranasal immunization of cattle which contained attenuated enteric strains is recommended to minimize the risk of BoCoV-associated pneumonia. A few attenuated and inactivated vaccines against BoCoV

enteric disease have been developed (Saif, 2010; Brownlie, 2017; Tizzard, 2020). There are scarce data from epidemiological studies conducted on the BRDC suggesting that serum Ab levels against BoCoV may be a marker for the level of protection against respiratory infections (Saif, 2010). Cattle that shed respiratory BoCoV at the beginning of the epizootic of BRDC/pneumonia have low levels of HV and HI Ab, while those animals that have been shown to have high levels of Ab against HE and S virus glycoproteins remain negative for BoCoV, the cause of respiratory disease (Lin et al., 2001, 2002). Levels of HV, HI, IgM, IgG1 and IgG2-BoCoV serum Ab were highly correlated with protection against respiratory BoCoV infection. Only IgM Ab responses were detected in cattle suffered of fatal respiratory infections caused by BoCoV. Ideal vaccines yet to be produced against pneumoenteric pathogens that damage mucous membranes should be effective at both of the above-mentioned viral replication sites (respiratory and intestinal tract), in order to provide optimal protection for immunized cattle. Most of these vaccines available on the world market fail to elicit sterilizing immunity or prevent subsequent reinfections, as observed in natural or experimental BoCoV infections (Cho et al., 2001; Heckert et al., 1990). Vaccinations should provide the widest and strongest possible protection against severe forms of enteric/respiratory disease that require treatment or are fatal. Vaccination of calves on farms gives the best results before transport to auction barns or fattening factories, because BoCoV infections that occur in fattening farms require the rapid appearance of protective immune responses. There is strong evidence that vaccination of young dairy calves with parenteral or intranasal (IN) modified live (MLV) vaccines is effective providing clinical protection against BRDC after experimental challenge with respiratory viruses (Kurćubić et al, 2021). Plummer et al. (2004) reported that IN vaccination of feedlot calves with a MLV-BoCoV calf vaccine on entry to a fattening facilities reduced the risk of medication for BRDC in calves. Recent research from Uruguay demonstrated that vaccination of dams against BoCoV reduced his shedding in their calves (Castells et al., 2019). A few licensed MLV against BoCoV are currently available on the world market (Bovilis, Merck/PBS Animal Health; Guardian, Merck; Calf-Guard, ScourGuard 4KC and BoviShield Gold 5, Zoetis; CattleWin BC, KyotoBiken Laboratories, Kyoto, Japan). Field studies evaluating the protective efficacy against NCD/WD remain very poor. Some of studies suggest that above mentioned vaccines induce weak protection against viruses that causes BRDC (Fulton et al., 2016). Most studies evaluating candidate BoCoV vaccines report on their safety and immunogenicity but not about protective efficacy (Welter, 1998; Takamura et al., 2002; Decaro et al., 2009). Intricate management of vaccine use (variable age of target groups of cattle, production status, newly arriving animals) and unfavorable cost-benefit ratio of vaccination suggest that exist a high level of need for

evaluation and employment of alternative control and prevention strategies for control of BoCoV infection. Biosecurity control program for BCoV and BRSV has been implementing exclusively in Norway, like a joint program, since 2016 (strict biosecurity measures and financial reward system for compliant farmers/industries). Vaccination not included in this program, which basis is separation of animals of different statuses, different transport vehicles, the building of loading areas, the provision of a sluice and clean clothes and footwear for visitors of farms, hotline to report outbreaks of disease to limit further spread of BoCoV and BRSV (Stokstad et al., 2020). Mass medication (metaphylaxis) effectively contributes to the reduction of morbidity through the prevention of bacterial co-infections in BRDC, using antimicrobial drugs (AMD) selected on the basis of susceptibility testing, mainly with prolonged action, for the treatment of cattle who are suspected on BRDC or which have been in contact with infected cattle (Edwards, 2010; Urban-Chmiel and Grooms, 2012; Anholt et al., 2017). Because multiple factors can cause or contribute to BRDC, there is no single method to prevent this serious syndrome. Like most other diseases that occur in animals that are under stress, a combination of measures to minimize stress and boost immunity is the best approach to prevent illness. As alternatives are proposed: preconditioning programs, dietary manipulations and feed supplements, probiotics and prebiotics, remote early disease identification systems and immunomodulatory medication.

## **Conclusions**

In the etiology of three serious clinical syndromes of cattle, coronaviruses (CoV) appear as the causes. CoV have zoonotic potential, reinforces the need for constant surveillance and intensive research with close cooperation between veterinarians and medical doctors, as both are responsible for the preservation of human health. The current state of affairs indicates that the knowledge gained so far about BoCoV infections and its consequences should be consolidated, expanded and enriched in order to timely predict and prevent new fatal pandemics such as COVID-19.

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## RECENT TRENDS IN RESEARCH AND TECHNOLOGY OF DIFFERENT BERRY SPECIES

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### ABSTRACT

*Berry production is a very important branch of agriculture in the Republic of Serbia. Fruit production accounts for about 11% of agriculture production value (Development Strategy of Agriculture of the Republic of Serbia 2014-2024). Natural conditions of our country, especially some of the regions, are very favorable for the cultivation of fruit, including berry fruit. It is known that Republic of Serbia belongs to the group of the world's leading producers of raspberries and blackberries, while the level of strawberry production is an economically extremely important factor. Because of the biological properties associated with berry fruits, the identification of their phenolics content is necessary for the evaluation of berries consumption on human health. They are globally known and consumed, and berries' phytochemical are used as functional food ingredients. Several studies have shown that the amount of phytochemicals depends on the species (genetic factors), one of the key factors, and environmental factors, agronomic factors as well as agricultural practices, that is organic versus conventional agricultural practices. In this regard, organic (fruit) production has become very popular due to the 'sustainable intensification' of the production with less pesticide and heavy metal residues, pronounced aroma, better soil health and higher biodiversity. The rhizosphere inoculation with foliar spraying by biofertilizer could be useful tool in organic berry growing technology contributing at the same time to the production of healthy fruits with good quality and providing the reduction of environmental pollution.*

*Therefore, the objective of this study was to show evaluate the effects of biofertilizer (Biovermix) which designed at the Fruit Research Institute Čačak, on the phytochemical profile of blackberry, blueberry, and strawberry fruit, as well as effect of rain-shield cultivation blackberry on biological properties.*

**Key words:** berry fruit, state, growing technology, Republic of Serbia,

## BLACKBERRY PRODUCTION

The economic significance of the European blackberry (*Rubus* subg. *Rubus* Watson) makes it an important berries in Serbia, where it is positioned immediately after raspberry and strawberry (Nikolić and Milivojević, 2015). Blackberry production began after the World War II with no significant increase in production volume though, as in the case of raspberry. According to Milutinović *et al.* (1999), in 1997 around 12.000 t of blackberry was produced in Serbia. The production was tripled by 2005 amounting to 28.000 t on a total area of about 5.000 ha (Nikolić *et al.*, 2012). According Strik *et al.* (2014), the blackberry production constitutes 69% of the European and 18% of the world's production, which places Serbia among the four leading global producers of this fruit (Fig. 1).

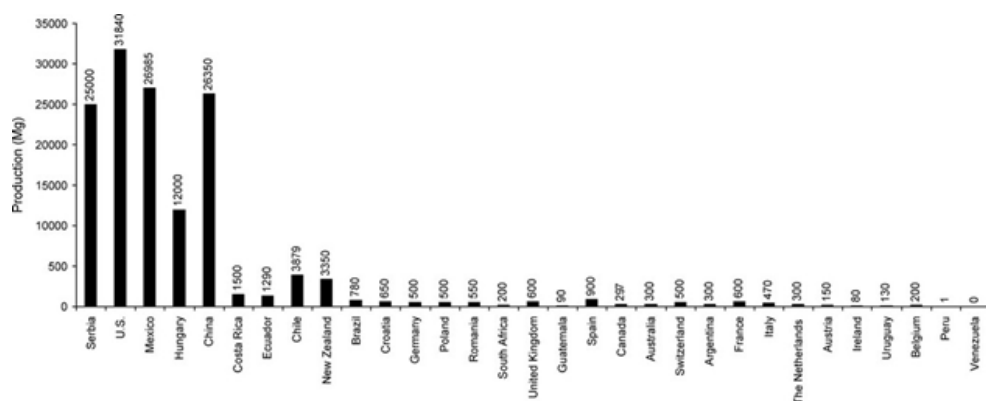


Figure 1. Worldwide cultivated blackberry area in 1995, 2005, and 2015 (projected); 1 Mg = 1.1023 t.

Analyzing data of the Chamber of Commerce of RS relating to the structure of exported fruit, 21,451,375 t of blackberry fruits were exported in 2014 in the amount of 39,432, 601 \$. In recent years (2012–2020), according to the data from the Republic Statistic Department ([www.stat.gov.rs](http://www.stat.gov.rs)), blackberry production in the Republic of Serbia significantly oscillated. According to the same source, in 2017, realized production reached 28,334 t and around 29,000 tons of fresh and frozen of blackberries were exported to the EU in 2018.

The blackberry plantings in Serbia are dominated by the ‘Čačanska Bestrna’ and ‘Thornfree’ cultivars, with more than a 95% share, followed by the ‘Black Saten’, ‘Dirksen Thornless’ and some more recent cultivars, such as ‘Loch

Ness', 'Chester Thornless', 'Triple Crown' (Nikolić et al., 2012). In order to achieve and maintain optimal production level for the Republic of Serbia (25–30.000 t), as well as to eliminate variations in terms of yield, fruit quality, purchase price and market demand, application of newer growing technologies and technological processing methods as well as partial assortment change are required (Karaklajić-Stajić et al., 2016). Analyzing blackberry production from the previous period, we can observe a delay in its placement, as a result of domination of the cultivar 'Čačanska Bestrna', characterized by an exceptional yield potential, though its fruits are exclusively intended for processing. Fruits of the abovementioned cultivar are placed in domestic and international market solely in frozen state, though the level of processing facilities is low and thus the economy of the Republic of Serbia is suffering big financial losses. In order to overcome the existing situation in blackberry production, it is necessary to manage the production in a direction that will be much more profitable considering the modern nutrition concept is ever more imposing the usage of innovative food with added value, which has a beneficial effect on preservation of human health.

In highlands of blackberry of the Republic of Serbia, trellis cultivation system is predominant which implies combination of vertical and horizontal three-wire systems (Nikolić, 2001). According to Petrović et al. (2007) advantages of a trellis system are easier application of mechanization for inter-row tillage and protection from disease causal agents and pests, even ripening of fruits and better lignification of canes for subsequent vegetation which improves resistance to low temperatures.

In the world largest blackberry production regions, there are different types of growing system, which is mostly due to differences between cultivars regarding canes architecture. In recent years, blackberry production system in a protected and semi-protected area have been used, primarily for the protection fruit from the adverse effect of environmental factors, which represents a significant advantage in comparasion to open-field production. The primary reason for growing blackberry in a protected area is protection from excessive precipitation, low winter and high summer temperatures, increase of relative air humidity, preventing causative agents of disease as well as reducing ultraviolet radiation. Also, growing blackberry in greenhouses and screenhouse prolongs maturity stage and thus increases production profitability. For such type production, it is necessary to introduce high yielding quality primocane and floricanes cultivars which, besides freezing, can be used for fresh consumption. In this way, placement of fresh fruits would be enabled representing a new challenge in blackberry production. Depending on the type of training system, growing blackberries in protected, and semi-protected areas can be carried out in different ways. Blackberry cultivation under rain shield (one or two) cultivation system is

easier and highly profitable production system in semi-protected areas. Based on the results of comparative studies between growing blackberry 'Čačanska Bestrna' in an open field and in rain shield cultivation system conducted at Fruit Research Institute, Čačak, Karaklajić-Stajić et al. (2021) confirmed differences in bio-productive traits. Namely, eliminating harmful effects of abiotic factors, primarily precipitation, significantly contributed to increasing yield and enhancing fruit quality. Growing blackberry in rain shield cultivation system have had positive effects on vegetative and generative potential, manifestation of better nutritional quality of fruit as well as lower incidence of grey mold.

The results of examining generative parameters i.e. number of fruiting braches, inflorescence, fruits (Table 1), and yield per cane (Table 2) are shown in the function of the cultivation techniques over the three-year period. The cultivation system and year of study, as well as their interaction effect, statistically significantly influenced on the number of inflorescences and fruits per cane, while the number of fruiting branches per cane in blackberry was significantly influenced by year and interaction of the cultivation system/year. Average values of the number of fruiting branches, inflorescence and fruits per cane ranged in the interval from 12.81 to 16.33, from 98.25 to 143.16 and from 246.90 to 364.32, respectively. The application of the rain-shield cultivation system during the three-year experiment period significantly influenced the most important parameters of the blackberry generative potential. A significant increase compared to the standard cultivation system was found in the number of inflorescence and fruits per cane, whereas for the fruiting branches number, higher values were also noted, but with no significance. Significant differences in the number of fruiting branches and inflorescence per cane were also found between the years of study, while in terms of number of fruits per cane, the significance of differences was recorded only in relation to the first year. These differences in the number of fruiting branches and inflorescence per cane between the years of study occurred most likely as an outcome of different climatic conditions in the period of flower bud differentiation, since blackberry belongs to the late flower bud differentiation cultivar group. If we observe the result tendency related to the number of fruiting branches for each cultivation system in each year of the study, we will notice number of fruiting branches, which indicates that the value of the mentioned generative potential parameter was the result of the interaction effect between cultivation system and cultivation conditions in certain years. According to Strik (2012), the differentiation of flower buds in blackberries, raspberries, strawberries and blueberries is conditioned to a considerable extent by the complex activity of abiotic factors, among which temperature conditions, light regime, soil moisture, and also the applied cultivation technology should be highlighted.

Table 1. Effect of cultivation techniques on parameters of generative potential of blackberry 'Čačanska Bestrna'

| Treatment                             |       | Number of<br>fruiting<br>branches per<br>cane | Number of<br>inflorescence<br>per cane | Number of<br>fruits per cane |
|---------------------------------------|-------|-----------------------------------------------|----------------------------------------|------------------------------|
| Cultivation techniques (A)            |       |                                               |                                        |                              |
| Rain shield                           |       | 14.60 ± 0.51 a                                | 123.59 ± 2.90<br>a                     | 323.72 ± 10.34<br>a          |
| Standard                              |       | 14.05 ± 0.38 a                                | 112.61 ± 4.50<br>b                     | 285.90 ± 13.34<br>b          |
| Year (B)                              |       |                                               |                                        |                              |
| 2012                                  |       | 16.28 ± 0.26 a                                | 99.65 ± 2.49<br>c                      | 362.99 ± 13.67<br>a          |
| 2013                                  |       | 13.89 ± 0.38 b                                | 131.31 ± 3.29<br>a                     | 281.74 ± 8.88 b              |
| 2014                                  |       | 12.81 ± 0.57 c                                | 123.35 ± 4.32<br>b                     | 269.70 ± 10.86<br>b          |
| Cultivation techniques × Year (A × B) |       |                                               |                                        |                              |
| Rain shield                           | 2011. | 16.24 ± 0.46 a                                | 98.25 ± 3.91<br>c                      | 364.32 ± 21.33<br>a          |
|                                       | 2012. | 12.91 ± 0.49 c                                | 143.16 ± 4.92<br>b                     | 304.33 ± 13.27<br>b          |
|                                       | 2013. | 14.67 ± 0.46 b                                | 129.37 ± 2.94<br>a                     | 302.50 ± 8.39<br>b           |
| Standard                              | 2011. | 16.33 ± 0.26 a                                | 101.04 ± 3.26<br>c                     | 361.66 ± 18.58<br>a          |
|                                       | 2012. | 14.87 ± 0.33 b                                | 119.46 ± 3.42<br>b                     | 259.15 ± 4.02<br>c           |
|                                       | 2013. | 10.95 ± 0.44 d                                | 117.32 ± 5.57<br>b                     | 246.90 ± 11.32<br>d          |
| ANOVA                                 |       |                                               |                                        |                              |
| A                                     |       | ns                                            | *                                      | *                            |
| B                                     |       | *                                             | *                                      | *                            |
| A × B                                 |       | *                                             | *                                      | *                            |



Values within each column followed by the same small letter are insignificantly different at the  $p \leq 0.05$  by LSD test; ns - non significant differences.

The yield per cane of the examined blackberry cultivar was statistically significantly influenced by cultivation system. Average yield values per cane ranged from 2,805.56 to 3,395.81 g. Significantly higher values of yield per cane were found in semi-closed conditions of blackberry cultivation system.

Table 2. Effect of cultivation techniques on parameters of generative potential of blackberry 'Čačanska Bestrna'

| Treatment                             |      | Yield per cane (g) |
|---------------------------------------|------|--------------------|
| Cultivation techniques (A)            |      |                    |
| Rain shield                           |      | 3,395.81 ± 45.84 a |
| Standard                              |      | 2,843.45 ± 46.35 b |
| Year (B)                              |      |                    |
| 2012                                  |      | 3,199.06 ± 58.29 a |
| 2013                                  |      | 3,109.20 ± 66.64 a |
| 2014                                  |      | 3,072.85 ± 79.35 a |
| Cultivation techniques × Year (A × B) |      |                    |
| Rain shield                           | 2012 | 3,543.37 ± 70.48 a |
|                                       | 2013 | 3,351.11 ± 61.20 a |
|                                       | 2014 | 3,292.93 ± 56.33 a |
| Standard                              | 2012 | 2,805.56 ± 80.45 a |
|                                       | 2013 | 2,867.29 ± 51.33 a |
|                                       | 2014 | 2,852.78 ± 50.84 a |
| ANOVA                                 |      |                    |
| A                                     |      | *                  |
| B                                     |      | ns                 |
| A × B                                 |      | ns                 |

Values within each column followed by the same small letter are insignificantly different at the  $p \leq 0.05$  by LSD test; ns - non significant differences.

Gaskell (2004) states that the cultivation of raspberries in closed area is widespread in the US and is primarily intended for the production of fruits for fresh consumption, since modification and elimination of harmful effects of abiotic factors, primarily precipitation, enables better fruit quality and, on the other hand, extends the harvest period, which all together increases production profitability.

## **STRAWBERRY PRODUCTION**

The average production of strawberries in Serbia in the last five years was 25–30,000 t and the area under plantations was about 7,000 ha (FAOSTAT), which indicates stable production in the observed period. The strawberry fruit are mostly used fresh and places on the domestic and foreign markets while a small part goes to further processing. The export of strawberries is dominated by primary products (fresh and frozen fruits) with lowest price. The amount of exported strawberries in the last five years was approximately 10,000 t while the value of export was about 20,000,000\$ (FAOSTAT). In export marketing channels, fresh strawberries produced in Serbia compete with quality (Zarić et al., 2008). Export of fresh strawberries is mainly oriented towards the Russian market, while frozen strawberries are exported to EU countries and Russia.

The most common cultivars in strawberry plantations are ‘Clery’, ‘Joly’, ‘Alba’, ‘Asia’, ‘Arosa’ and ‘Roxana’ (Milivojević, 2018). These cultivars are economically important, while the everbearing cultivars and cultivars for processing are grown on a smaller scale with the predominantly represented cultivar ‘Senga Sengana’. Therefore, it is necessary to monitor changes in the assortment in the world and introduce new cultivars in order to meet the needs to producers, consumers and processors.

The most common growing technology of strawberry in the open field on beds covered with foils and drip irrigation system. However, strawberry production in Serbia lags behind global innovations in strawberry production in the world. Strawberry growing in a protected condition (tunnels, greenhouses) is less represented due to the high costs of establishing such plantation. In addition, extensive strawberry plantation without mulch (foil or organic mulch) and drip irrigation are still present. Bearing in mind all the above mentioned, the average yield per area are very low compared to countries with developed strawberry production. Low yields are a consequence of frequent occurrence of late spring frosts during flowering and rainy periods during ripening of fruits. Large numbers of strawberry plantations are based on plants originating from native orchards or with insufficient application of agrotechnical practices, in

which yields are low and fruit quality varies from year to year which also affects the reduction of commercial strawberry yields. Bearing in mind the large and growing market suitable for the placement of fresh strawberries in EU countries that seek strawberries throughout the year, it is necessary to introduce new cultivars and growing technology in protected condition in order to prevent damage caused by adverse environmental factors and extend the season of fresh strawberry production.

### **EFFECTS OF BIOFERTILIZER BIOVERMIX ON QUALITY OF FRESH BERRIES**

Several studies have shown that the amount of phytochemicals depends on the cultivar (genetic factors), one of the key factors, and environmental factors (light, temperature, water regimes, atmospheric carbon dioxide), agronomic factors (water management, mineral nutrition, grafting, application of elicitors, stimulating agents, plant activators) as well as agricultural practices, that is organic versus conventional agricultural practices (Wang et al., 2009). In this regard, organic (fruit) production has become very popular due to the ‘sustainable intensification’ of the production with less pesticide and heavy metal residues, pronounced aroma, better soil health and higher biodiversity (Lockie et al., 2006). You et al. (2011) showed that organically grown berries have significantly higher levels of bioactive compounds compared to berries from integrated production. It is widely acknowledged that using composts and vermicomposts as amendments, rather than industrialized fertilizer and raw manure, could improve soil nutrients and promote soil health (Jack and Thies, 2006).

The improvement of plant growth using vermicompost may result by impact its nutrients and biologically active substances (Warman and AngLopez, 2010). Many plant growth regulators of microbial origin are found in vermicompost (Atiyeh et al., 2002), and the application of vermicompost significantly contribute to the activity of soil enzymes (Albiach et al., 2000). The microbial biomass also plays an important role in plant growth in the field. Leachates thus derived from vermicomposting are regarded as beneficial and can be used as liquid fertilizer due to high concentration of plant nutrients (Jarecki et al., 2005; Gutierrez-Miceli et al., 2008; Tejada et al., 2008). Since these alternatives are not clearly reported in the literature at present, it is not recommended to introduce abovementioned liquid fertilizer into berries production without prior study of its effect on the fruit quality. Due to limited quantities of the mentioned bioresource on the market, there is a need to use a vermicompost-based bioproduct.

Taking these facts into consideration, this study aimed to evaluate the effects of biofertilizer Biovermix which designed at the Fruit Research Institute Čačak, and obtained from vermicompost on the phytochemical profile of blackberry, blueberry, and strawberry fruit.

The results obtained from this study could help growers to expand the application of biofertilizers in berry production and thus positively affect the minimal environmental pollution with improved nutritional properties of berry fruit.

Fruits from three berry species (blackberry ‘Čačanska Bestrna’, highbush blueberry ‘Aurora’, and strawberry ‘Senga Sengana’) were used in this study. All examined berries, were grown at the experimental station of the Fruit Research Institute, located in central Čačak, Western Serbia.

The study involved watering of biofertiliser Biovermix in orchards of examined berry species. Biovermix were applied three times during growing period: at the beginning of growing period, flowering (5–10% flowers open) and ripening (Table 3). The trial involved two treatments i.e. with Biovermix application (0.5 dm<sup>3</sup> per bushes/runners) and untreated plants served as control. The biofertiliser Biovermix specially obtained as liquid extract of vermicompost of organic origin, enriched with selected strains of beneficial microorganisms (bacteria strains of the genus *Azotobacter* sp., *Bacillus* sp., and *Pseudomonas* sp. as well as *Trichoderma* sp. fungus).

Table 3. Overview of biofertiliser application time during investigation

| Species                          | Foliar applications of Biovermix biofertilizer |                           |                            |
|----------------------------------|------------------------------------------------|---------------------------|----------------------------|
|                                  | 1 <sup>st</sup>                                | 2 <sup>nd</sup>           | 3 <sup>rd</sup>            |
| Blackberry<br>‘Čačanska Bestrna’ | 11 <sup>st</sup> of May                        | 30 <sup>th</sup> of May   | 10 <sup>th</sup> of July   |
| Blueberry<br>‘Aurora’            | 11 <sup>st</sup> of April                      | 10 <sup>th</sup> of June  | 15 <sup>th</sup> of August |
| Strawberry<br>‘Senga Sengana’    | 15 <sup>th</sup> of March                      | 15 <sup>th</sup> of April | 20 <sup>th</sup> of May    |

Fully ripe blackberries were black, glossy and easily picked from the branches (Karaklajic-Stajic et al., 2017). In order to obtain uniform samples of blueberry, berries were visually chosen from the same pool of each bush at the

same development stage, but for strawberries harvest date established based on sensory properties (colour, flavor, and firmness of berries). Samples of each species (each replication containing 0.5 kg of berries) were immediately frozen in liquid nitrogen and stored at -20°C until chemical analyses.

Table 4. Fruit weight, soluble solids content of berry species treated with biofertilizer Biovermix

| Berry species      | Application         | Fruit weight (g)<br>content (°Brix) | Soluble solids |    |
|--------------------|---------------------|-------------------------------------|----------------|----|
| Strawberry         | Biovermix           | 15.53±0.31                          | 9.77±0.95      |    |
| ‘Senga Sengana’    | Without application | 12.47±0.85                          | 9.40±0.85      | ns |
| Blueberry          | Biovermix           | 2.18±0.36                           | 10.15±1.27     |    |
| ‘Aurora’           | Without application | 2.00±0.21                           | 10.10±0.64     | ns |
| Blackberry         | Biovermix           | 8.80±0.44                           | 8.90±0.85      |    |
| ‘Čačanska Bestrna’ | Without application | 8.23±0.40                           | 8.40±1.01      | *  |

\* and ns mean respectively significance and not per  $P < 0.05$  (Student’s t-test)

We used three berry species (strawberry, blueberry, and blackberry) in the study, which are interesting because of their good nutritive potentials. The public health experts are recommending increased consumption of berries because they are rich in natural antioxidants that have been linked to reduced risk of various health maladies, including cancer, coronary heart disease, metabolic disorders, and inflammatory responses (Obrenovich., 2011). In general, the berries weight was lower in treatment without application biofertilizer (Table 4). Berries weight increased by 7% (blackberry ‘Čačanska Bestrna’), 9% (blueberry ‘Aurora’), and 24,54% (strawberry ‘Senga Sengana’) in conditions of application Biovermix. The same trend in terms of berry weight identified in the present study correspond to the intention reported by Hassan et al. (2018) for the black cherry tomato treated with vermicompost leachates. Moreover, our results contrary with Budiastuti et al. (2012) who reported that melon fruits obtained under organic or natural fertilization generally have lower weight and yield than those produced using conventional chemical fertilization. The positive effect of applied

biofertilizer was confirmed on the fruit weight, which will directly affect the increase to yield. On the other hand, production efficiency will be higher due to the production of berries in ecosafe protective system. Interestingly, Biovermix application was significantly affected only on soluble solids content in blackberries (8.40–8.90 °Brix), but in strawberries (9.40–9.77 °Brix) and blueberries (10.10–10.15 °Brix) higher value on abovementioned parameter obtained without application. About that, Budiastuti et al. (2012) emphasizes that compost-tea treatments in their study were superior with respect to very important criteria, such as soluble solids content in melon fruit.

Table 5. Effects of biofertilizer Biovermix on phenols content (mg100<sup>-1</sup> g FW) in fresh blackberry ‘Čačanska Bestrna’

| Compounds            | Blackberry ‘Čačanska Bestrna’ |                 |
|----------------------|-------------------------------|-----------------|
|                      | Biovermix                     | Control         |
| I Phenolic acids     |                               |                 |
| Total phenolic acids | 82.75±3.21a                   | 50.28±1.24 b    |
| Total flavanols      | 61.44±1.58 a                  | 46.54±1.11 b    |
| Total flavonols      | 4.81±0.18 a                   | 3.06±0.03 b     |
| Total anthocyanins   | 126.12±2.34<br>a              | 77.77±1.78<br>b |
| TOTAL PHENOLS        | 275.12                        | 177.65          |

<sup>a</sup> Data expressed as the mean ± SD (n=4). b Means within the same column followed by different letters were significantly different at  $p < 0.05$ . \* and ns, significant or nonsignificant at  $p < 0.05$ .

Analysis of phenols content in blackberries indicated significant effect of Biovermix application on all studied phenols group (Table 5). Values of the studied phenols group were higher in treatment with Biovermix compared to treatment without application. Content of total phenolic acids, flavanols, flavonols, anthocyanins, and finally total phenols ranged from 50.28 to 82.75, 46.54 to 61.44, 3.06 to 4.81, 77.77 to 126.12 and 177.65 to 275.12, respectively.

Table 6. Effects of biofertilizer Biovermix on phenols content (mg 100<sup>-1</sup> g FW) in fresh blueberry ‘Aurora’

| Compounds            | Blueberry ‘Aurora’ |               |
|----------------------|--------------------|---------------|
|                      | Biovermix          | Control       |
| I Phenolic acids     |                    |               |
| Total phenolic acids | 6.89±0.09 a        | 8.98±0.11 a   |
| Total flavanols      | 8.93±0.05 a        | 9.93±0.13 a   |
| Total flavonols      | 18.52±0.07 a       | 17.93±0.05 a  |
| Total anthocyanins   | 153.26±1.01 a      | 136.96±0.42 b |
| TOTAL PHENOLS        | 187.60             | 176.80        |

<sup>a</sup> Data expressed as the mean ± SD (n=4). <sup>b</sup> Means within the same column followed by different letters were significantly different at  $p < 0.05$ . \* and ns, significant or nonsignificant at  $p < 0.05$ .

Total phenols content in blueberries ranged from 176.80 to 187.60 mg 100g<sup>-1</sup> FW and were higher in fruits untreated with biofertilizer Biovermix (Table 6). The application of abovementioned biofertilizer resulted in higher values of total flavonols and anthocyanis content, except the total phenolic acids and flavanols. Among the phenolic compounds detected in the fruit of the tested blueberry cultivar, total anthocyanins were found in high concentrations (153.26 mg 100<sup>-1</sup> g FW) in treatment with biofertilizer Biovermix.

Table 7. Effects of biofertilizer Biovermix on phenols content (mg 100<sup>-1</sup> g FW) in fresh strawberry ‘Senga Sengana’

| Compounds            | Strawberry ‘Senga Sengana’ |               |
|----------------------|----------------------------|---------------|
|                      | Biovermix                  | Control       |
| I Phenolic acids     |                            |               |
| Total phenolic acids | 7.47±0.11 a                | 6.82±0.21 a   |
| Total flavanols      | 37.71±0.33 a               | 38.73±0.65 a  |
| Total flavanons      | 20.06±0.96 a               | 17.44± 1.01 b |
| Total flavonols      | 0.89±0.02 a                | 0.83±0.02 a   |
| Total anthocyanins   | 27.54±1.56 a               | 30.47±1.11 a  |
| TOTAL PHENOLICS      | 93.67                      | 94.29         |

<sup>a</sup>Data expressed as the mean ± SD (n=4). <sup>b</sup> Means within the same column followed by different letters were significantly different at  $p < 0.05$ . \* and ns, significant or nonsignificant at  $p < 0.05$ .

Growing strawberries in intensive production conditions, i.e. using biofertilizers had a positive effect on the nutritional composition of the fruit that has been confirmed through higher the content of total phenolic acids, flavanones and flavonols (Table 7). However, content of total phenolic components in the fruits of the examined strawberry cultivar grown according to standard production criteria was higher, but without statistically confirmed significance of obtained average values.

The results obtained from this study could help growers to expand the application of biofertilizers in berry production and thus positively affect the minimal environmental pollution with improved nutritional properties of berry fruit.

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## ROLE OF QUANTITATIVE GENETIC IN SHEEP AND GOAT BREEDING

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**Abstract:** Quantitative genetic methods have a variety of applications in understanding the covariance of variations between natural and managed populations. Today, the combination of molecular or genomic selection in combination with the traditional is a very reliable method for faster, more accurate assessment of the breeding value of animals. Important features of body conformation in the meat industry and good growth performance in sheep and goats, including accelerated growth rate are of economic importance. Quantitative genetics methods aim to identify genetic variants associated with quantitative traits in order to achieve genetic improvement in growth performance. Quantitative genetics remains the most reliable tool for estimating the breeding value of domestic animals. The application of practical methods which are used to assess the phenotype, requires excellent theoretical knowledge of the genetic of animals.

**Key words:** quantitative genetics, sheep, goat, inherited traits

### INTRODUCTION

Genetic basis underlying the phenotypic variation among the population and each animal focussed primarily with the traits having a continuous range of values that it compasses with height, weight, longevity, and so on. Animal breeding aimed to improve animals by changing their genetic abilities for imperative traits. Quantitative genetics could use to understand how traits will respond to artificial selection regimes, and therefore, could be a vital tool in the development of selective breeding programs for animal improvement (Wolf, 2008). Although

animal breeding practice long before the science of genetics and the relevant disciplines of population and quantitative genetics are known, the breeding programs are relied mainly on simply selecting and mating the best individuals on their own or relative performance (Hill, 2014). Quantitative genetics is one of the genetics disciplines dealing with the mechanism of quantitatively inherited traits, where classical quantitative genetics is known as statistical genetics or biometrical genetics, used for statistical analysis for detecting possible genetic models for designed genetic populations (Gai and Lu 2013; Caro Petrović et al., 2013a, 2013b). Quantitative genetics is a conceptual and methodological framework, allowing biologists to study the genetics of complex phenotypic traits (Falconer Mackay, 1996; Lynch & Walsh, 1998; Caro Petrovic et al., 2012; Petrović et al., 2012;) i.e., traits transformed by a large number of genes.

Breeds of Sheep and goat are selected worldwide for meat, wool, and dairy production, and breeding objectives also include other functional traits such as reproductive performance and disease resistance (Rupp et al., 2016). Consequently, new methods of prediction of merit in breeding programs are again based on essentially numerical methods, but incorporating genomic information with long-term selection responses are revealed in laboratory selection experiments, and prospects for continued genetic improvement are high (Hill, 2010). The approach of quantitative genetics has diverse applications: it is fundamental to an understanding of variation and covariation among relatives in natural and managed populations. Hence, new methods of prediction of merit in breeding programs are based again on essentially numerical methods but incorporating genomic information. (Hill, 2010).

## **THE IMPORTANCE OF QUANTITATIVE GENETICS IN ANIMAL BREEDING**

The quantitative genetic approach has a diverse application in its fundamentals to the understanding of variation covariation among populations of the natural and its managed populations. Today, the combination of molecular or genomic selection combined with traditional is a very reliable method for a faster, more accurate assessment of the breeding value of animals (Petrović et al., 2018). Gu et al. (2022) informed that body conformation traits are economically important in the goat meat industry and good growth performance in goats, including an accelerated growth rate which could improve carcass weight and meat yield, and an identification of the genetic variants associated with these traits provides the basis for the genetic improvement of growth performance.

Designing effective breeding programs requires quantitative information about the nature and scale of genetic and environmental sources of the variation and correlation for components of performance (Toghiani, 2012). In the study of Petrovic et al. (2020), a quantitative genetic analysis of the variability and relationship of lamb body weight in the indigenous Pirot sheep population shows that the genetic potential of the lambs is characterized by high variability, which is very suitable for selection to higher growth.

Though most traits associated with reproduction have low heritability, genetic improvement will be slow if the selection is based on one or a few phenotypic records. The advances in molecular genetic techniques may serve as an alternative to increasing genetic progress in prolificacy. Various techniques have been developed to elucidate mechanisms involved in phenotypic expression at the DNA level. The development of the next generation of molecular tools to identify genomic genetic variants has made it possible to apply whole-genome scanning techniques, genome-wide association studies, and genomic selection to improve goat prolificacy (Luciano et al 2020).

### **Realibility of quantitative genetics**

Validation of the equations for predicting the reliability of genomic breeding values without all genotyping data was performed by Vadenplas et al. (2017), based on simulated data of a two-way crossbreeding program, using either two closely-related breeds or two unrelated breeds, to produce crossbred animals and also informed, that the proposed equations can be used for an easy comparison of the reliability of genomic estimated breeding values across many scenarios especially if all genotyping data are available.

The direct observable characteristics of an animal population that concentrates in a way that individual variation in genotype contributed by the environment to phenotype variance are on its quantitative traits. Many essential traits such as weight gain, milk yield, fat content, meat, and more depend on desirable traits to quantify are the quantitative traits. Dekhili (2014) the kind of selection based mainly on visual qualitative traits. According to Gutierrez-Gil et al. (2009), for the first time in a commercial population of dairy sheep, a whole genome scan using microsatellite markers revealed a genome-wise significant QTL, along with other suggestive genetic effects that influence milk production related traits. Quantitative genetics may guide an important way of understanding the genetic nature of populations. Genetic variation is assumed for many genetic phenomena such as inbreeding, selection, and genetic drift. On the otherhand, quantified genetic variation serves a variety of purposes in related disciplines (Lee 2002).

## RELATIONSHIP BETWEEN INHERITANCE FEATURES

Recent progress in quantitative genetics have contributed to more efficient animal production through a better understanding of the underlying genetic model, genetic parameters, breeding objectives, selection methods, and breeding systems necessary to engineer useful genetic changes in animal performance (Dickerson and Willham, 1983).

Application of mathematical model describing lactation curves of individual ewes is a useful tool to describe milk production of sheep which can then be used for QTL mapping (Raadsma et al., 2009).

The rate of adaptation can also be greatly compromised by the absence of a strong positive genetic correlation between the sexes for fitness, have suggested from recent studies of natural populations (Kirkpatrick, 2009).

### Genetic parameters of the population

Gai and Lu (2013), stated that development of molecular quantitative genetics has provided a way of genetic dissection of a quantitative trait.

Classically, the quantitative geneticists have envisioned DNA sequence variants as the only source of heritable phenotypes (Johannes et al., 2008).

According to Gipson (2019), for the most part, biometrics has concentrated upon the refining of estimates of heritability and genetic correlations. While heritabilities are instrumental in the calculation of estimated breeding values, genetic correlations are necessary in the construction of selection indices that account for changes in multiple traits under selection at one time.

Although most animal behaviors are associated with some form of inherited genetic variation, we still do not understand how genes shape behavior over time, either directly or indirectly (York, 2018). The said author compiled a data set comprised of over 1000 genomic loci representing a spectrum of behavioral variation across animal taxa. Comparative analyses reveal that courtship and feeding behaviors are associated with genomic regions of significantly greater effect than other traits, on average threefold greater than other behaviors. Investigations of whole-genome sequencing and phenotypic data for 87 behavioral traits from the *Drosophila* Genetics Reference Panel indicate that courtship and feeding behaviors have significantly greater genetic contributions and that, in general, behavioral traits overlap little in individual base pairs but increasingly interact at the levels of genes and traits. These results provide

evidence that different types of behavior are associated with variable genetic bases and suggest that, across animal evolution, the genetic landscape of behavior is more rugged, yet predictable, than previously thought (York, 2018).

According to Ma et al. (2021) genetic evaluation for a trait with group records can be further improved by a multiple-trait model including correlated traits with individual records and finally, for efficient use of group records in genetic evaluation, a relatively small group size, and close relationships between individuals within one group are needed.

Phenotypic information of both parents can predict relatively how the offspring will perform. However, the observed performance of each animal in each trait is the result of the heredity that it receives from both parents and the environment in which it raised, and even when an attempt is made to provide a uniform environment, there are still accidental and unknown environmental differences between animals (Babar et al., 2004, Caro Petrović et al., 2020).

There is a different level of correlation between lamb weights, which gives us a reason to say, that many paragenetic factors are crucial to the growth of lambs from birth to weaning. The multiple correlation coefficient indicates that any increase in lamb body weight during the observed periods is associated with an increase in the results of the dependent variable. In particular, any increase in lamb weight at birth is associated with a 90-day increase in lamb weight (Petrovic et al., 2020).

Phenotypic variation is produced through interactions between genotype and the environment (McManus 2011).

The economically important traits in the small ruminants (weight at various ages, growth rate, carcass characteristics) are subject to genetic and environmental influences. Effective breeding requires the selection of elite animals from flocks with better genetic worth. Growth, especially the pre-weaning growth rate in small ruminants, is influenced not only by the animal's own genetic makeup, but by environmental factors, that is, age of dam, birth weight, sex of lamb, and lambing season. Therefore, to have maximum genetic progress through selection, it is critical to devise effective selection indices and reliable estimates of non-genetic and genetic parameters (Ali et al., 2020).

The rate of adaptation can also be greatly compromised by the absence of a strong positive genetic correlation between the sexes for fitness, as suggested from recent studies of natural populations (Kirkpatrick, 2009).

## **IMPORTANCE OF BREEDING VALUE IN ANIMAL BREEDING**

Animal breeding value is estimated to be twice the expected performance of its progeny, expected progeny performance calling transmitting ability, of which it's the genetic advantage of an individual transmit to offspring. The breeding value can be estimated based on animal own record and performance of own relative (Toghiana, 2012).

The success of genetic improvement was based on expectations that the descendants by their phenotypic values will be above the parents' average values. Expected genetic progress-selection success is valued depending on the heritability of a given property and selection differential. The degree of inheritance which we call heritability is the ratio of genetic and total phenotypic variance. That means it is not a biological constant, while its value can be different in populations (Hill 2014; Petrović and Pantelić 2015; Caro Petrović et al., 2018).

Incorporating the measures of variation in intermediate phenotypes with genetic variation in molecular markers and quantitative phenotypic variation will provide a biological context in which to interpret the phenotype (Mackay 2009; Mackay et al., 2009).

## **CONCLUSION**

Despite advances in molecular genetics and genomic selection, quantitative genetics remains the most reliable tool for estimating the breeding value of domestic animals. The application of methods and software, which are used to assess the phenotype, requires excellent theoretical knowledge of the principles and methods of genetic improvement of animals.

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## **DEFICIT OF WATER FROM THE REDUCED ANNUAL RAINFALL IN THE EXISTING IRRIGATION SYSTEMS, LOCATED IN THE PELAGONIJA REGION**

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**Summary:** *Climate change that is a reality in the countries of the Balkan Peninsula is manifested by the problem of lack of irrigation water. Existing H.M.S. are supplied with irrigation water through built reservoirs, where rainwater accumulates in the rainy season and is used in the vegetative period, when it is most needed by crops. This scientific paper analyzes the hydrological data from 4 rain gauge stations (Kicevo, Krushevo, Prilep and Bitola), which gravitate to the Pelagonija region. With the hydrological analysis of the annual amounts of rainfall and with the application of the theoretical curves from the theory of probability, the values of the required quantities of rainfall are obtained, which are sufficient to ensure sufficient water capacity in the existing reservoirs.*

**Keywords:** *Annual rainfall amounts, Rain intensity, Gumble curve, Rainfall distribution, Hydromelioration systems.*

### **➤ Introduction**

Climate change that has become a reality in recent years, in the countries of the Balkan Peninsula, is increasingly manifested by increased droughts, which are repeated from year to year and are characterized by reduced rainfall and distribution of rainfall in periods outside the vegetation period. When water quantities are most needed for the normal development of agricultural crops, they contribute to a reduced yield of agricultural products. This situation especially refers to the existing hydro amelioration systems in the Pelagonija region, HMS Strezevo-Bitola and HMS Prilepsko pole-Prilep.

The reduced amounts of rain in the agricultural areas, which are intensively used for agricultural production, directly affect the efficient operation of the existing hydro-amelioration irrigation system, in terms of providing the required volume of irrigation water during the growing season.

The spatial and temporal distribution of the quantities of rain directly affects the following preconditions for planned and organized agricultural production.

1. Selection of agricultural crops to be planted, in a specific area.
2. The size of the agricultural area to be irrigated.

The existing agricultural bases that have been in use for decades, are undergoing changes according to the definition of the two preconditions that directly depend on climatic factors and they dictate the mode of operation of hydromelioration systems.

For the accurate definition of the variations of the rain in the Pelagonija agricultural regions, a statistical analysis of the available hydrological data for the rain in a certain period of time was performed, through which we can get an overview of the change in the amount of rain in the considered time interval. strings of 59 years from 1951 to 2010.

In order to get an answer to the fall of the rain in the past years, in relation to the average values of the rain, seen in a longer period of time, an analysis of the hydrological strings in the length of 59 was performed for four rain stations, Kicevo, Krushevo, Prilep. and Bitola.

The analysis of the 4 hydrological sequences includes the processing of statistical parameters through which the data for the occurrence of total precipitation in the range with a probability of 99.99% to 0.01%, ie the time range for the occurrence of total rainfall from 1 year to 10 000 years.

#### ➤ **Methods and Materials**

The processing of the hydrological sequences for the 4 rain gauge stations provides an opportunity to get an answer to the possible occurrence of rains in the period from 1 year to 10,000 years, which will cover all possible scenarios for the movement of rainfall in the Pelagonija region where belonging to rain gauges.

By obtaining the necessary data from the statistical processing of the hydrological data, the answer to the question of the required additional quantities of water needed for provision in the existing hydromelioration irrigation systems (Strezevo and Prilep field) that perform their function for more than 40 years.

Table 1 shows the hydrological sequences for the 4 rain gauge stations Kicevo, Krushevo, Prilep and Bitola from 1951 to 2010.

Table 1 [1]

| Year | KRUSEVO | KICEVO | BITOLA | PRILEP |
|------|---------|--------|--------|--------|
| 1951 | \       | 847,7  | 644,4  | 643,2  |
| 1952 | 368,7   | 485,0  | 469,9  | 393,2  |

|      |        |        |       |       |
|------|--------|--------|-------|-------|
| 1953 | 619,1  | 442,6  | 358,8 | 343,6 |
| 1954 | 1231,1 | 865,0  | 682,2 | 661,1 |
| 1955 | 1239,7 | 852,6  | 740,0 | 658,0 |
| 1956 | 735,4  | 710,5  | 575,1 | 531,6 |
| 1957 | 706,8  | 702,1  | 760,3 | 598,8 |
| 1958 | 645,4  | 733,1  | 595,6 | 602,5 |
| 1959 | 935,7  | 823,4  | 732,5 | 652,4 |
| 1960 | 666,4  | 823,4  | 565,4 | 609,3 |
| 1961 | 463,3  | 591,3  | 409,0 | 440,8 |
| 1962 | 950,4  | 991,9  | 786,6 | 710,5 |
| 1963 | 996,1  | 1126,3 | 649,4 | 751,5 |
| 1964 | 839,9  | 972,8  | 605,9 | 670,3 |
| 1965 | 494,3  | 738,1  | 337,5 | 492,8 |
| 1966 | 668,4  | 879,5  | 588,9 | 567,6 |
| 1967 | 719,8  | 670,7  | 601,1 | 470,7 |
| 1968 | 734,5  | 741,9  | 519,2 | 551,2 |
| 1969 | 746,7  | 905,9  | 545,6 | 488,1 |
| 1970 | 626,4  | 858,7  | 584,6 | 520,8 |
| 1971 | 804,1  | 874,4  | 552,8 | 515,2 |
| 1972 | 867,8  | 834,9  | 717,1 | 618,8 |
| 1973 | 903,8  | 912,2  | 659,6 | 690,0 |
| 1974 | 950,7  | 815,5  | 622,7 | 587,7 |
| 1975 | 758,5  | 682,1  | 532,6 | 524,0 |
| 1976 | 961,2  | 778,9  | 703,3 | 643,6 |
| 1977 | 619,6  | 629,5  | 365,0 | 413,9 |
| 1978 | 917,4  | 727,6  | 622,4 | 573,9 |
| 1979 | 1131,6 | 918,4  | 817,5 | 698,5 |
| 1980 | 889,1  | 678,6  | 619,6 | 652,4 |
| 1981 | 973,7  | 768,0  | 851,9 | 808,1 |
| 1982 | 814,2  | 550,5  | 688,6 | 476,2 |
| 1983 | 917,5  | 679,4  | 756,6 | 633,1 |

|                      |                   |                   |                   |                   |
|----------------------|-------------------|-------------------|-------------------|-------------------|
| 1984                 | 660,9             | 644,1             | 516,5             | 474,1             |
| 1985                 | 791,9             | 823,6             | 607,4             | 518,1             |
| 1986                 | 889,0             | 798,1             | 632,4             | 530,6             |
| 1987                 | 774,2             | 677,7             | 564,3             | 419,9             |
| 1988                 | 679,7             | 558,9             | 466,4             | 297,7             |
| 1989                 | 774,8             | 656,7             | 554,6             | 464,6             |
| 1990                 | 638,5             | 580,1             | 515,7             | 371,3             |
| 1991                 | 752,0             | 673,9             | 698,0             | 453,1             |
| 1992                 | 666,6             | 559,5             | 592,2             | 410,1             |
| 1993                 | 605,0             | \                 | 426,3             | 342,5             |
| 1994                 | 680,6             | \                 | 540,5             | 349,6             |
| 1995                 | 1044,1            | \                 | 665,1             | 682,6             |
| 1996                 | 968,6             | \                 | 679,0             | 607,7             |
| 1997                 | 539,1             | \                 | 549,2             | 452,5             |
| 1998                 | 912,4             | \                 | 633,5             | 516,0             |
| 1999                 | 665,6             | \                 | 713,0             | 503,0             |
| 2000                 | 555,7             | \                 | 402,5             | 295,7             |
| 2001                 | 649,6             | \                 | 394,1             | 402,1             |
| 2002                 | 1152,7            | \                 | 863,8             | 697,0             |
| 2003                 | 1066,2            | 699,4             | 609,3             | 533,8             |
| 2004                 | 855,3             | 819,4             | 701,7             | 588,5             |
| 2005                 | 1008,4            | 953,9             | 649,0             | 517,7             |
| 2006                 | 870,2             | 792,6             | 695,4             | 444,9             |
| 2007                 | 948,9             | 780,7             | 529,8             | 598,5             |
| 2008                 | 679,0             | 534,1             | 523,5             | 431,9             |
| 2009                 | 1204,2            | 930,9             | 794,9             | 657,6             |
| 2010                 | 1204,1            | 1077,3            | 788,0             | 747,6             |
| <b>Sum</b>           | <b>48134,6 mm</b> | <b>38173,4 mm</b> | <b>36567,8 mm</b> | <b>32502,1 mm</b> |
| <b>Average value</b> | <b>829,91 mm</b>  | <b>779,05 mm</b>  | <b>619,79 mm</b>  | <b>550,88 mm</b>  |

The intensity of the amounts of precipitation is determined according to Gorbachev's pattern. The required parameters for calculating the intensity of the rain are the following for the duration of the intense rain of 15 minutes:

1. Authoritative rain according to which the geometric characteristics of the water management facilities are dimensioned is  $qr = 166,7 \cdot i \left( \frac{l}{ha} \right)$ .
2. Intensity of rain  $i = \frac{\Delta}{\sqrt{t}} \left( \frac{mm}{min} \right) t = 15 \text{ min.}$
3. Rain intensity expressed through addiction  $\Delta = \mu \cdot \sqrt{p}$
4. p Probability of occurrence of computational rain. In the calculations, a period of repetition is taken three times during two years, ie  $p = 1.5$
5.  $\mu$  Climatic characteristic depending on the annual amount of precipitation  $\mu = \alpha \cdot \sqrt[3]{Hsr^2}$
6.  $\alpha = 0.046$  Geographic constant, which depends on latitude, at the location of the rain station.

Table 2 shows the calculated values of heavy rains for maximum, minimum and average annual rainfall for the 4 rain gauges.

Table 2

| Nu m. | Krusevo              | Kicevo               | Bitola               | Prilep               |
|-------|----------------------|----------------------|----------------------|----------------------|
| 1     | Hsr/g.=829,91<br>mm  | Hsr/g.=779,05<br>mm  | Hsr/g.=619,79        | Hsr/g.=550,88        |
| 2     | Hmax/g.=1239,<br>7   | Hmax/g.=1126,<br>3   | Hmax/g.=863,8        | Hmax/g.=808,1        |
| 3     | Hmin/g.=368,7        | Hmin/g.=442,6        | Hmin/g.=337,5        | Hmin/g.=295,7        |
| 4     | qrsg/god=200,1<br>5  | qrsg/god=197,2<br>4  | qrsg/god=169,1<br>9  | qrsg/god=152,3<br>1  |
| 5     | qrmax/god=261<br>,55 | qrmax/god=245<br>,35 | qrmax/god=208<br>,81 | qrmax/god=196<br>,63 |
| 6     | qrmin/god=116<br>,54 | qrmin/god=131<br>,63 | qrmin/god=113<br>,24 | qrmin/god=100<br>,59 |

Table 3 shows the rainfall intensities  $i = \frac{h}{t} \left( \frac{mm}{min} \right)$  for the maximum, minimum and average annual rainfall for the 4 rainfall stations.

Table 3



| N. | Krusevo             | Kicevo              | Bitola             | Prilep             |
|----|---------------------|---------------------|--------------------|--------------------|
| 1  | Hsr/g.=829,91<br>mm | Hsr/g.=779,05<br>mm | Hsr/g.=619,79      | Hsr/g.=550,88      |
| 2  | Hmax/g.=1239,7      | Hmax/g.=1126,3      | Hmax/g.=863,8      | Hmax/g.=808,1      |
| 3  | Hmin/g.=368,7       | Hmin/g.=442,6       | Hmin/g.=337,5      | Hmin/g.=295,7      |
| 4  | i sr/god=1,20       | i sr/god=1,183      | i sr/god=1,015     | i sr/god=0,913     |
| 5  | i<br>max/god=1,567  | i<br>max/god=1,472  | i<br>max/god=1,253 | i<br>max/god=1,179 |
| 6  | i min/god=0,699     | i min/god=0,789     | i<br>min/god=0,679 | i<br>min/god=0,603 |

From Table 3 can be seen the available water quantities obtained from precipitation (maximum, minimum and average annual amounts) that will be intended for irrigation of agricultural land in the Pelagonija region.

The annual amounts of precipitation measured at the rain gauge stations Bitola and Prilep, can partially provide water for irrigation of agricultural areas, which are covered by irrigation through the hydro-amelioration systems Strezevo and Prilep field, only as part of the total required volume for irrigation.

The required quantities of water for irrigation of agricultural areas for different crops are usually expressed according to the following pattern, which define the norm for irrigation.

$N_n = \Sigma P_v - \Sigma R_v$  where the Irrigation Norm is the difference between the required irrigation water and the available irrigation water.

The required water for irrigation of agricultural areas is the sum of the following quantities of water:

1. Evapotranspiration, ie a set of Evaporation and Transpiration of water in plants.  $E_t = E_v + T_r$ . Available volumes of irrigation water is a sum of the following components:
2. Rainwater that falls on agricultural land.
3. Capillary water located in the soil itself.

After the initial systematization of the hydrological sequences by years, which will be analyzed, the 4 basic parameters of the probability theory are determined, through which the necessary statistical parameters for processing the hydrological sequences will be obtained, from which the values of the large annual sums of rainfall.

In order to determine the percentage of rainwater that will participate in the irrigation of agricultural areas in the Pelagonija region, the processing of hydraulic arrays obtained from the 4 rainfall stations according to the methods of mathematical statistics has been prepared.

The parameters to be determined based on the data from the hydrological sequences are the following: [2]

1. Average value of the sequence, ie average annual precipitation  $Hsr = \frac{\sum Hi}{N} \left( \frac{mm}{god} \right)$
2. The mean square deviation of the sequence  $Sx, Sx = \sqrt{\frac{\sum (Hi - Hsr)^2}{N - 1}}$
3. The coefficient of variation  $Cv, Cv = \frac{Sx}{Hsr}$
4. Coefficient of Asymmetry  $Cs, Cs = \frac{\sum (Hi - Hsr)^3}{(N - 1) \cdot Sx^3}$
5. Coefficient of Asymmetry  $Cs, Cs = \frac{2 \cdot Cv}{1 - \frac{Hmin}{Nsre}}$

By defining the four parameters of mathematical statistics, which derive from the data of the hydrological sequences, it is possible to start testing the adaptability of the hydrological sequences with the theoretical curves, which are standard curves in the theory of probability.

Testing the adaptability of the hydrological sequence refers to the following 3 standard curves which are defined through the following functions. [2]

1. Gumble curve  $Hi = \frac{z}{\alpha} + \beta \left( \frac{mm}{god} \right)$ .
2. Pearson curve type III  $Hi = Hsr \cdot (1 + F \cdot Cv) \left( \frac{mm}{god} \right)$ .
3. Normal distribution, ie Gaussian curve  $Hi = Hsr + Sx \cdot Z \left( \frac{mm}{god} \right)$ .

By comparing the ratio between the coefficient of asymmetry and the coefficient of variation  $\frac{Cs}{Cv}$ , the best adaptability of one of the theoretical curves will be obtained, which will be used in the further analysis of the determination of the extreme values (maximum minimum) of the amounts of rainfall.

The values of the obtained statistical parameters, through which the values in the 3rd theoretical curves will be defined, are given in a table in Table 4.

Table 4

| Num. | Rain gauges given by cities | Average value Hsr (mm/god) | Medium square deviation Sx | Coefficient of Variation Cv | Coefficient of asymmetry Cs |
|------|-----------------------------|----------------------------|----------------------------|-----------------------------|-----------------------------|
| 1    | Kicevo                      | 779,05                     | 148,54                     | 0,191                       | 0,883                       |
| 2    | Krusevo                     | 829,91                     | 198,85                     | 0,240                       | 0,862                       |
| 3    | Prilep                      | 550,88                     | 121,51                     | 0,221                       | 0,952                       |
| 4    | Bitola                      | 619,79                     | 123,53                     | 0,1993                      | 0,875                       |

Table 5 shows the distributions of the sums of the total precipitation according to the Gumble curve, according to the following calculated parameters, necessary for defining the Gumble distribution curve. [2]

1.  $\alpha = \frac{1,2825}{Sx}$ ,  $\alpha 1 = 0,008634$ ,  $\alpha 2 = 0,00645$ ,  $\alpha 3 = 0,010555$ ,  $\alpha 4 = 0,010382$
2.  $\beta = Hsre - 0,45 \cdot Sx$ ,  $\beta 1 = 712,21$ ,  $\beta 2 = 740,43$ ,  $\beta 3 = 496,2$ ,  $\beta 4 = 564,2$
3.  $Z = \alpha \cdot (Hsre - \beta)$ ,  $Hi = \frac{Z}{\alpha} + \beta \left( \frac{mm}{god} \right)$

Table 5

| Nu m. | Sity     | 0,01 %  | 0,1 %   | 0,5 %  | 1 %    | 10 %   | 50 %   | 90 %   | 99 %   | 99,9 % |
|-------|----------|---------|---------|--------|--------|--------|--------|--------|--------|--------|
| 1     | Kice vo  | 1778,93 | 1512,3  | 1325,6 | 1244,9 | 975,12 | 754,72 | 615,62 | 535,35 | 455,09 |
| 2     | Krus evo | 2168,34 | 1811,44 | 1561,5 | 1453,6 | 1092,4 | 797,33 | 611,13 | 503,68 | 396,24 |
| 3     | Prile p  | 1368,77 | 1150,67 | 997,9  | 932,1  | 711,3  | 530,97 | 488,30 | 351,53 | 285,87 |
| 4     | Bitol a  | 1451,31 | 1229,58 | 1074,3 | 1007,3 | 782,85 | 599,55 | 483,87 | 417,12 | 350,37 |

Figure 1 shows the graphical representation of the obtained sums of precipitation distributions for the 4 rain gauge stations, according to the Gumble curve.

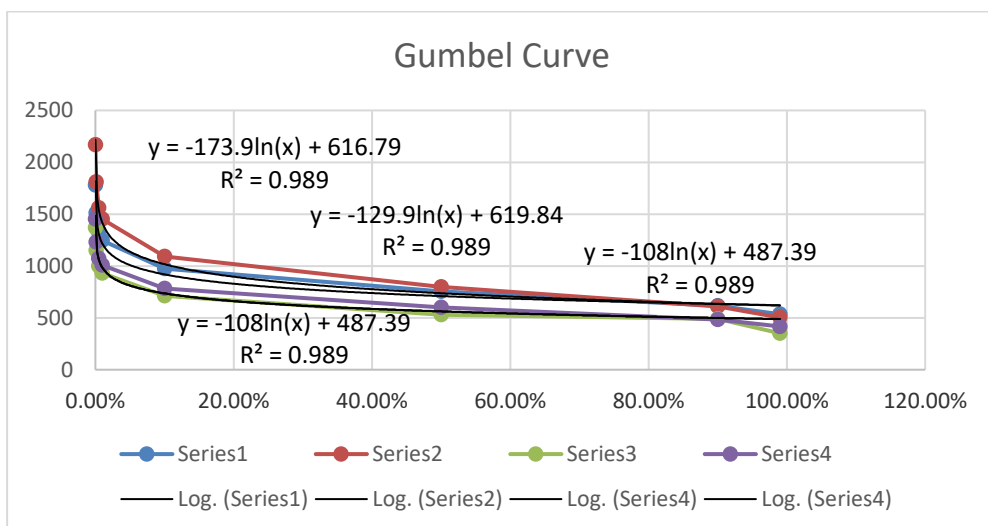


Figure 1

Table 6 shows the distributions of the sums of the total precipitation according to the Pearson curve type III, according to the following calculated parameters, necessary for defining the Pearson curve type III distribution. [2]

1.  $H_i = H_{sr} \cdot (1 + F \cdot Cv) \left( \frac{mm}{god} \right)$
2.  $H_{sr1}=779,05(mm/god), H_{sr2}=829,91 (mm/god), H_{sr3}=550,88 (mm/god), H_{sr4}=619,79$
3.  $Cv1=0,191, Cv2=0,240, Cv3=0,221, Cv4=0,1993$
4.  $Cs1=0,883, Cs2=0,862, Cs3=0,952, Cs4=0,875$

Table 6

| Nu m. | Sity    | 0,01 %  | 0,1 %   | 1 %     | 10 %   | 20 %   | 50 %   | 90 %   | 99 %   | 99,9 % |
|-------|---------|---------|---------|---------|--------|--------|--------|--------|--------|--------|
| 1     | Kicevo  | 1631,66 | 1430,78 | 1219,49 | 978,44 | 893,62 | 759,71 | 607,93 | 532,04 | 496,33 |
| 2     | Krusevo | 1925,39 | 1702,31 | 1405,54 | 1096,8 | 985,27 | 804,02 | 596,87 | 483,34 | 427,57 |
| 3     | Prilep  | 1276,48 | 1102,38 | 918,55  | 714,02 | 643,41 | 531,4  | 413,31 | 357,31 | 332,96 |
| 4     | Bitola  | 1327,58 | 1160,83 | 985,42  | 785,31 | 714,9  | 601,26 | 477,74 | 414,74 | 385,09 |

Figure 2 shows a graphical representation of the obtained sums of precipitation distributions for the 4 rainfall stations, according to Pearson curve III Type.

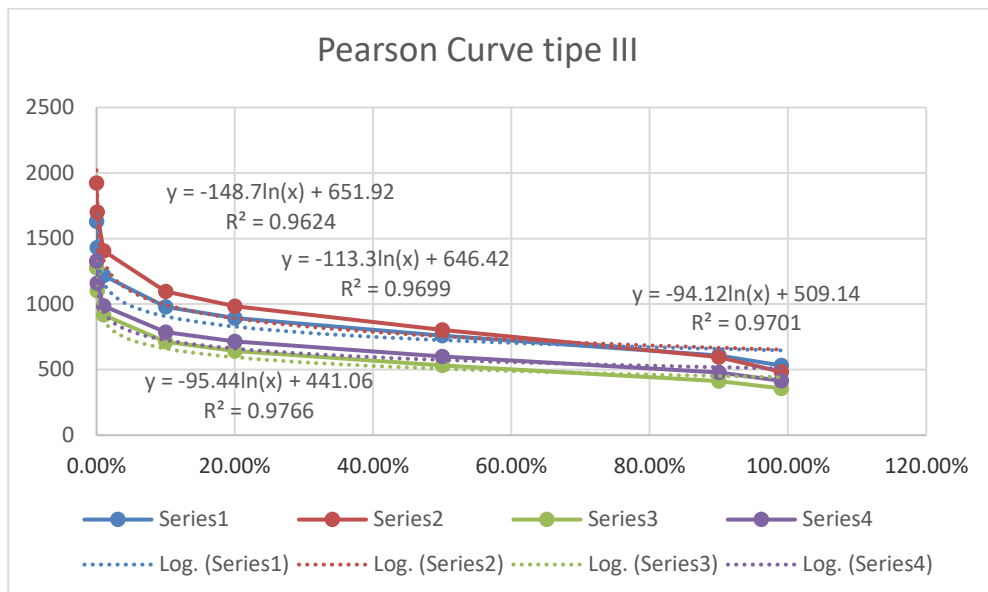


Figure 2

Table 7 shows the distributions of the sums of the total precipitation according to the Normal distribution, according to the following calculated parameters, necessary for defining the Normal distribution, ie the Gaussian curve. [2]

1.  $H_i = H_{sr} + S_x \cdot Z \left( \frac{mm}{god} \right)$
2.  $H_{sr1}=779,05(mm/god), H_{sr2}=829,91 (mm/god), H_{sr3}=550,88 (mm/god), H_{sr4}=619,79$
3.  $S_{x1}=121,51, S_{x2}=123,53, S_{x3}=148,54, S_{x4}=198,85$

Table 7

| Nu m. | Sity    | 0,01 %  | 0,1 %   | 1 %     | 10 %   | 20 %   | 50 %   | 90 %   | 99 %   | 99,9 %  |
|-------|---------|---------|---------|---------|--------|--------|--------|--------|--------|---------|
| 1     | Kicevo  | 1231,07 | 1154,52 | 1060,95 | 934,58 | 881,12 | 779,05 | 623,52 | 497,15 | 327,03  |
| 2     | Krusovo | 1289,44 | 1211,62 | 1116,50 | 988,03 | 933,67 | 829,91 | 671,80 | 543,32 | 370,38  |
| 3     | Prilep  | 1103,45 | 1009,86 | 895,50  | 741,01 | 675,65 | 550,88 | 360,75 | 206,27 | - 1,69  |
| 4     | Bitola  | 1359,51 | 1234,24 | 1081,12 | 874,32 | 789,82 | 619,79 | 365,26 | 158,46 | - 119,9 |

Figure 3 shows the graphic representation of the obtained sums of the precipitation distributions for the 4 rainfall stations, according to the Normal distribution, ie the Gaussian distribution.

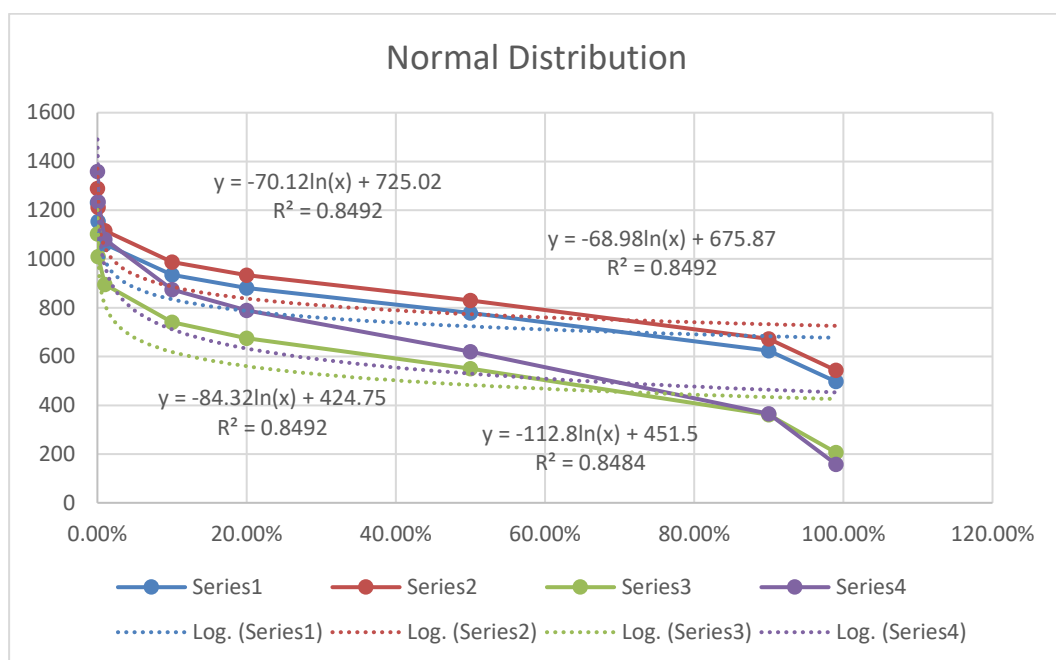


Figure 3

Table 8 shows the comparative values according to the two parameters for the four rain stations, located in the four cities Kicevo, Krushevo, Prilep and Bitola. The most adaptable distribution is the Gumble distribution with a ratio of coefficients of asymmetry and variation  $\frac{Cs}{Cv} = \frac{0,889}{0,213} = 4,174$ , as the mean value for all 4 curves.

Table 8

| Num . | Sity    | Mediu m annual rainfall | 50 %   | Deviation | Minimum amounts of precipitation | 99,99 % | Deviation |
|-------|---------|-------------------------|--------|-----------|----------------------------------|---------|-----------|
| 1     | Kicevo  | 779,05                  | 754,72 | 3,12 %    | 368,7                            | 455,09  | 18,98 %   |
| 2     | Krusevo | 829,91                  | 797,33 | 3,93 %    | 442,6                            | 396,24  | 10,47 %   |

|   |        |        |            |        |       |            |         |
|---|--------|--------|------------|--------|-------|------------|---------|
| 3 | Prilep | 550,88 | 530,9<br>7 | 3,61 % | 337,5 | 285,8<br>7 | 15,30 % |
| 4 | Bitola | 619,79 | 599,5<br>5 | 3,26 % | 295,7 | 350,3<br>7 | 15,60 % |

From Table 8 it can be easily seen that the values of the average annual precipitation amounts are close to the values of the distribution with 50% coverage (average deviation 3.48%), while the minimum amounts of precipitation registered at the rain stations deviate from the values of distribution with 99.99% coverage (average deviation 15.09%).

According to the obtained distributions of the magnitudes of the rain, it can be concluded that the dry periods are more and more present with all their negative consequences. This can best be stated by comparing the following parameters:

1. The ratio between the average annual rainfall amounts and the obtained distributions from the theoretical security curves of 50%.
2. The ratio between the minimum amounts of precipitation and the distributions of the theoretical security curves of 99.99%.

The maximum values, minimum values and mean values of the total precipitation obtained from the Gumbelot distribution for the 4 hydrological sequences are given in a table in Table 9.

Table 9

| N . | Sity    | Maximum amounts of precipitation | Average annual rainfall | Minimum amounts of precipitation | Ratio between Max and Min | Difference in percentages |
|-----|---------|----------------------------------|-------------------------|----------------------------------|---------------------------|---------------------------|
| 1   | Kicevo  | 1778,93                          | 754,72                  | 455,09                           | 3,91                      | 74,42                     |
| 2   | Krusevo | 2168,34                          | 797,33                  | 396,24                           | 5,47                      | 81,73                     |
| 3   | Prilep  | 1368,77                          | 530,97                  | 285,87                           | 4,79                      | 79,11                     |
| 4   | Bitola  | 1451,31                          | 599,55                  | 350,37                           | 4,14                      | 75,86                     |

From the initial processing of the 4 hydrological sequences and their adjustment to the Gumbel distribution, it can be seen that the minimum amounts of annual precipitation are present in the Pelagonija region Bitola and Prilep, exactly in the places where the precipitation is most needed.

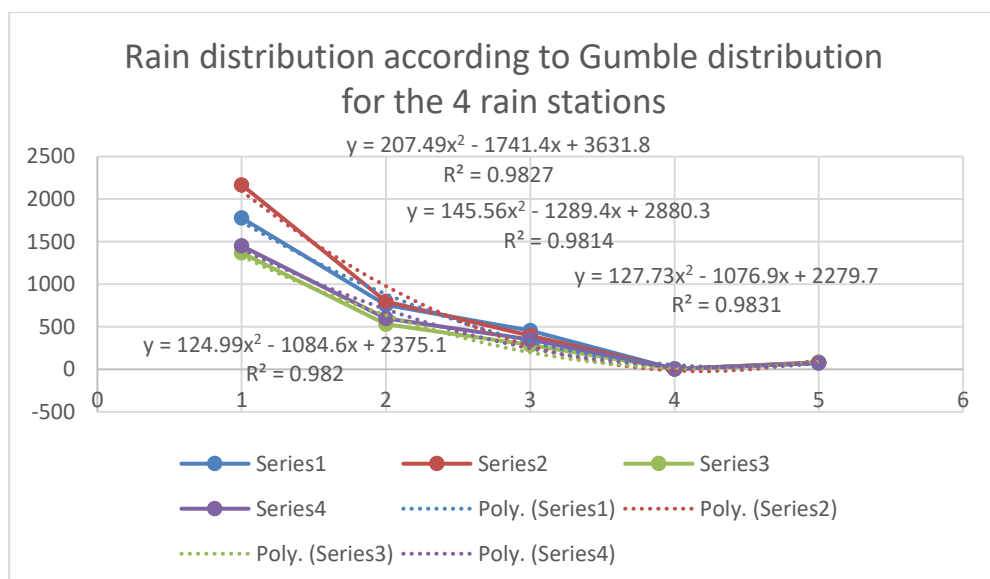


Figure 4

Figure 4 shows the graphic representation of the theoretical distribution of rainfall according to the Gumbel curve, after the statistical analysis.

#### ➤ Description of the Hydromelioration systems in the Pelagonija region

In the Pelagonija region there are two systems for irrigation of agricultural areas in Bitola and Prilep field, which have been operating for more than 40 years. In this scientific paper are analyzed the two existing hydro-ameliorative irrigation systems with the surfaces that are covered with irrigation:

1. H.M.S. Strezevo with a total area of  $A = 20,200$  Hectares.
2. Prilep field with a total area of  $A = 3,900$  Hectares.

The existing hydro systems for irrigation in the Pelagonija region, Strezevo and Prilep field, are systems that work on the principle of irrigation with artificial rain. Artificial rain is provided with various equipment for irrigation with artificial rain such as:

1. Sprinkler wings.
2. BUM rotary irrigation systems.
3. Typhoons, irrigation system with moving sleighs.



Both hydro-amelioration systems provide the required quantities of water from the following built reservoirs:

1. Accumulation Strezevo in total volume of  $=109 \cdot 10^6$  m<sup>3</sup>. Of which useful volume is  $W=89.5 \cdot 10^6$  m<sup>3</sup>.
2. Accumulation Prilep field with a total volume of  $W=6.3 \cdot 10^6$  m<sup>3</sup>. Of which useful volume is  $W=5.7 \cdot 10^6$  m<sup>3</sup>.

The Strezevo Reservoir, which has a useful volume of  $W=89.5 \cdot 10^6$  m<sup>3</sup>, provides irrigation water for the Bitola field at 4,430.69 (m<sup>3</sup> /ha/year).

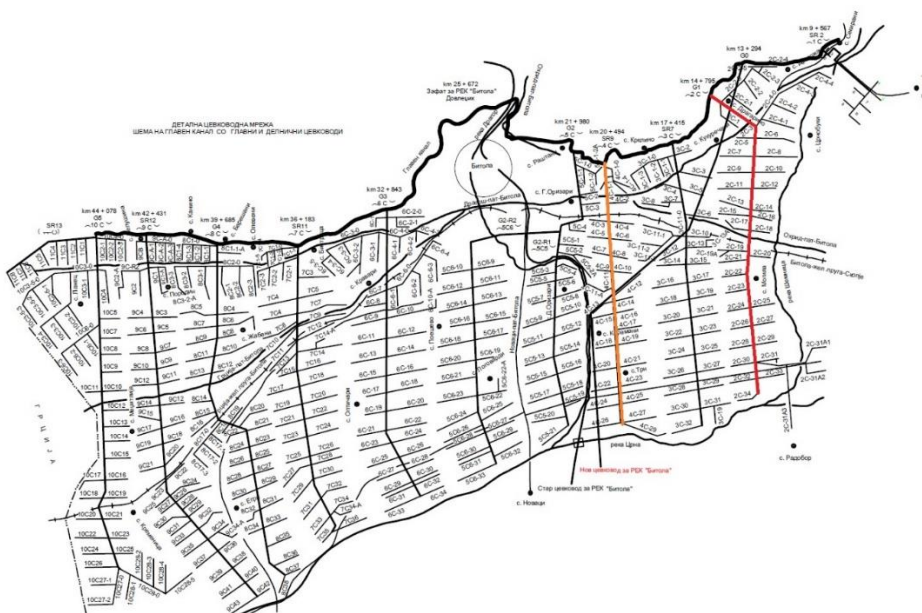


Figure 5 [3]

Figure 5 shows the schematic representation of HMS. Strezevo, with disposition of the main and dividing pipelines on the irrigated agricultural area.

HMS Strezevo provides the necessary quantities of water (volume of water) through the accumulation Strezevo with a total volume of  $W=109 \cdot 10^6$  m<sup>3</sup>. This volume of water is provided by two catchment areas:

1. Basin area of the river Semnica, on which the dam Stezevo is actually built with a volume of  $=67.47 \cdot 10^6$  m<sup>3</sup>.
2. Basin area of several rivers that distribute water in the reservoir through alimentation canal in the length of  $L=61.7$  Km. The volume of water provided through the alimentation canal is  $V=49.35 \cdot 10^6$  m<sup>3</sup>.

From the schedule of volume provision in the Strezevo reservoir, it can be concluded that the summary rains from the catchment area of the river Semnica participate in securing the volume of the reservoir with 57.76%. The remaining quantities of water are provided from other catchment areas. [3]

The accumulation that provides irrigation water for the Prilep field has a volume of  $W=5.7 \cdot 10^6 \text{ m}^3$ , and provides water at  $1,461.54 \text{ (m}^3 \text{ /ha/year)}$ .

Various crops are planted on the agricultural areas irrigated by HMS Strezevo, starting from cereals, vegetables and fruits. Different crops require a different irrigation regime, which is provided by HMS Strezevo, during the vegetative period.

Figure 6 shows a schematic representation of HMS. Prilep field, with disposition of the main and dividing pipelines on the irrigated agricultural area. [4]

HMS Prilepsko Pole specializes in irrigation of one dominant industrial agricultural crop and that is tobacco. The system itself is adapted to the needs of the tobacco irrigation regime and can be more easily adapted to the climatic factors that directly affect the irrigation regime.

From the picture itself you can see the layout of the pipelines in relation to the urban part of the city of Prilep.

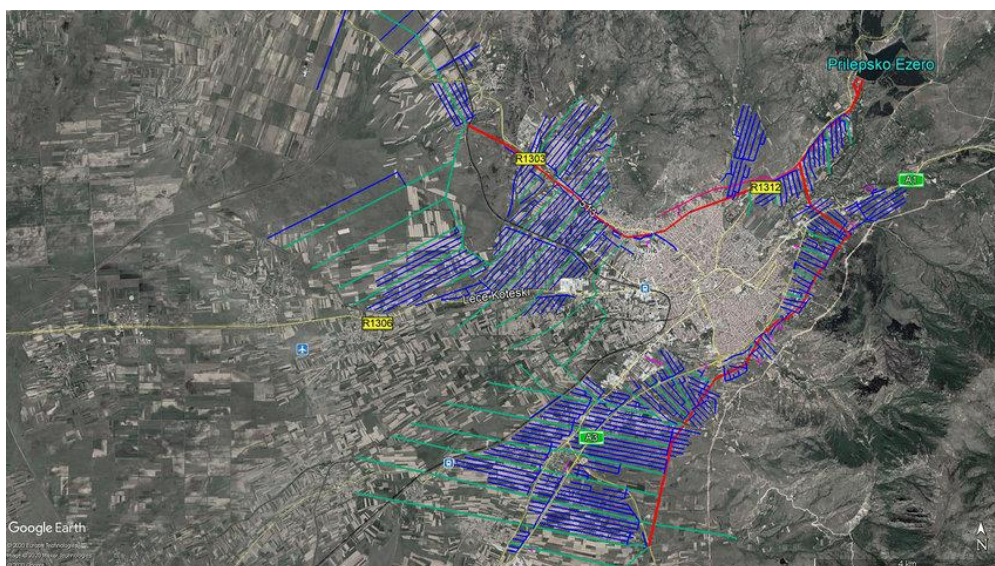


Figure 6 [4]

According to the initial data obtained from the ratio between the volume of the reservoirs and the irrigated areas, the share of rainwater in the filling of the reservoirs can be obtained. The calculations for the share of rainwater in providing the required volume of reservoirs are given in Table 10.

Table 10

| Nu<br>. | Useful<br>Accumulation Volume<br>(m <sup>3</sup> ) | Required<br>amounts<br>of water<br>per ha<br>(mm) | Maximum<br>amounts<br>of rain<br>(mm)<br>P=0,01 % | Annual<br>rainfall<br>amounts (mm)<br>P=50 % | Minimum<br>amounts<br>of rain<br>(mm)<br>P=99,99<br>% | Percentage<br>of<br>participation<br>in<br>minimal<br>rainfall |
|---------|----------------------------------------------------|---------------------------------------------------|---------------------------------------------------|----------------------------------------------|-------------------------------------------------------|----------------------------------------------------------------|
| 1       | $W=89.5 \cdot 10^6$                                | 443,7                                             | 1451,31                                           | 599,55                                       | 350,37                                                | 78,96 %                                                        |
| 2       | $W=5.7 \cdot 10^6$                                 | 146,15                                            | 1368,77                                           | 530,97                                       | 285,87                                                | 51,12 %                                                        |
| 3       | $W=89.5 \cdot 10^6$                                | 2,42                                              | 3,98                                              | 1,64                                         | 0,96                                                  | 60,33 %                                                        |
| 4       | $W=5.7 \cdot 10^6$                                 | 0,80                                              | 3,75                                              | 1,45                                         | 0,783                                                 | 2,13 %                                                         |

From the obtained tabular results for the variations of the rain in the Pelagonija region, it can be concluded that depending on which crops will be planted, ie the size of the Irrigation Norm required for crops, it can be concluded that in H.M. S. Strezevo, the minimum rainfall is not enough to cover the needs for irrigation of agricultural crops, while for Prilep field the minimal rains are sufficient to provide the necessary quantities of water for irrigation.

From the performed preliminary hydrological analyzes, it was concluded that the projected volumes of the reservoirs fully meet the needs for irrigation of crops planted in the Pelagonija region, but it was also concluded that if the trend of long dry periods continues, it will be necessary to provide additional sources for filling the reservoirs in order to provide the necessary volume for irrigation of the agricultural crops that are present in the Pelagonija region.

➤ **Conclusions from the performed hydrological analysis of the rain gauge stations**

From the performed analysis of the hydrological data for the 4 rain gauge stations (Kicevo, Krushevo, Prilep and Bitola), which are processed in the scientific paper, the following conclusions can be formulated regarding the hydrological processing and analysis of the available data.

1. From the performed hydrological analysis of the 4 hydrological sequences in the length of 59 years, it has been ascertained that there are wet and dry periods in certain time intervals.
2. The occurrence of dry periods is more pronounced in the past decades, which as a consequence has a negative impact on the cultivation of agricultural crops in the Pelagonija region.

3. In the Pelagonija region there are two major irrigation systems HMS Strezevo (Bitola) and HMS Prilepsko Pole (Prilep), which have been in operation for more than 40 years.
4. Both hydro-amelioration systems provide the required quantities of water with accumulated water from the two built dams.
5. The volume of accumulated water is sufficient for uninterrupted irrigation of crops in the vegetative period, but with the onset of the minimum amounts of rainfall per year, the first signs of water shortage are already felt.
6. From the schedule of volume provision in the Strezevo Reservoir, it can be concluded that the summary rains from the catchment area of the river Semnica participate in securing the volume of the reservoir with 57.76%, while the remaining volume of 42.24% is provided by other catchment areas.
7. Prilepsko Pole Reservoir can be more easily adapted to dry periods because it provides water for only one dominant industrial agricultural crop tobacco.
8. With the increase of the dry periods, it will be necessary to provide additional water sources that will accumulate for the needs of irrigation.

From the conclusions drawn, a single opinion can be deduced and that is that it is necessary to increase monitoring of hydrological phenomena in order to be able to respond in a timely manner by adjusting irrigation methods and planting crops that are resistant to droughts.

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## **CONDITIONS AND TRENDS IN THE SHEEP-BREEDING SECTOR IN R. MACEDONIA**

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### **Introduction**

Animal husbandry in R. Macedonia provides around 25% of the total agricultural production, the production of milk (cow's, sheep's and goat's) contributing to some 50% of its volume (MAFWE, 2020).

The numerous advantages (favourable climatic conditions, mountainous nature of the terrain, ability to maintain the rural population's economic capacities, as well as the tradition of sheep breeding) are ideal for the development of the sheep breeding sector in the country.

At the same time, sheep breeding is the only animal husbandry sector and branch of the economy where the natural resources of the country can be used most correctly and most completely.

This sector is mainly based on extensive rearing technologies, the use of natural pastures and doing its economic activities in rural areas with difficult production conditions. The attempts for achieving intensive way of rearing through the import of highly productive breeds (milch, in the first place) didn't enable development and progress of this sector, above all because of the poor acclimatization of the imported breeds as well as the unproviding of appropriate conditions for their rearing (Pacinovski et al., 2006).

Unfortunately, as a result of all these problems, from a country where 30 years ago around 2,500,000 head of sheep were bred and an annual income of more than USD 100 million from the export of lamb's meat was earned, today the Republic of Macedonia has fallen to the amount of some 600,000 head of sheep rendering an annual income in hard currency of approximately € 10 million.

Sheep breeding enables the production of various products (milk, meat, leather, wool, lard, fur, manure), where the main products bringing the largest amount of its incomes are the milk and lamb's meat. The largest part of the income from

milk comes from processed milk foods (white brined cheese, cashkwall/yellow cheese, sour milk and similar), sold on the home market, while lamb's meat is exported to many countries in Europe (Italy, Greece, Croatia, Serbia, Bulgaria, Bosnia and Hercegovina, etc.) and is the only traditional Macedonian sheep product enjoying recognition and reputation on those markets. Quite a large importance to the development of sheep breeding is given to the organic sheep breeding too, through certification of new as well as maintenance of the already existing organic farms through allocation of additional financial support from the national developmental programmes.

In this respect, efforts have been made in the past and are still made today, to maintain this sector of the economy at a steady, sustainable level. Sustainable sheep breeding is the only way for producing high-quality meat, milk and wool, while the preservation of the environment, the appreciation of the labour and the labourers' well-being are in line with the goals of achieving economic benefits and better social status of the farmers (Petrović et al, 2011).

## **Resources for development of the sheep-breeding sector**

Traditionally, the largest part of the sheep fund in Macedonia is reared by grazing (at the natural pastures, when possible). The pastures available are a good resource for development not only of the sheep breeding, but also of other animal husbandry branches, such as the cattle breeding and goat breeding.

The total agricultural area in R. Macedonia is 1,264,578 ha, of which 743,991 ha are pastures, and the other part (519,848 ha) is tillable land.

Of the total tillable land, 418,823 ha are ploughland and vegetable gardens, 16,784 ha are orchards, 24,468 ha are vineyards, and 59,773 ha are meadows. The remaining part of 739 ha are swamps, reed beds and fishponds.

Around 40% (208,000 ha) of the total tillable land and 80% (595,000 ha) of the pastures are state property, and are managed by MAFWE and the Public Company for Operating with the Pastures. The other available land resources are in the property of a large number of family-owned agricultural economies, and this is a limiting factor to the productivity and the economic results achieved.

According to a structural research by the State Statistics Office of the RM (SSO), made in 2016, the number of family-owned agricultural economies is 178,125, whereas according to the United Register of Agricultural Economies kept by the Ministry of Agriculture, Forestry and Water Economy (URAE of MAFWE), from 2020, this number is 172,663. On the other hand, according to the data from the Agency for Financial Support to the Agriculture and Rural

Development (AFSARD), from 2018, the number of beneficiaries of direct payments (financial aid) is 86,650 family-owned agricultural economies (MAFWE, 2013).

According to the data from SSO, the available resources per agricultural economy unit are, on the average, some 2,92 ha of tillable land and 4,18 ha of pastures. This fact indicates that the agricultural land fund is highly cleft into small pieces of land, a situation that makes difficult the organization of profitable and sustainable agricultural i.e. stock-breeding production.

Judging by the amount and the quality of the production, the pasture fund in Macedonia enables grazing 2–5 head of sheep/ha, in a year.

Beside the pastures, additional bulky foodstuff for feeding the sheep are alfalfa, clover, vetch, sweet pea and similar, and some cereals too of which the culture mostly used is the barley, while maize, wheat and triticale are used in lesser amounts. Both the former and the latter types of foodstuff are produced on the tillable pieces of agricultural land. The most of these cultures are produced domestically i.e. in Macedonia, and the amounts produced can satisfy the needs, except for the maize, wheat and triticale, which have to be imported in certain amounts.

### **The number of sheep reared**

The sheep breeding in our country, in the period after becoming an independent state (after 1990), has seen a significant decline in the number of sheep reared. This condition is mainly due to the massive migration of the rural population from the villages to the towns, looking for employment in the industrial sector since they regarded the sheep breeding as an unprofitable occupation (Pacinovski et al., 2012). Additional influence on this decline is exerted by the low profitability of this branch of the economy, the low rate of accumulation of money, and the opportunity of choosing other kinds of occupation which also offer better earnings, especially what concerns the young population. This is also influenced by the fact that, despite the attempts to modernize and intensify this branch via the application of modern production technologies, it still remains unchanged i.e. the attempts didn't achieve success, and that's why keeping sheep still requires the employment of a large amount of manual labour, a circumstance that makes sheep breeding quite an unattractive job.

Looking at the history, the largest number of sheep reared in the territory of R. Macedonia was recorded in 1989 – 2,490,000 head (Pacinovski et al., 2012).

Also looking at the records on the number of sheep head from 1960 onwards, we can see that the sheep-breeding sector suffered its greatest decline in the period 1990–2000, when the number of sheep dropped from 2,297,115 in 1990 to 1,250,686 in the year 2000, which is a decline by more than 1 million sheep. In the next decade, i.e. 2000–2010, another decline happened, when the number of sheep dropped to 778,404 head, which is a decline by half a million sheep (Table 1).

Analyzing the sheep-breeding sector in the region, Arsić et al., (2015), found that the number of sheep in Macedonia in 2007, compared to the situation in the year 2000, had decreased by 36.3%. Next in this infamous list is the Montenegro, with a drop by 12.8%, while Serbia recorded a drop by 0.3%. In the same period (2000–2007), an increase was recorded in Slovenia by 36.3% and in Bosnia and Hercegovina by 22.3%.

In the period after 2010, the number of sheep in Macedonia stabilized, ranging from 600,000 and 800,000 head (Table 1).

**Table 1.** The number of sheep in R. Macedonia in the period 1960–2021, by categories

| Year | Categories        |                     |                      | Total     |
|------|-------------------|---------------------|----------------------|-----------|
|      | Ewes for breeding | Lambs and weanlings | Rams and barren ewes |           |
| 1960 | 1 575 063         | 452 600             | 104 541              | 2 132 204 |
| 1970 | 1 391 085         | 380 577             | 91 075               | 1 862 737 |
| 1980 | 1 401 443         | 552 888             | 103 192              | 2 057 523 |
| 1990 | 1 612 527         | 522 507             | 162 081              | 2 297 115 |
| 2000 | 887 057           | 290 051             | 73 578               | 1 250 686 |
| 2010 | 568 301           | 180 173             | 29 930               | 778 404   |
| 2020 | 476 914           | 119 518             | 34 202               | 630 634   |
| 2021 | 489 579           | 108 756             | 34 946               | 633 281   |

**Source:** SSO (State Statistical Office)

Analyzing the situation in the last 10 years (2012–2021), a slight decline can be noticed, with small oscillations in certain years, and after that period, i.e. in 2021, the number of 633,281 sheep was recorded which is a drop by some



100,000 head i.e. by some 15%, which once again represents a significant drop in the national sheep flock (Table 2).

**Table 2.** The number of sheep in R. Macedonia in the period 2012–2021, by categories

| Year | Categories        |                     |                      |         |
|------|-------------------|---------------------|----------------------|---------|
|      | Ewes for breeding | Lambs and weanlings | Rams and barren ewes | Total   |
| 2012 | 520 767           | 173 605             | 37 966               | 732 338 |
| 2013 | 530 760           | 158 867             | 42 201               | 731 828 |
| 2014 | 531 160           | 164 625             | 44 672               | 740 457 |
| 2015 | 580 840           | 113 671             | 38 999               | 733 510 |
| 2016 | 555 932           | 123 426             | 43 937               | 723 295 |
| 2017 | 565 063           | 116 933             | 42 559               | 724 555 |
| 2018 | 579 747           | 113 690             | 33 553               | 726 990 |
| 2019 | 533 393           | 113 671             | 37 494               | 684 558 |
| 2020 | 476 914           | 119 518             | 34 202               | 630 634 |
| 2021 | 489 579           | 108 756             | 34 946               | 633 281 |

**Source:** SSO

The situation is similar in the other categories of sheep, with small variations upwards and downwards.

### **The size of sheep-breeding farms**

According to the data from the Food and Veterinary Agency (FVA), the number of sheep-breeding farms in Macedonia was 4,805 units in 2014, a number that decreased to 3,842 in 2019, which is a drop by some 1,000 farms.

Regarding the size, in our country the most widely spread are the farms keeping 101–200 sheep (30% of the total number), i.e. 1,153 farms (Table 3).

**Table 3.** Size and number of sheep farms in R. Macedonia

|                               | Number of sheep per farm |         |          |          |           |            |            |          |
|-------------------------------|--------------------------|---------|----------|----------|-----------|------------|------------|----------|
|                               | 1 to 5                   | 6 to 10 | 11 to 20 | 21 to 50 | 51 to 100 | 101 to 200 | 201 to 500 | Over 500 |
| <b>Share in per cents (%)</b> | 3                        | 2       | 3        | 13       | 21        | 30         | 24         | 4        |
| <b>Number of farms</b>        | 115                      | 77      | 115      | 499      | 807       | 1153       | 922        | 154      |
| <b>Total</b>                  | 3842                     |         |          |          |           |            |            |          |

**Source:** FVA (Food and Veterinary Agency)

At the second position in this list are the farms keeping 201–500 sheep (24%), then follow the farms keeping 51–100 sheep (21%). The least present are the farms keeping up to 21 head (8%), or 307 farms, which are deprived of the right to receive financial support from the government, a right enjoyed only by the farms keeping more than 30 head of sheep. Naturally, in this group fall some of the farms keeping from 21 to 50 sheep, which leads to the conclusion that approximately 300 farms are not entitled to the right of receiving financial support from the government. The number of the biggest farms keeping more than 500 sheep is 154 units, or around 4% of the total.

### Dynamics of sheep farms' enlargement

Analyzing the period 2014–2019, we can see that though small, there is a trend of enlargement i.e. consolidation of the sheep-breeding farms in Macedonia (Table 4).

**Table 4.** The share of sheep farms of larger size, by years

| Number of sheep per farm | Year |      |      |      |        |        |
|--------------------------|------|------|------|------|--------|--------|
|                          | 2014 | 2015 | 2016 | 2017 | 2018   | 2019   |
| 51-100                   | 24%  | 24%  | 22%  | 22%  | 25.65% | 25.47% |

|          |     |     |     |        |        |        |
|----------|-----|-----|-----|--------|--------|--------|
| 101-200  | 29% | 28% | 30% | 29.20% | 28.98% | 30%    |
| 201-500  | 19% | 20% | 22% | 24.47% | 25.65% | 25.47% |
| Over 500 | 2%  | 2%  | 3%  | 3.58%  | 4.01%  | 4%     |

**Source:** FVA

According to the data given in Table 4, in the period 2014–2019 a certain numeral increase can be seen in all these categories of farms. The highest increase (100%) is recorded in the biggest farms (over 500 sheep), and the lowest in those keeping 101 to 200 sheep. The reason for such trend in the enlargement is most probably the financial support from the state, a support which continuously, in a period of more than 10 years, the Ministry of Agriculture, Forestry and Water Economy, through the Agency for Financial Support to the Agriculture and Rural Development, gives to the farms by a head of sheep, and also some other conditions mentioned below.

In the next table (Table 5), the ratio between the sheep farms listed in the register as juridical persons (companies, i.e. firms) and those registered as physical persons (small farms) is shown, for the period 2014–2019.

**Table 5.** The share of sheep farms according to the ownership structure (physical/juridical persons), in percentages

| Business subject  | Year   |       |        |        |        |        |
|-------------------|--------|-------|--------|--------|--------|--------|
|                   | 2014   | 2015  | 2016   | 2017   | 2018   | 2019   |
| Juridical persons | 4.73%  | 4.5%  | 3.82%  | 3.06%  | 2.52%  | 1.54%  |
| Physical persons  | 95.27% | 95.5% | 96.18% | 96.94% | 97.48% | 98.46% |

In the last 50 years, the overwhelming part of the sheep farms in Macedonia have been in the property of small farmers while the percentage of farms in the property of juridical persons is very small, whereat in the period approximately up to 1995 the difference was lesser (90/10 ratio, i.e. 90% for physical persons and 10% for juridical persons).

According to the data given in Table 5, this ratio is a constant subject of change, and in 2019 it is 98.46% against 1.54%, in favour of physical persons.

It is considered that, at least in the last 10–15 years, the type of a farm's ownership hasn't influenced the production results of the particular farm, for the reason, as we think, that the way of a farm's organization is a matter of its owner's choice, i.e. whether with its registration the owner wants to obtain certain bigger rights and privileges in the process of getting financial support from the national or IPARD programmes intended for the stock-breeders. We must also have in mind the fact that juridical persons (companies) always have bigger chances for getting financial support either from the national programmes, either from foreign donors.

### Sheep fund's structure according to the breed

In the database of the Unit for Identification and Labelling the Domestic Animals, at the Food and Veterinary Agency, 14 sheep genotypes have been registered in our country: Ovchepolian, Sharplaninian, Karakachanian, Merinolandschaf, Merinolandschaf cross-breeds, Awassi, Awassi cross-breeds, Asaf, East-Friesian, East-Friesian cross-breeds, Sardinian cross-breeds, Romanov sheep, Black head Pleven sheep, and various cross-breeds. The number and the share of each genotype in the total number of sheep is shown in the following table (Table 6).

**Table 6.** Amount in numbers and percents of each sheep genotype in R. Macedonia

| Ranking | Breed                        | Percent | Number  |
|---------|------------------------------|---------|---------|
| 1.      | Ovchepolian                  | 26.98   | 196,142 |
| 2.      | Sharplaninian                | 26.42   | 192,071 |
| 3.      | Cross-breeds (Undefined)     | 19.40   | 141,036 |
| 4.      | Merinolandschaf cross-breeds | 12.59   | 91,528  |
| 5.      | Merinolandschaf              | 9.95    | 72,336  |
| 6.      | Awassi cross-breeds          | 2.82    | 20,501  |
| 7.      | Romanov sheep                | 0.88    | 6,398   |
| 8.      | Asaf                         | 0.50    | 3,635   |
| 9.      | Awassi                       | 0.30    | 2,181   |
| 10.     | East-Friesian                | 0.07    | 509     |

|              |                            |         |     |
|--------------|----------------------------|---------|-----|
| 11.          | Karakachanian              | 0.06    | 430 |
| 12.          | East-Friesian cross-breeds | 0.03    | 210 |
| 13.          | Black head Pleven sheep    | 0.01    | 73  |
| 14.          | Sardinian cross-breeds     | 0.00    | 0   |
| <b>Total</b> |                            | 726,990 |     |

**Source:** FVA

According to the official data shown in Table 6, the most widely present sheep breeds in our country are the local, autochthonous breeds, namely – Ovchepolian sheep (196,142) and Sharplaninian (192,071). In the third place are the various cross-breeds (undefined cross-breeds), then follow Merinolandschaf cross-breeds and purebred Merinolandschaf heads. The number of Awassi cross-breeds is 20,501 heads. All the other breeds (genotypes) are present in amounts below 10,000 head, which is an insignificant share in the total number of sheep in the country.

Anyway, a general assumption arises that the numbers for the autochthonous sheep populations presented in the table are incorrect, so it is necessary all the amounts of these populations beyond the official numbers of identified and recorded in the register of autochthonous sheep breeds to be transferred to the other populations.

Also, a large portion of the sheep population in Macedonia should be officially registered as cross-breeds of the Merinolandschaf breed, for the reason that starting from 1979 this breed has been used as a meliorator for improving the domestic sheep populations, a fact that also influenced the change of the sheep fund's structure according to the breed (Pacinovski et al., 2012).

### **Production and price of mutton and lamb's meat**

Lamb's meat is the only one strategic export sheep product in Macedonia. According to the long-time traditions in our country, the largest amount of lamb's meat is sold in spring, before the Christian holiday Easter (the so-called Easter lamb), and quite a small amount before the New Year's and Christmas holidays (the so-called Christmas lamb).

The amount of mutton produced in Macedonia, including lambs' and old and barren ewes' meat, is shown in Table 7.

**Table 7.** Production of mutton (including lamb's meat), annually

| <b>Year</b> | <b>Mutton produced (tons)</b> |
|-------------|-------------------------------|
| 2014        | 4727                          |
| 2015        | 3980                          |
| 2016        | 3737                          |
| 2017        | 3422                          |
| 2018        | 3446                          |
| 2019        | 3392                          |

**Source:** SSO

The production of mutton in Macedonia in the period 2014–2019 has been in a continuous “free fall”, with a small exception in 2018 when a slight growth was recorded. Namely, the annual production in 2014 was 4,727 tons whereas in 2019 it was only 3,392 tons, i.e. in a six years' period of time it recorded a nearly 30% decrease.

The largest amounts of lamb's meat produced in Macedonia are exported to EU countries (Italy and Greece mostly, and a small amount to Croatia), and some lesser amounts are sold in the countries from our region (Serbia, Montenegro, Bosnia and Hercegovina).

The total amount and worth of the lamb exported to these countries annually, is shown in the next table (Table 8).

**Table 8.** Lamb's meat exported (amounts and export share in percentages)

| <b>Parameter</b>                     | <b>Year</b> |             |             |             |             |
|--------------------------------------|-------------|-------------|-------------|-------------|-------------|
|                                      | <b>2014</b> | <b>2015</b> | <b>2016</b> | <b>2017</b> | <b>2018</b> |
| <b>EU, tons</b>                      | 2691        | 2235        | 2177        | 1971        | 1674        |
| <b>Export share</b>                  | 96.21%      | 94.62%      | 86.32%      | 87.48%      | 87.23%      |
| <b>Serbia, Montenegro, BiH, tons</b> | 106         | 127         | 345         | 282         | 245         |
| <b>Export share</b>                  | 3.79%       | 5.38%       | 13.68%      | 12.52%      | 12.77%      |

**Source:** SSO

Analyzed on annual level, a conclusion can be made that in the period 2014–2018 the amount of lamb's meat exported to EU countries (Italy, Greece, Croatia) continuously decreases, and this way in 2018 it was nearly 40% lesser than in the year 2014. Although in the same period there is a certain increase of the export of lamb's meat in the countries from our region, this amount is significantly smaller than the amount lost in the export to EU countries. Having in mind that in this period the number of sheep has dropped by some 7.5%, this fact can be regarded as a partial reason for the decreased export.

Regarding the proportion between the export to EU countries and the export to the countries from our region, the share of the export to EU countries has always been around 90%, consequently – the share of the export to the countries from our region has always been around 10% of the total export of lamb's meat.

In the next table (Table 9) the total worth of lamb's meat export is shown, and the buy-up prices for 1 kg of live weight for lambs weighing up to 18 kg and over 18 kg.

**Table 9.** The worth of lamb's meat export made and buy-up prices

| Parameter                                                  | Year                  |                       |                       |                       |                       |
|------------------------------------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
|                                                            | 2014                  | 2015                  | 2016                  | 2017                  | 2018                  |
| <b>Worth, €/000</b>                                        | 13323                 | 12448                 | 12358                 | 10227                 | 9726                  |
| <b>Buy-up price for 1 kg (lambs up to 18 kg), €/denars</b> | 2.64<br>(162.2 den.)  | 2.67<br>(164 den.)    | 2.63<br>(161.5 den.)  | 2.30<br>(141.25 den.) | 2.37<br>(145.45 den.) |
| <b>Buy-up price for 1 kg (lambs over 18 kg), €/denars</b>  | 2.31<br>(142.10 den.) | 2.37<br>(145.63 den.) | 2.48<br>(152.50 den.) | 2.15<br>(132.41 den.) | 2.21<br>(136.17 den.) |

According to the data given in Table 9, in the period 2014–2018 the worth of the export made and the amounts exported are in a continuous decrease. More precisely, the worth of the exported lamb of 13,323,000 euro in 2014, has fallen to 9,726,000 euro in 2018, which is a 27% drop.

Also, there is a slight decrease of the buy-up price for 1 kg of live weight (for the both categories of lambs. i.e. those weighing up to 18 kg and those

weighing over 18 kg), namely – for the first category it has fallen from € 2.64 for 1 kg in 2014 to € 2.37 in 2018, which is approximately a 10% decrease, while for the second category it has fallen from € 2.31 in 2014 to € 2.21 in 2018, which is approximately a 4% decrease. Most definitely this factor too had a big influence on the decrease of the national sheep flock, together with the rise of the price of the labor in the sheep-breeding sector and the certain rise of the price of the livestock feed.

### Production and price of sheep's milk

The largest amount (over 95%) of breeding ewes in our country are milked. Some of the sheep farms sell part of the milk to dairies, leaving some milk for processing in their own production facilities (mandri) into hard milk products (cheeses), of which the largest part is white brined cheese, and a small part is yellow cheese (Bieno & Kashkaval). Beside the cheese, sheep's milk is also used for making curd and fermented milk products (yoghurt and sour milk).

There are still no precise data on what is the percentage of the farms selling their milk in raw condition and those processing their milk in-house into white brined cheese or yellow cheese (Bieno & Kashkaval). The estimates are that 60–70% of the farms process the milk in-house (in their own dairy plant-mandri), and 30–40% of the farms sell the milk in collected raw milk to dairies (60–70% against 40–30% ratio). The milk products made in-house are sold on the market through the regular retail network (shops and bazaar stalls), and through direct sale by the farm.

The production of sheep's milk in our country is shown in Table 10.

**Table 10.** Production (in total and by a milch sheep) and price of sheep's milk, annually

| Year | Total/tons | Per sheep/lit. | Price/lit.        |
|------|------------|----------------|-------------------|
| 2014 | 35661      | 74             | 33.18 ден / €0.54 |
| 2015 | 40748      | 77             | 34.29 ден / €0.56 |
| 2016 | 41065      | 78             | 34.69 ден / €0.56 |
| 2017 | 35364      | 69             | 36.42 ден / €0.59 |
| 2018 | 36559      | 68             | 35.24 ден / €0.57 |
| 2019 | 35088      | 68             | 39.00 ден / €0.63 |

**Source:** SSO and AMIS (Agricultural market information system)



The numbers presented in the table about the sheep's milk produced in our country are in fact the amounts the farms had sold to the dairies. Therefore, the fact must be noted that these numbers do not include the amounts of milk processed by the farms in-house, in their own dairy plant (mandri).

Also, regarding the amounts of milk produced by a head of ewes, the average has been calculated only on the grounds of the amounts sold to dairies, not taking into account the amounts of milk processed by the farms in-house and the milk spent for suckling the lambs. But, having in mind that in ewes the ratio between the milk suckled and the milk milcher usually is 40:60 (depending on the length of the suckling period), the total amounts of milk given by a head of ewes can be roughly calculated.

Looking through the data given in Table 10, there can be seen that the total amount of registered milk production in our country has a trend of stagnation, except in the years 2015 and 2016, when a considerable growth in the production was registered. The reasons for this can be looked for from many aspects, but the most probable cause for this growth is that in fact it is a matter of some farms' attempt to do business via selling their milk to the dairies, but after a period of time when they realized that this way their profitability decreased as a consequence of the low buy-up price of the milk, they returned to the in-house processing of the milk into sheep cheese thus achieving a considerable increase of the income. This way the functionality and sustainability of the farms have improved.

The price for a liter of sheep's milk ranges from 33.18 den. or 0.54 euro in 2014 to 39.00 den. or 0.63 euro in 2019, thus noting a continuous growth in the period under observation. The farmers and their associations often react that the buy-up price is still quite low and doesn't enable sustainability and growth of the farms. On the other hand, the price of white brined cheese ranges from 300 to 500 denars (5 to 8 euro) per Kg, depending on its quality and the region it comes from (Porchu and Dzabirski, 2019). The price of sheep Kashkaval in R. Macedonia differs quite a lot, ranging from 600 to 1,200 denars (10 to 20 euro) per Kg, again depending on its quality and the geographic region it comes from.

## **Production of wool**

The annual production of wool in our country is shown in Table 11. According to the data presented in the table, the annual production of wool in the period 2016–2019 ranges from 843,637 kg in 2019 to 1,359,194 kg in 2017.

**Table 11.** Production of wool (total and per head of sheep)

| <b>Year</b> | <b>Amount of wool, kg</b> | <b>Number of sheared sheep</b> | <b>Wool per head, kg</b> |
|-------------|---------------------------|--------------------------------|--------------------------|
| 2016        | 850,968                   | 548,803                        | 1.55                     |
| 2017        | 1,359,194                 | 670,973                        | 2.03                     |
| 2018        | 990,476                   | 602,964                        | 1.64                     |
| 2019        | 843,637                   | 568,930                        | 1.48                     |

**Source:** SSO

For more than 20 years, the production of wool in our country is of very little or of almost no economic value. In some years, it happened certain wholesale traders to buy it up at very low prices, at about 0.30 euro per kilogramme in average. In our country nowadays there are almost no spinning-mills for processing the wool into yarn, because the import of cheap textile from China and Turkey had simply destroyed the domestic production of woollen products. The amounts of wool bought up by the wholesale traders are mainly exported to Turkey.

### **Financial support to the sheep-breeding sector**

The financial support to the sheep-breeding sector in our country is mainly realized through two programmes: The Programme for Financial Support to the Agriculture and The Programme for Financial Support to the Rural Development.

The subsidizing, as a direct financial support to the sheep-breeding sector, is implemented with the goal of neutralizing the risks constantly present in this sector of the economy. That's why after the proclamation of the Republic of Macedonia's independence, the subsidies in the stock-breeding sector, thereby in the sheep-breeding sector too, continuously increase.

The measures which for many years now have been used for subsidizing the sheep-breeding sector through the both programmes, are presented in the following table (Table 12).

**Table 12.** Kinds and amounts of financial support to the sheep-breeding sector from the both programmes (programmes from the year 2021)

| <b>Measure (kind of support)</b>                                                                                  | <b>Financial support Programme</b>                               | <b>Rural development Programme</b>       |
|-------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------|------------------------------------------|
| Direct payments for a marked head of sheep from all the categories                                                | 17 euro/head                                                     | /                                        |
| Additional direct payments for a female lamb kept                                                                 | 17 + 11 = 28 euro/f. lamb (11 euro in the first year)            | /                                        |
| Additional payments for a sheep head of the autochthonous Pramenka (Ovchepolian, Sharplaninian and Karakachanian) | /                                                                | 9 euro/head                              |
| Direct payments for produced and sold sheep's milk                                                                | 0.07 euro/litre sheep's milk (68 lit. $\times$ 0.07 = 4.76 euro) | /                                        |
| Aid for preservation of the rural areas and their traditional characteristics                                     | /                                                                | Yearly wage for workers in sheep farming |
| Organic sheep production                                                                                          | /                                                                | +30% of the support for a marked head    |

**Source:** MAFWE

The most of the sheep farms in our country use the first two measures of support mentioned above. Theoretically, the farms which want to use all the measures are required to keep sheep from the atochthonous populations and to be certified as organic farms. If they meet these requirements and use that opportunity, the financial support can reach 50 euro per head of sheep, an amount which gives good conditions for profitable and sustainable doing business in the sheep-breeding sector.

## **Measures for improving the sheep husbandry production**

The rearing of highly productive sheep breeds is one of the main preconditions for creating a highly developed sheep husbandry production, no matter that the experience with rearing some of these breeds has been negative in certain farms, and the cause for this negative experience should be subject of further analyses and researches in the future. This is also the case with a large number of countries of south-east Europe and the Mediterranean (Greece, Spain, Italy, France, and in the recent years Bulgaria and Romania too), where a large part of the sheep's milk produced comes exactly from the highly productive sheep breeds: Lacon, Asaf, and similar. The import of new breeds is in fact one of the main preconditions for improving the productivity in the sheep breeding, therewith providing further development of this sector (Antunović et al., 2012).

The production of sheep's milk in our country cannot be increased unless certain changes in the breed structure are made, at least in a certain percent.

The government's care about the protection and preservation of the autochthonous sheep breeds must continue in the future, since these breeds play a big role in the process of maintaining and continuing the tradition of production of traditional, autochthonous and branded sheep's milk and meat products (Ovchepolian lamb, Sharplaninian lamb, Macedonian lamb, Galichki cashkawall, Berovsko cheese, Mariovsko cheese, and many others).

Since so far no sheep product has been branded, it is necessary to complete the procedures for branding at least one or two Macedonian products made of sheep's milk.

It is necessary to improve the genetic potential of the domestic autochthonous sheep breeds via intensification of the selective activities (control of the production properties and selection of elite heads). For this purpose, the number of Recognized sheep breeders' organizations should be increased, so that a higher number of sheep head would be covered by the selective activities. There is still no certified organization of sheep breeders of any of the high-productive sheep breeds (Asaf, Merinolandschaf). This step would also require increase of the finance earmarked for the selection i.e. realization of the CBPAB (ZOPOD), (Common Basic Programme for Animal Breeding). The current finances are quite limited, thus not allowing some considerable increase of the work in this respect.

It is also necessary to increase the investments in construction of roads allowing good access to the sheep farms as well as the investments for intensification of the process of exploitation of renewable sources of energy through the construction of solar and wind-turbine powerplants on the high-mountain

pastures. Everywhere where favourable conditions exist, it is necessary to provide a connection to the electrical network, so that performing the sheep-keeping job would become much easier. The solar and wind powerplants that have been installed on the farms so far, at least for the time being cannot satisfy the needs for electric energy, especially in those farms where milking systems are used (the existing powerplants give electricity sufficient only for lighting).

Also, investments in modernization of the farms are necessary, through the construction of new lodging premises for the sheep. The fact that a large part of the sheep in R. Macedonia are reared in old, ruined and inappropriate premises, necessitates the construction of new or reconstruction of the existing premises. The joint financing in improving the premises for rearing sheep should amount up to 90% of an investment's total worth, with the only goal to modernize and up-grade this now unattractive sector of stock-breeding. Without new investments in this sector, its further development is simply impossible (Antunović et al., 2016).

Further, it is necessary to explore new markets where Macedonian lamb can be offered, which is otherwise a sheep product of exceptional quality.

Furthermore, it is necessary to pass a new law about the pastures, in order to solve the problems with the pastures. This law would also resolve some problems between the hunters' clubs and the sheep-breeders, which arise because there are certain locations used by the both groups. A clear delimitation must be made where the hunters are allowed to hunt, and where the sheep breeders can graze their sheep. Additionally, this law would enable more intensive development of the country tourism. Herein, the need must not be forgotten to take care about the more and more aggressive spread of the ploughland at the cost of the pastures which are steadily transformed into agricultural land for tillage.

Also, it is necessary to implement the law on the legalization of the objects built on agricultural land and intended for sheep farms, especially regarding those farms which have permanent whereabouts and implement a stationary rearing system. Of course, the law will be implemented only where there are appropriate conditions for this. Here the state can be of great help, through alleviating and speeding-up the legalization procedures, and through strengthening the capacities of the local units of the ministry in charge of urbanism i.e. the Ministry of Transportation and Communications (with appropriate personnel, in the first place) and the ministry itself. The experience has shown that the realization of these measures has been waiting for years now. All these measures are intended to enable the use of finances granted from the IPARD tranche.

And finally, it is necessary to improve the capacities of the advisory system in our country, through more intensive engagement of the scientific institutions without whose participation the development of the sheep-breeding sector and of all the other stock-breeding sectors is simply impossible.

## **Conclusions**

The sheep breeding, an important sector of the stock-breeding branch of the economy in R. Macedonia, is in stagnation in the last 10 years, with a bit negative trends.

The negative trends are noticed in the number of sheep head and in the total production (both in decrease).

The main reasons for this situation are the lack of sufficient number of workforce, the increase of the production costs and the decrease of this sector's profitability, and all these circumstances have serious influence on the drop in the interest for doing this kind of business.

All the measures that shall be taken in the future must be directed to the improvement of the working conditions in this sector.

Above all, this means:

- Modernization through the introduction of new technologies (mechanical milking of the ewes, etc.), which way the amount of manual labour would be reduced;
- Improvement and modernization of the technologies for feeding the sheep;
- Improvement of the production results, through the introduction of all the known rearing technologies;
- Improvement of the methods for the selection performed at the sheep farms (including the appropriate cooperation between the sheep farmers' associations in this respect, e.g. through exchange of quality animals for reproduction);
- Specialization of the sheep-breeding farms, through the introduction of new, more productive breeds (where there are appropriate conditions), and so on;
- Improvement of the animals' well-being, through the improvement of the health care given to the sheep. This means, in the first place, to resolve the problems with certain diseases which occasionally appear in the sheep population in certain regions of the country, wherein the crucial role belongs to the veterinarian services;

- Finding ways for motivating the young people to move back to the villages and find occupation there, through giving more substantial financial stimulations (by giving attractive grants for the young farmers, etc.);
- Branding of products made of sheep's milk and delicatessen made of sheep's meat;
- Popularization of country tourism, and so on.

The financial support to this sector, given by the Ministry of Agriculture, Forestry and Water Economy and the Government of the Republic of Macedonia through the national programmes, must continue in the future, wherein special importance must be given to the improvement of the working conditions, which way the work in this sector would become more attractive.

The sheep farmers should act in a much more organized manner in the process of defending their interests, through the creation of appropriate associations, which way the sale of their products would improve, and the purchase of all the materials and tools necessary for modern and profitable work would be eased.

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## **BIOCONTROL ABILITY OF *BACILLUS HALOTOLERANS* AGAINST STONE FRUIT PATHOGENS**

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### **Abstract**

*The biocontrol ability of *Bacillus halotolerans* strain B33 against the most significant stone fruit bacterial and fungal pathogens was investigated under in vitro conditions. The results indicate that the inhibition zone toward bacterial pathogen *Xanthomonas arboricola* pv. *pruni* originating from peach and apricot (strains Xp219 and Xp320, respectively) ranged from 20 to 45 mm in diameter, depending of the used *B. halotolerans* concentration. *B. halotolerans* B33 did not inhibit the growth of bacteria *Pseudomonas syringae* pv. *syringae* (strains RE05 and RE3 originating from sweet cherry), *P. s. pv. morsprunorum* race 1 (strain Pm5 originating from sweet cherry and Pm26 from plum) and *P. cerasi* (strain RE10 originating from wild cherry). In the case of fungal pathogen *Monilinia fructicola* strain 116, 41.66–61.00% growth inhibition was observed when pure culture of *B. halotolerans* was used. From the obtained results in this work, *B. halotolerans* strain B33 could be recommended to be potentially used as a suitable biocontrol agent for the control of *Xanthomonads* plant pathogenic bacteria and *Monilinia* spp. on stone fruit.*

**Keywords:** *fungi, bacteria, pathogen, stone fruit, biological control.*

## Introduction

Fruit growing is an important part of agriculture in Serbia, with stone fruit orchards occupying 67% of the area designated for this purpose. Stone fruit (*Prunus* spp.) cultivation depends on a wide range of natural factors, including soil characteristics, climate, diseases and pests (Keserović et al., 2014). However, many fungal and bacterial pathogens undermine stone fruit quality and yield, resulting in significant economic losses. In general, fungal pathogens from the genus *Monilinia* (species *laxa*, *fructigena*, and *fructicola*) are considered the most destructive for all types of stone fruits, as they cause blossom and shoot blight, as well as brown rot in the fruit ripening stage (Hrustić et al., 2015). Pathogen *M. fructicola* is listed as an A2 quarantine organism in EU (EPPO, 2020). Ample evidence also indicates that the most significant bacterial pathogens of stone fruit species *Pseudomonas syringae* pathovars (*syringae* and *morsprunorum*), *P. cerasi* and *Xanthomonas arboricola* pv. *pruni* are responsible for dieback, as well as leaf and fruit bacterial spots (Iličić, 2016; Balaž et al., 2016; Iličić et al., 2018; Iličić & Popović, 2021; Iličić et al., 2022). Bacterium *X. arboricola* pv. *pruni* is listed as a quarantine organism in EU (EPPO, 2021).

Pathogen control has traditionally relied on the use of synthetic pesticides. However, increasing adoption to integrated pest management (IPM) has created an urgent need to find alternative measures that are less dependent on chemicals in order to satisfy the growing food demand while maintaining fruit protection and food safety. The most promising strategies are based on the use of biocontrol beneficial bacteria (Jiménez-Gómez et al., 2021) with zero residues and no withdrawal period.

*Bacillus* species are plant growth promoting, root-associated bacteria (PGPR) and are thus promising biocontrol agents. Plant growth promotion of *Bacillus* species bacteria is usually a result of combined action of two or more mechanisms, such as plant yield enhancement, soil biofertilization, abiotic stress and drought mitigation (Slama et al., 2019). One of the most promising activities of these bacteria is their ability to inhibit many plant pathogenic fungi and bacteria (Siddiqui et al., 2001; Jošić et al., 2011; Berić et al., 2012; Zdravković et al., 2015; Milijašević-Marčić et al., 2018; Nikolić et al., 2019; Marković et al., 2020; Jelušić et al., 2021). Bacterium *Bacillus halotolerans* is beneficial rhizobacteria which recently started receiving extensive attention due to its antagonistic activity, physiological traits, and other beneficial effects such is potential to promote plant growth and tolerance to drought and salinity stress (Zhang et al., 2018; Slama et al., 2019; Wang et al., 2021; Wu et al., 2021). Different strains of this bacterium were recently described as potent antagonistic agents against plant pathogenic

fungi (Sagredo-Beltrán et al., 2018; Slama et al., 2019) root-knot nematodes (Xia et al., 2019), which implies its perspective in wide use. *B. halotolerans* is listed on Biosafety level 1 Risk group (=biological agents which are unlikely to cause disease human disease) (<https://bacdiv.dsmz.de/pdf.php?id=1095&doi=10.13145/bacdiv1095.20170829.2>).

Guided by this evidence, the aim of this study was to determine the biocontrol potential of *B. halotolerans* strain B33 in the control of stone fruit pathogens, i.e. bacteria *P. syringae* pv. *syringae*, *P. s.* pv. *morsprunorum* race 1, *P. cerasi*, *X. arboricola* pv. *pruni* and fungi *Monilinia fructicola*, *in vitro* assays.

## Materials and Methods

The strain of *B. halotolerans* coded as B33, obtained from the culture collection of the Institute for Plant Protection and Environment, was grown on Nutrient Broth (NB) for 24 h at 26 °C.

Its biological activity was tested against strains of bacteria *P. syringae* pv. *syringae* (strains RE05 and RE3 originating from sweet cherry), *P. s.* pv. *morsprunorum* (strains Pm5 and Pm26 originating from sweet cherry and plum, respectively), *P. cerasi* (strain RE10 originating from wild cherry), *Xanthomonas arboricola* pv. *pruni* (strains Xp219 and Xp320 originating from peach and apricot, respectively) and fungi *Monilinia fructicola* (strain 116 originating from peach). Bacterial strains were grown on Nutrient Agar (NA) for 48–72 h at 26 °C, while strain 116 was grown on Potato Dextrose Agar (PDA) for 10 days at 23 °C.

Inhibitory effect of B33 on the bacterial strains growth was evaluated by Agar-diffusion assay using holes in medium (Gojgić-Cvijović & Vrvic, 2003). Bacterial suspension of each tested strain was added to NA to obtain the final concentration of  $10^8$  cells mL<sup>-1</sup> and was poured into sterilized Petri plates (ø 90 mm). As soon as the medium had solidified, the holes (ø 7 mm) were made on agar in Petri plates. To obtain the minimum inhibitory concentration (MIC), six bacterial concentrations of the strain B33 were prepared ( $10^4$ – $10^9$  cells mL<sup>-1</sup>) and 10 µL of each of the tested concentrations was placed in the plate holes. Petri plates were then left to incubate at temperature 26 °C to promote bacterial development. The interaction between the tested bacterial pathogens and *B. halotolerans* strain B33 was expressed by the formation of the inhibition zone, the diameter (expressed in mm) of which was determined three days after incubation. Sterile distilled water served as a control treatment.

Antagonism of the strain B33 against the fungal pathogen *M. fructicola* (strain 116) was determined using the dual culture method. Agar discs (ø 7 mm)

of the tested fungi pathogen strain were placed in the center of PDA plates ( $\varnothing$  90 mm). Drops (8  $\mu$ l in volume) of bacterial suspension of the strain *B. halotolerans* B33 adjusted to  $10^8$  cells  $\text{mL}^{-1}$  were placed on four sides near the edge of the Petri plate. Sterile distilled water served as a control treatment. In order to quantify the antagonistic potential of *B. halotolerans*, pathogen growth area was measured after 14 days of incubation at 23 °C, and the percent of inhibition was calculated using the formula given by Zarrin et al. (2009).

Three replicates were conducted for each of the tested bacterial or fungal strains, and for each treatment/ *B. halotolerans* B33 concentration. All experiments were performed twice.

The results were statistically analyzed by conducting analysis of variance (ANOVA), whereby Duncan's Multiple Range Test ( $p < 0.05$ ) was performed for testing the differences between means related to different treatments.

## Results and Discussion

Due to the increasing health and environmental risks posed by the pesticide use in agriculture, less harmful alternatives such as plant growth-promoting bacteria to suppress phytopathogens must be explored. At present, *Bacillus* species are considered the most promising group of bacteria for plant pathogen control, as they have the capacity to act as biopesticides, biostimulators and biofertilizers. Most important bioactive molecules from the genus *Bacillus* are synthesized peptides and lipopeptides, polyketide compounds, bacteriocins and siderophores. In general, they exhibit a broad spectrum of antagonistic activity against plant pathogenic bacteria, fungi and viruses (Romero et al., 2007; Fira et al., 2018).

The results obtained in the present study indicate that *B. halotolerans* strain B33 has the capacity to suppress the growth of most common bacterial and fungal pathogens of stone fruit. The results of assays including bacterial pathogens, presented in Table 1, indicate that B33 was effective in the suppression of *X. arboricola* pv. *pruni*. Both strains Xp219 (originating from diseased peach leaves) and Xp320 (originating from diseased apricot fruits) were suppressed in the applied concentrations  $10^6$ – $10^9$  cells  $\text{mL}^{-1}$  of *B. halotolerans*, while lower concentrations ( $10^4$  and  $10^5$  cells  $\text{mL}^{-1}$ ) were ineffective. Thus,  $10^6$  was established as the MIC, resulting in an inhibitory zone of 20.00–20.33 mm diameter, which increased to 28.00–29.00 mm, 38.33–38.66 mm, and 45.00 mm as the concentration increased to  $10^7$ ,  $10^8$  and  $10^9$ , respectively. However, in this study, no inhibitory effect was obtained when strains of *P. syringae* pv. *syringae*

(RE05 and RE3) isolated from diseased sweet cherry, as well as strains of *P. syringae* pv. *morsprunorum* race 1 (Pm5 and Pm26, isolated from sweet cherry and plum, respectively) and *P. cerasi* (RE10, isolated from wild cherry) were used, irrespective of the *B. halotolerans* B33 concentration.

The inhibitory activity of *B. halotolerans* strain B33 against fungal pathogen *M. fructicola* strain 116 was assessed using the dual culture method. Based on the obtained results, *B. halotolerans* achieved 41.66–61.00% inhibition of *M. fructicola* growth. Referring to the obtained results, it could be concluded that applied treatments with *B. halotolerans* were effective in pathogen control, indicating that this strain should be considered for use in organic agriculture, and especially in product storage.

If bacterium *B. halotolerans* is used as a biocontrol agent, it could effectively inhibit many plant pathogenic fungi such are *Fusarium* spp., *Alternaria alternata*, *Botrytis cinerea*, *Rhizoctonia bataticola* and *Phytophthora infestans* (Slama et al., 2019; Wang et al., 2021). According to Wang et al. (2021) treatment with *B. halotolerans* strain KLBC XJ-5 controlled mycelial growth as well as conidial germination of *B. cinerea* (grey mould) *in vitro*. The disease in strawberries inoculated with *B. halotolerans* KLBC XJ-5 was lower in comparison with that in the control fruit. Slama et al. (2019) found four bacterial isolates of *B. halotolerans*, designated BFOA1, BFOA2, BFOA3 and BFOA4 that proved very active against *Fusarium oxysporum* f. sp. *albedinis*; active against *Fusarium* isolates belonging to four species: *F. oxysporum*, *F. solani*, *F. acuminatum* and *F. chlamydosporum* and exhibited strong activities against another four major phytopathogens: *Botrytis cinerea*, *Alternaria alternata*, *Phytophthora infestans*, and *Rhizoctonia bataticola*. Authors also proved *in vivo* antifungal activity of strain BFOA4 against *F. oxysporum* f. sp. *radicis-lycopersici* infection on tomato fruits. *B. halotolerans* strain MS50-18 A can inhabit the growth of plant pathogens and is good for pepper plantlet roots (Zhang et al., 2018).

In this study for the first time we demonstrated antibacterial activity of *B. halotolerans* strain B33 against xanthomonads plant pathogenic bacteria. According to Jelušić et al. (2021) two bacteria from the genus *Bacillus*, *B. velezensis* and *B. megaterium* were found to be effective against crucifers pathogen *Xanthomonas campestris* pv. *campestris* *in vitro* and *in vivo* when applied as a whole-culture and as a cell-free supernatant. Rabbee et al. (2022) reported that *B. velezensis* Bv-21 can be used as an effective and eco-friendly biocontrol agent either by itself or as an active compound against the wild-type and streptomycin-resistant *Xanthomonas citri* subsp. *citri*, which causes bacterial canker disease in citrus fruit. Nikolić et al. (2019) demonstrated significant

biocontrol potential of the crude lipopeptide extracts and cell culture of *Bacillus amyloliquefaciens* SS-12.6 in suppression of leaf spot disease (*P. syringae* pv. *aptata*) severity on sugar beet plants. Some *Bacillus* species were reported as an antagonist of *P. s. pv. syringae* pathogen of the citrus blast in the study of Mougou and Boughalleb-M'hamdi (2018).

The evidence obtained in the present study confirms the importance of the developed *in vitro* antagonistic assays for screening different bacteria for their antagonistic activity. Finally, the *B. halotolerans* strain B33 constitutes a promising biocontrol agent which produces antibacterial compounds against *X. arboricola* pv. *pruni*, as well as exhibits antifungal activity against pathogen *M. fruticola*. Future studies will thus be performed *in planta* under different production conditions.

**Table 1.** Inhibition zone caused by *Bacillus halotolerans* against different bacterial pathogens of stone fruits

| <i>B. halotolerans</i> strain B33 –<br>Bacterial concentration | 10 <sup>9</sup>      | 10 <sup>8</sup>    | 10 <sup>7</sup>    | 10 <sup>6</sup>    | 10 <sup>5</sup> | 10 <sup>4</sup> |
|----------------------------------------------------------------|----------------------|--------------------|--------------------|--------------------|-----------------|-----------------|
| Plant pathogenic bacteria /<br>Strains                         | Inhibitory zone (mm) |                    |                    |                    |                 |                 |
| <i>X. arboricola</i> pv. <i>pruni</i> , strain<br>Xp320        | 45.00 <sup>e</sup>   | 38.33 <sup>d</sup> | 29.00 <sup>c</sup> | 20.33 <sup>b</sup> | 0 <sup>a</sup>  | 0 <sup>a</sup>  |
| <i>X. arboricola</i> pv. <i>pruni</i> , strain<br>Xp219        | 45.00 <sup>e</sup>   | 38.66 <sup>d</sup> | 28.00 <sup>c</sup> | 20.00 <sup>b</sup> | 0 <sup>a</sup>  | 0 <sup>a</sup>  |
| <i>P. syringae</i> pv. <i>syringae</i> , strain<br>RE05        | 0 <sup>a</sup>       | 0 <sup>a</sup>     | 0 <sup>a</sup>     | 0 <sup>a</sup>     | 0 <sup>a</sup>  | 0 <sup>a</sup>  |
| <i>P. syringae</i> pv. <i>syringae</i> , strain<br>RE3         | 0 <sup>a</sup>       | 0 <sup>a</sup>     | 0 <sup>a</sup>     | 0 <sup>a</sup>     | 0 <sup>a</sup>  | 0 <sup>a</sup>  |
| <i>P. syringae</i> pv. <i>morsprunorum</i> ,<br>strain Pm5     | 0 <sup>a</sup>       | 0 <sup>a</sup>     | 0 <sup>a</sup>     | 0 <sup>a</sup>     | 0 <sup>a</sup>  | 0 <sup>a</sup>  |
| <i>P. syringae</i> pv. <i>morsprunorum</i> ,<br>strain Pm26    | 0 <sup>a</sup>       | 0 <sup>a</sup>     | 0 <sup>a</sup>     | 0 <sup>a</sup>     | 0 <sup>a</sup>  | 0 <sup>a</sup>  |
| <i>P. cerasi</i> , strain RE10                                 | 0 <sup>a</sup>       | 0 <sup>a</sup>     | 0 <sup>a</sup>     | 0 <sup>a</sup>     | 0 <sup>a</sup>  | 0 <sup>a</sup>  |

\*Mean values of inhibition zone diameters are shown. Values followed by the same letter are not significantly different ( $p < 0.05$ ) according to the Duncan's multiple range test results.

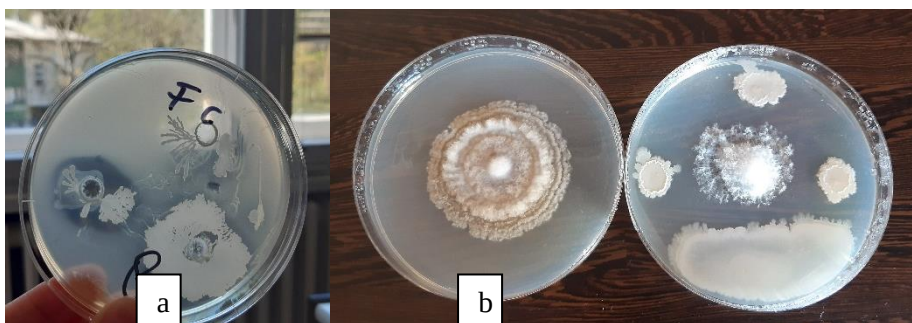


**Figure 1.** Biocontrol ability of *B. halotolerans* strain B33 with respect to:  
 (a) inhibition of *X. arboricola* pv. *pruni* bacterial strain Xp219; and  
 (b) *M. fructicola* strain 116 control treatment (left) and treatment with B33 (right).

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## **CORRELATION BETWEEN BODY WEIGHT OF LAMBS FROM BIRTH TO WEANING IN VARIOUS STRAINS OF SHEEP PRAMENKA**

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### **Abstract**

*Insight into the results of the research on the connection between the traits of physical development of lambs unequivocally points to the fact of the existence of very significant correlations ( $P < 0.01$ ) between all examined traits, in all four examined genotypes of sheep. The highest correlation coefficient of 0.525 was in lambs of Pirot pramenka, between body weight at 30 and 90 days of age. The lowest coefficient in the Pirot genotype was 0.164 between body weight at 60 and 90 days of age. In Svrlijig pramenka lambs, the highest correlation coefficient was 0.482 between body weight at 30 and 60 days of age, the lowest coefficient in the Svrlijig genotype was 0.299 between body weight at 60 and 90 days of age. Sjenica pramenka lambs, the highest correlation coefficient was 0.517 between body weight at birth and at 90 days of age, while the lowest coefficient was 0.430 between body weight at birth and at 30 days of age. For the Sharplanina pramenka genotype, the highest correlation coefficient was 0.472 between body weight at birth and 90 days of age, with lowest coefficient of 0.361 between body weight at birth and 60 days of age.*

**Key words:** *Pramenka sheep, body weight, correlation*

### **INTRODUCTION**

Significant research has been conducted to investigate the linear association and define the genetic flexibility of lamb growth traits (Olivier et al., 2001; Snowden, 2002; Cloete et al., 2004; Lambe et al., 2006). The aim of the findings is to introduce a more efficient organization of procedures for increasing the body weight of lambs through selection (Bradford et al., 1999; Olivier et al.,

(2001; Snowden, 2002; Cloete et al., 2004). Genetic parameters of growth traits of lambs of different breeds were examined by Safari et al., 2005; Miraei-Ashtiani et al., 2007; Rashidi et al., 2008; Gowane et al., 2010; Mohammadi et al., 2010, Caro Petrovic et al., 2012, however, research of this kind in populations of domestic pramenka is insignificant. (Petrović et al., 2011). Caro Petrović et al., (2013) who state that Pearson's correlations in Sharplanina sheep lambs were highest between 30 and 90 days of age (0.72), and lowest between lambs at birth and 60 days of age (0.179). Results similar to the previous ones were obtained by Mohammadi et al. (2012), Dugma (2002), Naser et al., (2002), Baneh et al., (2010), Borg et al., (2009). Mokhtari (2012) who found that the values of correlations of body weight of nits at birth and at 90 days of age were 0.37 and 0.49. Similar values are reported by Fogarty (1995), who found correlations between lamb body weight at birth and weaning of 0.39. Somewhat higher correlation values were obtained by Naser et al. (2001) in the Dorper sheep breed. Positive correlations between the growth traits of lambs are cited by Snyman et al. (1997). Lower values were calculated by Momoh et al., (2013) in whom the correlation between the body weight of lambs at birth and weaning at 90 days was 0.28. The association between lamb body weight at weaning and growth during previous age periods has been examined by many authors. Ghafouri-Kesby et al., (2011) cite positive and medium correlations of body mass traits of lambs in the zandi population. Higher values of correlation between weaning weight and lamb growth of 0.83 are reported by Duguma et al., (2002) in merino sheep, and Rashidi et al., (2008) in kermani sheep. Less values of the relationship between lamb body weight are given by Maria et al., (1993). Gowane et al., (2010) examined the association of growth characteristics of lambs of the malpur breed up to the age of 12 months. Caro Petrović et al., (2012) state that phenotypic correlations are positive and very highly significant between the characteristics of lamb growth. Genetic correlations were significant, but not between all observed periods of lamb age. A group of authors (Schinckel et al., 2003, 2004; Wang and Zuidhof, 2004) state that researching the linear correlation of lamb growth traits greatly increases the chance of improving lamb body mass performance through selection, which is in line with our research goals. Lewis and Brotherstone (2002) as well as Fischer et al., (2004) state that the body weight of lambs in the third month of age is conditioned by body weight in previous stages of development. Other authors including Lewis and Brotherstone (2002), Fisher et al., (2004); Safari et al., 2005; Miraei-Ashtiani et al., (2007); Rashidi et al., (2008); Gowane et al., (2010); Mohammadi et al., (2010), Petrović et al., (2012) Caro Petrović et al., (2012) state the influence and significance of the connection between the growth characteristics of lambs. The importance of this problem and very interesting results are also presented by Bromley et al., (2001). Petrović et

al., (2012) are in the Württemberg population sheep examined the association between physical development traits. Genetic correlations vary, ranging from 0.728 to 0.976. The results obtained for phenotypic correlations ranged from 0.183 to 0.421. Caro Petrović et al., (2012) state that more important phenotypic and genetic trends in lamb growth traits were examined. The research included lambs of two strands: Lipka and Svrljig, from birth to 90 days of age. Phenotypic correlations are positive and very highly significant between growth traits. Genetic correlations were significant, but not between all observed periods of lamb age. The association of body weight with other body weight traits was examined by Mohammadi et al., (2010) in sanjabi sheep and Mohammadi et al., (2011) in zandi sheep. Positive correlations were reported by Duguma et al., (2002) in tigerhoek merino sheep, Gowane et al., (2010) in malpura sheep. Baneh et al. (2010) also obtained positive correlations between the body weight of lambs. Momani et al. (2002) state that regression analysis showed that there is a significant correlation between lamb body weight at birth and other growth traits during the observation period. Mohammadi et al., (2010) found that the association of lamb growth traits was positive and ranged from a very wide range of 0.00 to 0.99, depending on the traits compared. In other studies, Mohammadi et al., (2011) state that phenotypic correlations between lamb growth traits were variable and ranged from low to high, ranging from -0.23 to 0.88. Gowane et al., (2010) state that genetic correlations between body mass traits were positive, ranging from 0.40 to 0.96. Neser et al., (2001), in the Dorper population of lambs, determined the correlation between the characteristics of the body mass of lambs, where the values of bond strength were at the level of medium strength. Hasan et al., (2013) found that there is an association between lamb growth traits. The values of the correlation coefficient ranged from 0.57-0.97. The association between lamb body weight traits has been published in various other publications, where in most cases the existence of a relationship between the examined lamb traits has been shown (Meibodi et al., 2001; Ozcan et al., 2005; Miraei-Ashtiani et al., 2007). Gizaw et al., 2007, Rashidi et al., 2008, Mokhtari et al., 2008, Vatankhah and Talebi, 2008).

Mousa et al., (2013) state that genetic and phenotypic correlations between lamb body weight at birth and weaning were  $0.52 \pm 0.01$  and  $0.37 \pm 0.03$ . Variations in phenotypic correlations between body weight of lambs at birth and weaning ranged from 0.25 according to Jafaroghli et al., (2010) to 0.49 according to Prakash et al. (2012). In addition, genetic correlations between birth weight and rejection also vary, and data from 0.049 to 0.88 are reported in the literature. (Rashidi et al., 2008; Roshanfekr et al., 2011; Prakash et al., 2012).

Caro Petrović et al., (2013) examined lambs of the Sharplanina sheep

breed. The following characteristics were observed: birth weight, at 30, 60 and 90 days of age (BWB, BW30, BW60 and BW90). Correlations and multivariate linear regression were calculated. This meant calculating all possible and best subsets of regression equations. Each equation was evaluated as well as its coefficient of determination ( $R^2$ ). The results showed that the weight of lambs increased by about six times from birth to weaning. Specifically, the lambs achieved an average total gain of 17.66 kg, or 196 grams per day. A very significant correlation ( $R < 0.01$ ) was found between BWB and BW30, BW60 and BW90. Also, a very significant correlation ( $R < 0.01$ ) between BW30-BW60, BW30-BW90 and BW60-BW90 was shown. Also, the obtained coefficient of multiple determination ( $R^2$ ) showed that 50.7% of the variance was BW90, determined by the variance of the predictor variables presented in the model. The corrected coefficient of multiple determination ( $R^2$  adjusted) is 0.506, which means that 50.6% of the BW90 variance is due to the variance of the predictor variables in the model. Any increase in lamb weight during the observed period of life is associated with an increase in the dependent variable BW90. Virtually every increase in BW30 by 1 kg is associated with an increase in BW90 by 1,928 kg.

The review of the literature shows the existence of a connection between the characteristics of the body weight of lambs in the period from birth to weaning. This connection is characterized by certain variations in direction and strength, which further opens the space for new research in different areas of breeding and in different breeds of sheep.

## **MATERIAL AND METHODS**

The research was carried out in eastern, southern, and south-western Serbia, more precisely in the areas where most of Pramenka's are grown. The mating was in season from June to September, and ewes lambing has started in December and prolonged further during the winter period.

Technology applied in the raising of lambs was that, after ten days after birth, the offspring have separated from their mothers but with the possibility of sucking twice a day. Then, has started feeding with hay and concentrate mixture for lambs, and these nutrients were available up to the age of 90 days.

Material for the research included four strains (Piriot, Svrlijg, Sjenica, Sharplanina) of Pramenka sheep. In each strain, the mothers have divided into three groups according to ages (1- two years old; 2- three years old, 3-four years old). All experimental animals had properly marked and recorded. The data processing included a total of 1800 lambs (450 lambs per strain of Pramenka sheep).

The research was conducted over periods of three years, conforming to the pre-established plan. In each year of the experiment, the bodyweight of lambs of all genotypes had measured at the time of birth and 30, 60, and 90 days of age. The measurement has carried out by scale with an accuracy of 0.10 kg, and all data was used for statistical analysis by using SPSS (Statistical Package for the Social Sciences) statistical software, 20 (2012).

## RESULTS AND DISCUSSION

Insight into the results of the research leads to the fact that there are very significant correlations ( $P < 0.01$ ) between all examined traits, in all four examined genotypes of sheep.

The highest correlation coefficient of 0.525 was in lambs of Pirot pramenka, between body weight at 30 and 90 days of age. The lowest coefficient in the Pirot genotype was 0.164 between body weight at 60 and 90 days of age.

Table 1. Correlation between the characteristics of the body mass of lambs of Pirot pramenka

| Genotype                                                 |      | PI1    | PI30   | PI60   | PI90   |
|----------------------------------------------------------|------|--------|--------|--------|--------|
| PI1                                                      | r    | 1      | .397** | .197** | .438** |
|                                                          | Sig. |        | .000   | .000   | .000   |
|                                                          | N    | 450    | 450    | 450    | 450    |
| PI30                                                     | r    | .397** | 1      | .195** | .525** |
|                                                          | Sig. | .000   |        | .000   | .000   |
|                                                          | N    | 450    | 450    | 450    | 450    |
| PI60                                                     | r    | .197** | .195** | 1      | .164** |
|                                                          | Sig. | .000   | .000   |        | .000   |
|                                                          | N    | 450    | 450    | 450    | 450    |
| PI90                                                     | r    | .438** | .525** | .164** | 1      |
|                                                          | Sig. | .000   | .000   | .000   |        |
|                                                          | N    | 450    | 450    | 450    | 450    |
| **Correlations significant at the level of $P \leq 0.01$ |      |        |        |        |        |

Table 2. Correlation between the characteristics of the body mass of lambs of the Svrlijig pramenka

| Genotype                                                   |      | SV1    | SV30   | SV60   | SV90   |
|------------------------------------------------------------|------|--------|--------|--------|--------|
| SV1                                                        | r    | 1      | .405** | .387** | .385** |
|                                                            | Sig. |        | .000   | .000   | .000   |
|                                                            | N    | 450    | 450    | 450    | 450    |
| SV30                                                       | r    | .405** | 1      | .482** | .442** |
|                                                            | Sig. | .000   |        | .000   | .000   |
|                                                            | N    | 450    | 450    | 450    | 450    |
| SV60                                                       | r    | .387** | .482** | 1      | .299** |
|                                                            | Sig. | .000   | .000   |        | .000   |
|                                                            | N    | 450    | 450    | 450    | 450    |
| SV90                                                       | r    | .385** | .442** | .299** | 1      |
|                                                            | Sig. | .000   | .000   | .000   |        |
|                                                            | N    | 450    | 450    | 450    | 450    |
| **. Correlations significant at the level of $P \leq 0.01$ |      |        |        |        |        |

In Svrlijig pramenka lambs, the highest correlation coefficient of 0.482 was between body weight at 30 and 60 days of age. The lowest coefficient in the Svrlijig genotype was 0.299 between body weight at 60 and 90 days of age.

Table 3. Correlation between the body weight characteristics of the lambs of Sjenica pramenka

| Genotype |      | SJ1    | SJ30   | SJ60   | SJ90   |
|----------|------|--------|--------|--------|--------|
| SJ1      | r    | 1      | .430** | .498** | .517** |
|          | Sig. |        | .000   | .000   | .000   |
|          | N    | 450    | 450    | 450    | 450    |
| SJ30     | r    | .430** | 1      | .439** | .439** |
|          | Sig. | .000   |        | .000   | .000   |
|          | N    | 450    | 450    | 450    | 450    |



|                                                            |      |        |        |        |        |
|------------------------------------------------------------|------|--------|--------|--------|--------|
| SJ60                                                       | r    | .498** | .439** | 1      | .497** |
|                                                            | Sig. | .000   | .000   |        | .000   |
|                                                            | N    | 450    | 450    | 450    | 450    |
| SJ90                                                       | r    | .517** | .439** | .497** | 1      |
|                                                            | Sig. | .000   | .000   | .000   |        |
|                                                            | N    | 450    | 450    | 450    | 450    |
| **. Correlations significant at the level of $P \leq 0.01$ |      |        |        |        |        |

Table 4. Correlation between body mass characteristics of Sharplanina pramenka lambs

| Genotype                                                   |      | SP1    | SP30   | SP60   | SP90   |
|------------------------------------------------------------|------|--------|--------|--------|--------|
| SP1                                                        | r    | 1      | .386** | .361** | .472** |
|                                                            | Sig. |        | .000   | .000   | .000   |
|                                                            | N    | 450    | 450    | 450    | 450    |
| SP30                                                       | r    | .386** | 1      | .424** | .399** |
|                                                            | Sig. | .000   |        | .000   | .000   |
|                                                            | N    | 450    | 450    | 450    | 450    |
| SP60                                                       | r    | .361** | .424** | 1      | .456** |
|                                                            | Sig. | .000   | .000   |        | .000   |
|                                                            | N    | 450    | 450    | 450    | 450    |
| SP90                                                       | r    | .472** | .399** | .456** | 1      |
|                                                            | Sig. | .000   | .000   | .000   |        |
|                                                            | N    | 450    | 450    | 450    | 450    |
| **. Correlations significant at the level of $P \leq 0.01$ |      |        |        |        |        |

In the population of lambs of Sjenica pramenka, the highest correlation coefficient was 0.517 between body weight at birth and at 90 days of age. The lowest coefficient in the Sjenica genotype was 0.430 between body weight at birth and at 30 days of age.

In the Sharplanin pramenka genotype, the highest correlation coefficient of 0.472 was between birth weight and 90 days of age. The lowest coefficient in the Pirot genotype was 0.361 between body weight at birth and 60 days of age.

The results we obtained in our research are in accordance with the data of Caro Petrović et al. (2013), who state that Pearson correlations in Sharplanina sheep lambs were highest between 30 and 90 days of age (0.72), and lowest between lambs' body weight at birth and 60 days of age (0.179). By the way, the average values of correlations were similar to our results.

Results similar to ours were obtained by Mohammadi et al., (2012), Dugma (2002), Naser et al., (2002), Baneh et al., (2010), Borg et al., (2009).

Our research is in agreement with the results of Mokhtari (2012) who found that the values of correlations of body weight of lambs at birth and at 90 days of age were 0.37 and 0.49. Similar values are reported by Fogarty (1995), who found correlations between lamb body weight at birth and weaning of 0.39. Somewhat higher correlation values were obtained by Naser et al., (2001) in the Dorper sheep breed. Positive correlations between lamb growth traits are reported by Snyman et al., (1997).

Lower values than ours were calculated by Momoh et al., (2013) in whom the correlation between the body weight of lambs at birth and weaning at 90 days was 0.28.

The relationship between lamb body weight at weaning and growth during previous age periods is examined are many authors. Ghafouri-Kesby et al., (2011) cite positive and medium correlations of body mass traits of lambs in the zandi population. Higher values of correlation between weaning weight and lamb growth of 0.83 are reported by Duguma et al., (2002) in merino sheep, and Rashidi et al., (2008) in kermani sheep. Less values of the relationship between lamb body weight are given by Maria et al., (1993). Gowane et al., (2010) examined the association of growth characteristics of lambs of the malpur breed up to the age of 12 months.

## CONCLUSION

Insight into the results of the research on the connection between the traits of physical development of lambs unequivocally points to the fact of the existence of very significant correlations ( $P < 0.01$ ) between all examined traits, in all four examined genotypes of sheep.

Based on our research and derived conclusions, it can be stated with confidence that the rearing of domestic lambs from birth to weaning is under the influence and interaction of various factors. The effects of the observed influences are a

very important factor of successful production in sheep breeding. This undoubtedly indicates the need to include all the above factors in the procedure of implementing breeding programs

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## STRATEGIC MODEL IN OPTIMIZATION OF AGRICULTURAL PRODUCTION

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**Summary:** *The subject of the research is the analysis of the state and basic problems of development in the optimization of crop and livestock production in the Nisava district of Serbia, as well as the economic analysis of farms in the agricultural conditions of production. The research included concrete case studies of the proposed model of optimization of the warfare production, leaving the possibility for the developer to set an infinitely large number of restrictions and requirements that were not included in the case in which the model was presented in accordance with the requirements and specifics of a particular agricultural organization or the conditions of the territory. The application of the model can be for the optimization of the production of individual in-house households, agricultural enterprises and cooperatives, a specific region or optimization of national agriculture. Problems of regional disposition of agricultural production can and should be addressed using modern planning methods. It provides optimal spatial proportional production and maximum consumption of available production resources. In addition to optimizing the development of agriculture, the model is also important for its functioning. By resolving the national model for the planning of agricultural development and the application of regional models, the following objectives can be determined: strategic - securing self-esteem and economically-maximizing the economic results of agriculture. The realization of these goals creates preconditions for achieving the economic and social goals, that is, the total developmental villages and labor in agriculture.*

**Key words:** Optimization of production, crop, livestock, meat, prices,



## Introduction

The proposed model for optimizing agricultural production has a great application potential. Knowing the available resources plays an extremely important role in the quality of the optimization results. A complete and accurate database that includes all the resources available to the developer, combined with knowledge of the technical and technological requirements of the type of production, enables the application of this model to reach the most optimal production structure in a quick and simple way, which will satisfy the requirements set through the limits and as a result give the maximization get or minimize production costs. The difference is in a totma which will be set for a dozen of optimization of individual farms, and for the optimization of national postulation on thousands of constraints, with the same relationship in numerous changes in Vico (2012).

## Materials and method

The contribution of the applied optimization model is presented on the example of optimization of cattle breeding and cattle production on the territory of the Nisava District. In order to demonstrate the economic viability of the proposed models, we will maximize the gross profit by production, which includes those crop and livestock for which we have data from the Statistical Office of the Republic of Serbia, in order to determine the advantage of using the proposed model on the example of 2015 by the comparative method, Čomić (1968), Mitrinovic (1983), Milovanovic and Milovanovic (2000). The model will set a relatively small number of constraints to show efficiency. With the increase in the number of requests to optimize, by increasing the number of organ-evaluations, the advantages of applying the proposed optimization model are growing and Furundžić and Radović (1966), Krstić (1988).

Overview of the cultures and types of cattle to be observed (unknown):

|                      |                   |
|----------------------|-------------------|
| X1 - Wheat           | X6 - Sugar beet   |
| X2 - mercantile corn | X7 - oilseed rape |
| X3 - Silage corn     | X8 - alfalfa      |
| X4 - sunflower       | X9 - cows         |
| X5 - strain          | X10 - pigs        |

Goal function:

If we assume that the coefficients in unknown amounts are gross profits by cultures.

Livestock, then a set of gross profit per production line represents the total gross profit.

$$Z (\text{mak}) = 2218 X_1 + 1371X_2 + 574X_3 + 1282X_4 + 4120X_5 + 4118X_6 + 324X_7 + 414X_8 + 1989X_9 + 84X_{10}$$

In the territory of the Nisava District, the total area under arable land and gardens is 93786 ha.

$$X_1 + X_2 + X_3 + X_4 + X_5 + X_6 + X_7 + X_8 \leq 93786$$

Due to the correct cultivation, the areas under wheat should be equal to the surface under all other cultures.

$$X_1 - X_2 - X_3 - X_4 - X_5 - X_6 - X_7 - X_8 = 0$$

All production lines must be included in production:

$$x_1 > 0$$

$$x_2 > 0$$

$$x_3 > 0$$

$$x_4 > 0$$

$$x_5 > 0$$

$$x_6 > 0$$

$$x_7 > 0$$

$$x_8 > 0$$

$$x_9 > 0$$

$$x_{10} > 0$$

The need for cattle species for nutrients is expressed by mathematical formulation.

The needs of dairy cows for food are:

$$5,55x_1 - x_9 \leq 0$$

$$5,92x_2 - x_9 \leq 0$$

$$12,19x_3 - x_9 \leq 0$$

$$11,24x_4 - x_9 \leq 0$$

$$12,82x_6 - x_9 \leq 0$$

$$10x_7 - x_9 \leq 0$$

$$5,32x_8 - x_9 \leq 0$$

The needs of fattening pigs for fodder are:

$$62,5x_1 - x_{10} \leq 0$$

$$31,25x_2 - x_{10} \leq 0$$

$$12,19x_3 - x_{10} \leq 0$$

$$128,20x_5 - x_{10} \leq 0$$

$$48,78x_6 - x_{10} \leq 0$$

$$3333,33x_8 - x_{10} \leq 0$$

In 2015 under the crops there were 63194 ha:

$$62,5x_1 - x_{10} \leq 0$$

$$31,25x_2 - x_{10} \leq 0$$

$$12,19x_3 - x_{10} \leq 0$$

$$128,20x_5 - x_{10} \leq 0$$

$$48,78x_6 - x_{10} \leq 0$$

$$3333,33x_8 - x_{10} \leq 0$$

In 2015, under industrial plants:

$$x_4 + x_5 + x_6 + x_7 \leq 419$$

In 2015, fodder plants were:

$$x_8 \leq 26614$$

Active agricultural population in Nisava district number 21745 inhabitants according to the 2012 Population Census. The production of planned plant crops requires constant labor force, expressed in the needs of permanent workers per ha, in months, respectively:

$$0,971x_1 + 1,971x_9 + 0,041x_{10} \leq 21745$$

$$1,313x_9 + 0,041x_{10} \leq 21745$$

$$0,060x_1 + 0,388x_4 + 0,476x_5 + 0,969x_6 + 0,054x_7 + 0,060x_8 + 1,197x_9 + 0,041x_{10} \leq 21745$$

$$\begin{aligned}
 &0,729X_2 + 0,574X_3 + 0,402X_4 + 0,571X_5 + 0,761X_6 + 3,333X_7 + 1,310X_9 + \\
 &0,041X_{10} \leq 21745 \\
 &0,007X_1 + 0,384X_2 + 0,290X_3 + 0,296X_4 + 0,133X_5 + 0,912X_8 + 1,161X_9 + \\
 &0,041X_{10} \leq 21745 \\
 &0,009X_1 + 0,080X_2 + 0,242X_3 + 0,940X_6 + 0,577X_8 + 1,251X_9 + 0,041X_{10} \\
 &\leq 21745 \\
 &0,762X_1 + 0,131X_3 + 0,184X_5 + 0,785X_6 + 0,133X_7 + 0,385X_8 + 1,183X_9 + \\
 &0,041X_{10} \leq 21745 \\
 &0,043X_1 + 2,267X_3 + 3,931X_6 + 0,550X_8 + 1,259X_9 + 0,041X_{10} \leq 21745 \\
 &0,486X_1 + 1,216X_2 + 1,133X_3 + 0,359X_4 + 0,281X_5 + 1,996X_6 + 0,467X_7 + \\
 &0,178X_8 + 1,260X_9 + 0,041X_{10} \leq 21745 \\
 &1,156X_1 + 0,653X_2 + 0,046X_4 + 0,122X_6 + 0,223X_8 + 1,257X_9 + 0,041X_{10} \\
 &\leq 21745 \\
 &0,111X_8 + 1,298X_9 + 0,041X_{10} \leq 21745 \\
 &1,507X_9 + 0,041X_{10} \leq 21745
 \end{aligned}$$

The number of universal combines in the Nisava district is 156 or otherwise, in September we have 7020, and in October 7254 working days, the universal harvester is available. Production of planned crops has demands on weekdays of universal harvesters in September and October respectively:

$$\begin{aligned}
 &0,230X_1 \leq 7020 \\
 &0,267X_2 + 0,280X_4 + 0,250X_5 \leq 7254
 \end{aligned}$$

## Results

After setting the limits and their expression by mathematical, we are going to optimize production using the LINDO software package.

From the optimization request when writing the program in Lindu, we exclude some at the beginning of the limit, because optimization is done only for a part of livestock production, that is, cattle breeding production where it is trying to provide food for optimal livestock production capacities at the level of the Nisava District. Following such limitations, much of the resources remain free to optimize crop production independent of cattle breeding, as well as the capacity of cattle breeding for other species and categories of domestic animals.

$$\text{MAX } 2.218 X_1 + 1.371X_2 + 0.574X_3 + 1.282X_4 + 4.120X_5 + 4.118X_6 + \\
 0.324X_7 + 0.414X_8 + 1.989X_9$$

**ST**

$$X1 + X2 + X3 + X4 + X5 + X6 + X7 + X8 \leq 93786$$

$$X1 > 0$$

$$X2 > 0$$

$$X3 > 0$$

$$X4 > 0$$

$$X5 > 0$$

$$X6 > 0$$

$$X7 > 0$$

$$X8 > 0$$

$$X9 > 0$$

$$X10 > 0$$

$$5.55X1 - X9 \leq 0$$

$$5.92X2 - X9 \leq 0$$

$$12.19X3 - X9 \leq 0$$

$$11.24X4 - X9 \leq 0$$

$$12.82X6 - X9 \leq 0$$

$$10X7 - X9 \leq 0$$

$$5.32X8 - X9 \leq 0$$

$$X1 + X2 + X3 \leq 63194$$

$$0.971X1 + 1.971X9 + 0.041X10 \leq 21745$$

$$1.313X9 + 0.041X10 \leq 21745$$

$$0.060X1 + 0.388X4 + 0.476X5 + 0.969X6 + 0.054X7 + 0.060X8 + 1.197X9 \leq 21745$$

$$0.729X2 + 0.574X3 + 0.402X4 + 0.571X5 + 0.761X6 + 3.333X7 + 1.310X9 \leq 21745$$

$$0.007X1 + 0.384X2 + 0.290X3 + 0.296X4 + 0.133X5 + 0.912X8 + 1.161X9 \leq 21745$$

$$0.009X1 + 0.080X2 + 0.242X3 + 0.940X6 + 0.577X8 + 1.251X9 \leq 21745$$

$$0.762X1 + 0.131X3 + 0.184X5 + 0.785X6 + 0.133X7 + 0.385X8 + 1.183X9 \leq 21745$$

$$0.043X1 + 2.267X3 + 3.931X6 + 0.550X8 + 1.259X9 \leq 21745$$

$$0.486X1 + 1.216X2 + 1.133X3 + 0.359X4 + 0.281X5 + 1.996X6 + 0.467X7 + 0.178X8 + 1.260X9 \leq 21745$$

$$1.156X1 + 0.653X2 + 0.046X4 + 0.122X6 + 0.223X8 + 1.257X9 + 0.041X10 \leq 21745$$

$0.111X8 + 1.298X9 \leq 21745$   
 $1.507X9 \leq 21745$   
 $0.230X1 \leq 7020$   
 $0.267X2 + 0.280X4 + 0.250X5 \leq 7254$

**END**

Lindo daje optimalno rešenje ovog sistema ograničenja i predlog optimalne strukture ratarske i stočarske proizvodnje Nišavskog okruga, po čijem smo uslovima za organizuje poljoprivredne proizvodnje i postavili ograničenja.

*NO LIKELY SOURCES OF ERROR WERE FOUND*

*LP OPTIMUM FOUND AT STEP 7*

*OBJECTIVE FUNCTION VALUE*

1) 130084.7

| <i>VARIABLE</i> | <i>VALUE</i> | <i>REDUCED COST</i> |
|-----------------|--------------|---------------------|
| <i>X1</i>       | 681.170837   | 0.000000            |
| <i>X2</i>       | 0.000000     | 3.271764            |
| <i>X3</i>       | 0.000000     | 0.594521            |
| <i>X4</i>       | 0.000000     | 2.848869            |
| <i>X5</i>       | 29016.000000 | 0.000000            |
| <i>X6</i>       | 294.890656   | 0.000000            |
| <i>X7</i>       | 0.000000     | 6.461156            |
| <i>X8</i>       | 710.619995   | 0.000000            |
| <i>X9</i>       | 3780.498291  | 0.000000            |
| <i>X10</i>      | 0.000000     | 0.000000            |

## **Discussion**

From this solution, we see that the maximum utilization of agricultural resources in the production of 3780 dairy cows and the structure of crop production in the cattle breeding service of 681 ha under wheat, 29016 ha under the soy, 295 ha under the sugar beet and 710 ha under alfalfa.

In relation to the available resources, this leaves us 6,3084 ha for independent crop production, for which optimization again comes from the goal function for maximizing gross profit.

$$\text{MAX } 2.218X1 + 1.371X2 + 0.574X3 + 1.282X4 + 4.120X5 + 4.118X6 + 0.324X7 + 0.414X8$$

**ST**

$$X1 + X2 + X3 + X4 + X5 + X6 + X7 + X8 \leq 63084$$

$$X1 - X2 - X3 - X4 - X5 - X6 - X7 - X8 = 0$$

$$X3 + X2 \geq 20000$$

$$X1 > 0$$

$$X2 > 0$$

$$X3 > 0$$

$$X4 > 0$$

$$X5 > 0$$

$$X6 > 0$$

$$X7 > 0$$

$$X8 > 0$$

$$0.230X1 \leq 7020$$

$$0.267X2 + 0.280X4 + 0.250X5 \leq 7254$$

**END**

Lindo provides the optimal structure of independent crop products: wheat 30521 ha, corn 20,000 ha, soybean 7656 ha and sugar beet 2865 ha.

LP OPTIMUM FOUND AT STEP 4

OBJECTIVE FUNCTION VALUE

1) 138461.0

| VARIABLE | VALUE        | REDUCED COST |
|----------|--------------|--------------|
| X1       | 30521.738281 | 0.000000     |
| X2       | 20000.000000 | 0.000000     |
| X3       | 0.000000     | 0.794864     |
| X4       | 0.000000     | 2.838240     |

|    |             |          |
|----|-------------|----------|
| X5 | 7656.000977 | 0.000000 |
| X6 | 2865.738281 | 0.000000 |
| X7 | 0.000000    | 3.794000 |
| X8 | 0.000000    | 3.704000 |

The basic characteristic of the proposed model is universality. As mentioned in the previous chapter, the application of the model is possible and equally efficient, as in the optimization of the production of individual farms, as well as in the optimization of national agriculture Radović (2002), Mičić et al. (2017). A certain number of constraints set in the model represent universal knowledge and know-how in the requirements of individual crop cultivations, ie, the type of livestock for permanent and occasional work force, the needs of tractors and other machinery, as well as the requirements of certain types of cattle for field crops in the case of independent food provision.

Tabela 1. Tabela osnovnih proizvodnih i ekonomskih karakteristika linije i vrsta produkta

| LINE<br>(TYPE OF<br>PRODUCT<br>ON)) | MARK<br>ING<br>(NEPO<br>ZNATA<br>) | PRODUCT INDICATORS   |                             |                            |                        | ECONOMIC INDICATORS<br>(€) |                    |                         |            |                      |
|-------------------------------------|------------------------------------|----------------------|-----------------------------|----------------------------|------------------------|----------------------------|--------------------|-------------------------|------------|----------------------|
|                                     |                                    | Yield<br>mtc /<br>ha | Annual needs<br>on weekdays |                            |                        | Costs                      |                    |                         |            | Value<br>prod<br>uct |
|                                     |                                    |                      | Stan<br>dig<br>work<br>ers  | Mediu<br>m<br>machin<br>es | Heavy<br>machin<br>ery | Dir<br>ect                 | Indir<br>e<br>ktni | Gros<br>s<br>profi<br>t | Profi<br>t |                      |
| Wheat                               | x <sub>1</sub>                     | 38,<br>70            | 1,59<br>3                   | 1,09<br>3                  | 0,583                  | 3.0<br>85                  | 1.2<br>85          | 2.21<br>8               | 93<br>3    | 5.27<br>6            |
| Mercantile corn                     | x <sub>2</sub>                     | 61,<br>82            | 3,06<br>2                   | 2,04<br>1                  | 0,624                  | 4.4<br>86                  | 1.2<br>87          | 1.3<br>71               | 84         | 5.85<br>7            |
| Seed corn                           | x <sub>3</sub>                     | 21,<br>13            | 3,84<br>3                   | 3,31<br>8                  | 0,724                  | 9.9<br>88                  | 2.8<br>98          | 9.8<br>52               | 6.9<br>54  | 19,8<br>50           |
| Silage corn                         | x <sub>4</sub>                     | 305<br>,03           | 4,63<br>7                   | 4,12<br>31                 | 1,246                  | 4.4<br>13                  | 1.3<br>20          | 57<br>4                 | 74<br>6    | 4.98<br>7            |
| Sunflower                           | x <sub>5</sub>                     | 16,<br>15            | 1,49<br>1                   | 0,90<br>1                  | 0,624                  | 11.<br>227                 | 2.4<br>43          | 4.1<br>18               | 1.6<br>75  | 15.3<br>45           |



|                        |          |        |        |       |       |        |       |       |       |        |
|------------------------|----------|--------|--------|-------|-------|--------|-------|-------|-------|--------|
| Soya                   | $x_6$    | 20,00  | 1,807  | 1,080 | 0,582 | 5.698  | 1.856 | 4.120 | 2.264 | 9.818  |
| Sugar beet             | $x_7$    | 548,63 | 9,657  | 6,195 | 0,624 | 11.227 | 2.443 | 4.118 | 1.675 | 15.345 |
| Oil beet               | $x_8$    | 362,69 | 3,987  | 3,366 | 0,471 | 3.652  | 217   | -324  | 541   | 3.328  |
| Lucerka                | $x_9$    | 87,77  | 2,996  | 2,623 | -     | 3.865  | 1.185 | 414   | 771   | 4.279  |
| Cows (milk production) | $x_{10}$ | 43,30  | 15,070 | 1,580 | -     | 9.051  | 1.233 | 1.989 | 756   | 11.040 |
| Breeding younger       | $x_{11}$ | -      | 3,960  | 1,056 | -     | 8.153  | 988   | 1.956 | 868   | 10.009 |
| These cattle           | $x_{12}$ | 3,34   | 3,093  | 0,672 | -     | 4.232  | 148   | 294   | 146   | 4.526  |
| Your pig               | $x_{13}$ | 0,87   | 0,550  | 0,025 | -     | 944    | 47    | 84    | 37    | 1.028  |
| Mixed animal feed      | $x_{14}$ | -      | 0,432  | 0,900 | -     | 2.526  | 325   | 625   | 300   | 3.151  |

Source: Radović (2002) Application of Linear Programming in Agriculture, Potez, Belgrade, p. 229. - 249.

In addition to the limitations given in Table 1, in the chapter available to the organizer of production in terms of additional restrictions, is the entire database of agricultural resources of the observed farm, enterprise, cooperative or territory. A database containing resources is an unlimited source of demand that should be respected in optimizing the application of the proposed model and take into account Rodić (1991), Radović and Furundžić (1997), Todorovic (1999).

Setting limitations in terms of utilizing available resources, one should bear in mind the strategic commitments in terms of production to meet their own needs within the holding, agricultural organizations and territories, or the production that should provide export and surplus products, market demands, placement capability, capacity processing industry, food industry needs for raw materials, etc. A wide array of potential limitations, the flexibility of the model and the capacity of the model and the LINDO software package to process huge

systems of equations, that is, inequalities, give an unlimited possibility of applying the proposed model for the optimization of crop and livestock production both in terms of the size of the optimization object, and in terms of the structure of production, or type of optimization object.

## Conclusion

From the mentioned solution, we see that the maximum utilization of favorable resources in the production of 3780 dairy cows and the structure of rocket production in the cattle breeding service of 681 ha under wheat, 29016 ha under the soy, 295 ha under the sugar beet and 710 ha under alfalfa. The obtained optimization solutions are also one of the basis for the formulation of long-term agrarian policy. Model elements can be used for annual planning of more favorable production, as well as for creating short-term measures of agrarian policy, which practically optimize the functioning of more favorable production, at the level of more favorable farm, enterprise, regional, or national level. The limitation of production factors and the constant demand for agricultural products imposes the need to activate all available potentials. Regionalization and regional planning of agriculture are only part of an attempt to exploit the potential for agricultural production to the best possible extent. The application of mathematical models in planning regional development is not a newer date. The biggest obstacle, in addition, is to provide an adequate database, since the accuracy of the individual parameters involved in the model largely depends on the variability of the solutions obtained. Different methods are used to solve the model, but the simplest method of linear programming is simplex method, which, in spite of the lack, provides the possibility of relatively simple obtaining of several variants of optimal solutions, changing of individual parameters in the initial model, thus understanding the consequences of the change, which makes it easy to make decisions. In this paper we have given a mathematical model of linear programming for optimal regional planning of crop and livestock production, as well as the results of applying the model on the example of Nišavski okruga.

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## REGIONAL AND INFRASTRUCTURE DEVELOPMENT IN THE AREA OF VOJVODINA

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**Abstract:** *Vojvodina has always been the most advanced and developed part of Serbia. The reason for that should certainly be sought in the extremely fertile land, good geographical determination, but also in the numerous factories that were built and opened en masse in the time of the old Yugoslavia.*

*Periods of great crises in our country have left a significant mark on the whole of Serbia, including Vojvodina. Infrastructural backwardness became more and more evident, especially in the area of Srem and Banat, in which the least investment was made. In the past few years, significant progress has been made in this area as well. Infrastructure projects in Vojvodina have experienced their full prosperity, which was accompanied by the opening of new factories, construction of road infrastructure, renovation and rehabilitation of old buildings that are in the function of education, culture, sports and other socially useful activities.*

*The city of Novi Sad, as the administrative center of Vojvodina, enjoys a special place in these infrastructural investments and progress. In addition to the economic, Novi Sad is an inviolable cultural center, not only of Vojvodina, but also of the whole of Serbia. Exactly for these reasons, Novi Sad was declared the capital of European culture in 2022.*

**Key words:** *infrastructure, investments, Vojvodina, roads, facilities.*

## **Introduction**

Vojvodina is a northern Serbian autonomous province, made up of three regions: Srem, Banat and Backa. Historically, Vojvodina became an integral part of Serbia only in 1918, after the disintegration of Austria-Hungary, although in 1848 there were strong currents for Vojvodina to declare itself Serbian and to secede from the then Austro-Hungarian Empire. In 1848, at the May Assembly in Sremski Karlovci, Serbian Vojvodina was proclaimed, which consisted of Srem, Banat, Bačka and Baranja, and Stevan Šupljikac was elected its duke. In addition to the political one, they also worked on church autonomy, so the Karlovac metropolitanate received the status of a patriarchate, and Josif Rajačić was enthroned as a patriarch.

The Hungarian government tried to conquer Serbian Vojvodina militarily, and sent military troops to Vojvodina, where the war between the rebel Serbs began, supported by the motherland, the Principality of Serbia and the Hungarian army. The war was won on the side of the Serbs only thanks to the intelligent policy of the Serbian leaders, who, knowing the relations in the Austro-Hungarian monarchy and the important relations between the Hungarians and the Austrians, supported Austria in suppressing the Hungarian revolution. Gaining for themselves a special autonomy called the Serb Duchy and the Tamiš Banat. Despite that, this region was not ruled by Serbs, but by Austrians through their governor and emperor himself.

Nowadays, Vojvodina is an integral part of the Republic of Serbia and makes up 27.9% of the total territory of this country. With the administrative center in Novi Sad, Vojvodina, according to the data from the last census, it has 1,991,809 inhabitants. Of that number, the presence of as many as 25 national minorities was noted: Hungarian, Romanian, Ruthenian, Slovak, Croatian, Yugoslav, Montenegrin, Roma, Bunjevac, Russian, Macedonian, Ukrainian, Bulgarian, Muslim, German, Slovenian, Albanian, as well as other smaller nations, more related to the given locality in Vojvodina.

In order to best illustrate the composition of the population in the territory of Vojvodina, an overview is given by narrower regions, which can be seen in Figure 1. "Demography of Vojvodina", taken from the official website of the Republic Bureau of Statistics:



**Figure 1:** Demography of Vojvodina

Thanks to the participation of so many peoples and ethnic groups, it should come as no surprise that Vojvodina is the cradle of culture, because each nation carries its own authentic customs and traditions, and their nurturing only further contributes to its spread and adoption by members of other nations. This process of diffusion of cultural contents and cultural patterns contributes to the creation of a broader picture and better acceptance and understanding of traditions, customs and foreign culture, which raises tolerance towards other peoples and contributes to the condensation and compression of cultural patterns of different origins in a narrow geographical area.<sup>1</sup>

<sup>1</sup> Matić, S. *Kulturno nasleđe Vojvodine*, Turistička organizacija Vojvodine, 2014, str. 14.

## **Infrastructure projects**

Infrastructure projects are related to capital investments in the construction and maintenance of capital facilities of public and state interest. Infrastructure should include technical systems that are necessary for the functioning of the state and local self-government units. This primarily refers to the water supply and sewerage network, traffic network, electricity network, investment in educational - scientific institutions and sports facilities.

The issue of investment is regulated by the Law on Investments<sup>2</sup>, which seeks to encourage investment in infrastructure in order to strengthen economic and economic development and increase the employment rate. These are direct investments in tangible and intangible assets of the domestic economy. In a special chapter, the legislator singled out the provisions concerning investments of special importance. This investment, in terms of the provisions of the Law, should be understood as investments that would significantly improve the competitiveness of the entire economy or individual industries of the Republic of Serbia.<sup>3</sup> When it comes to the value of the investment, it cannot be less than 5 million euros, and when it comes to the number of new jobs, the investment must create a minimum of 500 new jobs to have the status of investment of special importance. If the criterion is the amount of investment in fixed assets, then they cannot be less than two million euros. Investments in extremely underdeveloped municipalities and areas of our country are especially appreciated.

Nowadays, Serbia is in a large investment cycle, worth billions of euros, whose goal is to raise infrastructure to a significantly higher level and become the engine of development of the Serbian economy: fast roads, highways, high-speed railways, utilities in as many as 65 municipalities. In Serbia. In the previous period, only the most important road, railway and water infrastructure projects were defined, which are rapidly connecting Serbia with the region in terms of infrastructure, and thus creating a base for accelerated economic development and strengthening. After several lost decades, the southern branch of Corridor X to Northern Macedonia and Greece was completed, successfully overcoming all construction and urban problems posed by the Grdelica gorge. Also, the eastern branch of Corridor X was completed, which connects Serbia with Bulgaria by highway.

Serbia has never connected with BiH in terms of infrastructure in a way that suits the 21st century, despite the fact that the Serbian people have their own

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<sup>2</sup> "Službeni glasnik RS" broj 89/2015 i 95/2018.

<sup>3</sup> Chapter No. III "Investment of Special Importance and Forms of State Aid".

entity within BiH. Based on a clear plan and vision of the Serbian and Turkish governing structures, MGSI is currently working on the construction of the Belgrade-Sarajevo highway in the direction of Kuzmin-Sremska Raca-Bijeljina in the length of 18 km where works are already underway, as well as the section Požega-Kotroman in length of 60 km. Also, even with Montenegro, a country so close to Serbia, there has never been a connection with modern infrastructure and thus no preconditions have been created for a greater degree of use of the Port of Bar, with which the Serbian economy is traditionally well connected. And things started to change there, a part of the Miloš Veliki highway from Belgrade to Čačak was completed, and after taking responsibility for the work of MGSI, work was accelerated on the section of this highway from Preljina to Požega, where work is expected to be completed by the end of 2021. After that, only the section from Požega to Boljar remains to be built, which is being negotiated with the Chinese side. Also, works on the reconstruction of the Belgrade-Bar railway are in progress, but there is room for significant improvement here.

In addition to these most important works on road infrastructure and in the field of railway infrastructure, several important projects are being implemented, of which the most important for the regional connection, but also for the entire European railway transport network, is the reconstruction of Corridor X, together with partners from China and Russia. The federations on the northern branch, as well as the EU partners on the southern branch, are making serious progress, and they expect that we will have a high-speed railway to Novi Sad built by the end of next year, and that we will have a completely modernized and reconstructed railway Corridor X.

Also, after several decades, preparations have been completed and the implementation of the investment plan for the improvement of the transport infrastructure of the Danube, Sava and Tisza, worth half a billion euros, has begun. The Đerdap 1 and Đerdap 2 shipyards, which are the backbone of the Danube, are being reconstructed, the wider capacities of ports in Smederevo, Belgrade, Prahovo, Bogojevo, Sremska Mitrovica are being built, critical sectors for navigation are being removed and on that way enabling total Danube transport in Europe to increase from 40 million tones to more than 55 million tones.

In order to achieve the logistical connection of the Western Balkans region, the concepts of the so-called "Dry ports" through the construction of an intermodal terminal in Batajnica, and through the design of a new port in Belgrade on the model of "dry port" which will be logistically connected with the largest seaports in the region, first Thessaloniki, Piraeus, Rijeka and Constanta.

The projects that are currently being implemented are: high-speed railway Belgrade - Novi Sad, over which trains will run at a speed of 200 kilometers per



hour, construction of a bypass around Belgrade, section of the bridge on the Sava near Ostružnica - Bubanj Potok, construction of road Ruma - Šabac - Loznica , construction of the section of the road "Milos Veliki" Preljina - Požega, housing construction for members of the security services, construction of the Moravian Corridor (highway 110.3 kilometers long from Pojat to Preljina), construction of the Fruška Gora corridor (47.7 kilometers long) connecting three municipalities: Novi Sad, Irig and Ruma), starting the construction of the "Belgrade Metro" and expanding the capacity of the Port of Smederevo.

### **Infrastructure investments in Vojvodina**

The Directorate for Capital Investments in Vojvodina was established on the territory of the Autonomous Province of Vojvodina. The Provincial Assembly Decision No. 025-6 / 2014 of 23 December 2014 established the Capital Investment Directorate of the Autonomous Province of Vojvodina, the legal successor of the Capital Investment Fund of the Autonomous Province of Vojvodina, to finance capital investments in areas of interest to the Autonomous Province of Vojvodina. the province of Vojvodina, and in Article 3 of the same, its competencies are defined:

"The Administration finances programs and projects of interest to AP Vojvodina, especially in the areas of: 1) spatial planning and development, agriculture, waterpower engineering, forestry, hunting and fishing, tourism, 2) catering, spas and health resorts, environmental protection, industry and crafts, road, river railway traffic, road arrangement, education, sports, culture, health and social protection and 3) public information, energy and other areas in the territory of AP Vojvodina."

Programs and projects of interest to AP Vojvodina, in terms of the decision of the Provincial Assembly, include projects of construction, reconstruction, adaptation, rehabilitation, extension and capital maintenance of buildings and structures, infrastructure, including project planning services that are part of the project, land for construction, as well as projects involving investments in equipment, machinery and other non-financial assets, on the territory of AP Vojvodina. Further powers and obligations of the Administration for Capital Investments of AP Vojvodina are defined by the Provincial Assembly decision.

In accordance with the Provincial Assembly decision on the formation of the Administration for Capital Investments of the Autonomous Province of Vojvodina, the Administration is focused on the users of the budget of the

Autonomous Province of Vojvodina. The Capital Investment Administration of the Autonomous Province of Vojvodina provides services to other bodies and organizations of the Autonomous Province of Vojvodina, direct and indirect users of the budget of the Autonomous Province of Vojvodina. Funds for project financing are allocated to direct and indirect users of the budget of AP Vojvodina in accordance with special decisions of the Provincial Government, as well as conducting public tenders for financing and co-financing projects in waterpower engineering and environmental protection, local and regional economic development, transport infrastructure, education, student standards and sports development.

Financial resources for financing program activities and projects are provided in accordance with the budget of the Autonomous Province of Vojvodina for 2021, within the Regional Development Program, which contains the following program activities and projects on 31.08.2021. Years<sup>4</sup>, as can be seen from Table 1:

| <b>PROGRAM ACTIVITY</b>                                                                                                                                                    | <b>AMOUNT</b> |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
| Support for projects in the field of water management and environmental protection                                                                                         |               |
| <b>456.000.000,00</b>                                                                                                                                                      |               |
| Support for projects in the field of local and regional economic development                                                                                               |               |
| <b>383.631.102,00</b>                                                                                                                                                      |               |
| Support for transport infrastructure projects                                                                                                                              |               |
| <b>2.234.854.015,70</b>                                                                                                                                                    |               |
| Support for projects in the field of education, student standards                                                                                                          |               |
| <b>280.941.956,13</b>                                                                                                                                                      |               |
| Support for projects in the field of sports development                                                                                                                    |               |
| <b>539.000.000,00</b>                                                                                                                                                      |               |
| Project planning                                                                                                                                                           |               |
| <b>93.792.800,00</b>                                                                                                                                                       |               |
| Procurement of 2 linear accelerators with planning system, RV system, dosimetry and immobilization set, with space adaptation and planning system, IOV in Sremska Kamenica |               |

<sup>4</sup> Information on the work of the Administration for Capital Investments in AP Vojvodina, available at: <https://kapitalnaulaganja.vojvodina.gov.rs/>, visited on 30.12.2021. year at 11 o'clock.

|                                                                                                                                                                                        |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>6.475.950,48</b>                                                                                                                                                                    |
| Continuation of adaptation, reconstruction and extension of the building National Theater - Nepsinhaz in Subotica<br><b>400.000.000,00</b>                                             |
| Construction of the student cultural center in Novi Sad<br><b>150.000.000,00</b>                                                                                                       |
| Construction of a central wastewater treatment plant in the municipality of Mali Idoš<br><b>231.626.450,21</b>                                                                         |
| Construction of a wastewater treatment plant in the settlement of Bačka Topola<br><b>184.773.314,45</b>                                                                                |
| Reconstruction and extension of existing buildings and construction of new buildings within the Hertelendi castle complex in Bočar, Municipality of Novi Bečej<br><b>82.801.207,84</b> |
| Reconstruction, extension and construction of the facility within the complex of the home for the mentally ill in Curug<br><b>150.000.000,00</b>                                       |
| Construction of a cultural center in Ravno selo<br><b>160.000.000,00</b>                                                                                                               |
| Arrangement of the old core of Sremski Karlovci<br><b>200.000.000,00</b>                                                                                                               |
| Construction of the local road Stapar - Šivac<br><b>150.000.000,00</b>                                                                                                                 |
| <b>TOTAL FOR THE REGIONAL DEVELOPMENT PROGRAM:<br/>5.703.896.796,81</b>                                                                                                                |

**Table 1.** Regional Development Program of Vojvodina

The Administration for Capital Investments of the Autonomous Province of Vojvodina, with its activities, is focused on the users of the budget of the Autonomous Province of Vojvodina. The Capital Investment Administration of the Autonomous Province of Vojvodina provides services to other bodies and organizations of the Autonomous Province of Vojvodina, direct and indirect users of the budget of the Autonomous Province of Vojvodina. There are no activities

of the Capital Investment Administration of the Autonomous Province of Vojvodina in connection with which, based on laws and other regulations, natural and legal persons would have the right or opportunity to ask the Administration to act in a certain way.

In the period from 01.01.2021. to 31.08.2021. 992,284,517.47 dinars were realized in 2006, which is 16.67% of the plan. Out of that amount, 640,974,902.86 dinars were realized from source 01 00 - General revenues and budget revenues, 351,309,614.61 dinars were realized from source 13 00 - unallocated surplus of revenues and incomes from previous years.

### **Significant infrastructure projects in Vojvodina**

Infrastructure projects in Vojvodina can be divided into projects in waterpower engineering and environmental protection, local and regional development, transport infrastructure, energetics and energetic efficiency, health and social protection, protection of cultural heritage, education, student standards, as well as sports development.

Significant infrastructure projects in the field of water management and environmental protection in 2021 were in the city of Pančevo, where the second phase of construction of the Potamis collector began, including the construction of part of the collector from the Crveni Magacin pumping station to the existing main pumping station FCS Luka Dunav. The project includes the construction of 1,748 meters of network, which improves the wastewater management system on the territory of the City of Pancevo and provides better conditions for business entities in the collector zone. Then, in Sremski Karlovci, the project of draining atmospheric water from Branko Radičević Square and inflow streets was realized, which is a continuation of the previously started project of reconstruction of the sewage system of used water. This phase of the project will build about 200 m of atmospheric sewage in the center of the settlement, which will significantly contribute to preserving the environment and improve the quality of life of citizens and many tourists who visit Sremske Karlovce every day.

In Kikinda, the pipeline on the part of the water supply network was reconstructed, which means the replacement of the existing distribution pipeline pipes with adequate high-density polyethylene pipes. The water supply network was reconstructed in the total length of 5,849.50 meters in Feješ Klare Street, Nićifor Brandić Street, Distrička Street, Dimitrije Tucović Street, Branko Radičević Street, Đuro Daničić Street and Vojin Zirojević Street. In the municipality of Novi Kneževac, a part of the water supply network was repaired

when the existing asbestos cement pipes were replaced, in order to ensure an orderly supply of drinking water on the territory of the settlement. The project envisages the rehabilitation of the network in the streets of Kralja Petra Prvog, Žarko Zrenjanin, Trg Moše Pijade, Krsturski put, Nemanjina and Jelena Bajić. A plant for the preparation of drinking water was built in Novi Bečej, which provided healthy drinking water for all residents of Novi Bečej. The first phase of construction includes the construction of a filter station with filtration equipment. The gross area of the building is 385.06 m<sup>2</sup>. The location of the plant is at the existing spring in Novi Bečej. In Alibunar, a sewerage network has been built on about 60% of the territory of Alibunar. With the construction of 2,672 meters of sewage network in Block 5, users who still use septic tanks were connected to the sewage network of wastewater, which greatly contributed to improving the quality of life of citizens. The first phase includes the construction of the network in Nemanjina Street and in the part of Žarko Zrenjanin Street until the connection to the pumping station, as well as the pumping station and the discharge line.

The settlement of Šajkaš is one of the most organized places in the municipality of Titel, and with the construction of the sewerage network, the level of quality of life of the population was additionally raised and it contributed to the preservation of the environment. The first phase envisages the construction of a sewerage network in the streets Radnička, Zmaj Jovina, Petra Drapšina and Braće Krkljuš. The water supply network in Stara Pazova has already been arranged in three phases, and this year the fourth phase of the water supply network of the Vojka settlement began, which includes the construction of about 3,200 meters of street water supply network, which will enable connection of about 250 new households and businesses to the public water supply system. The project includes the construction of a water supply network in Sestara Grujić, Stevana Popova, M. Đorđevića Neša, Karađorđeva, Nikola Filipina and Majur streets. In the municipality of Pećinci, a water supply system has been built in the town of Ashani, which consists of a well, a hydrophore station and a water supply network. Hydromechanical and electrical equipment was worn out, so the project envisages rehabilitation and replacement of hydraulic equipment in the hydrophore station, equipping wells and installing chlorination equipment at the source, in order to ensure uninterrupted and safe water supply to the population.

In Novi Bečej, there was also a partial construction of the sewerage network in the settlement "Šušanjki sliv". The project for the construction of the secondary sewerage network envisages the construction of about 1,588 m of the network in the streets of Vlade Kolara, Miloja Čipčić, Tapai Šandor and Brigadira Ristića. With the construction of the secondary sewerage network, the citizens of the "Šušanj basin" will stop using septic tanks, which will contribute to the

improvement of the quality of life, but also to the protection of the environment. The population of Nova Crnja and Vojvode Stepe is supplied with water from springs rich in natural organic matter, and trihalomethanes are formed during chlorine treatment. The realization of the project will procure and install new equipment for the production and dosing of mixed disinfectant at the point of consumption with an automatic dosing system and a system for monitoring the concentration of residual chlorine in the treated water. In Kikinda, the water supply network was also rehabilitated, which includes the reconstruction of 2,513.80 meters of network in Albertova, Kumanovska and Veljka Petrovića streets in Kikinda. The realization of this project creates conditions that will contribute to raising the quality of the water supply network and the construction of a water factory.

In the Vrbas settlement "Vrbas Sever" near the Kudeljara colony, wastewater is currently being drained through septic tanks and absorption wells, which leads to pollution of the land and the environment in general. The project envisages the construction of 192 meters of sewerage network, and connection to the previously built sewerage system that leads to the Central Wastewater Treatment Plant. The second phase of reconstruction and expansion of the atmospheric sewerage of the "Dunavac" basin is being carried out in the municipality of Apatin, which is a continuation of the project started in mid-2017, which aims to solve the problem of draining atmospheric water from the urban area and prevent of this part of the settlement. The project envisages the reconstruction of the collector in the streets of Nikola Tesla and Duško Trifunović, in the length of about 522 meters. In Kupinovo, Pačinci municipality, water supply takes place through wells, hydrophore stations and water supply network. Hydromechanical and electrical equipment is worn out, so due to the age and corrosion of the equipment, there is a constant danger of accidents and water loss. The project envisages the installation of new hydrotechnical equipment in the hydrophore station facility, complete rehabilitation of equipment in well shafts, as well as the installation of chlorination equipment.

In the municipality of Beočin, in the settlement of Rakovac, 14 years ago, a part of the sewage network of wastewater was built. The realization of this project envisages the construction of a sewerage network in the areas of "Salaksija", "Belegir" and in the part of Pašićeva Street, with a total length of about 2900 meters. By building a sewerage network, the preservation of the environment, prevention of water and soil pollution is achieved, and thus the life of the inhabitants in this part of the Beočin municipality will be of better quality. The fifth stage of the reconstruction of the water supply network is taking place in Choka, which includes the replacement of worn out asbestos-cement pipes with polyethylene pipes in the length of 2,565 m, as well as the construction of new

shafts. With the realization of this project, households in the streets of Branko Radičević, Braće Vujić, Marshal Tito, Vuk Karadžić, Petar Drapšin, Jadranska and Sutjeska will be provided with a stable water supply with hygienically correct water. In Indija, the construction of a collecting pipeline and two wells with accompanying equipment will provide the capacity of the Indija spring of 150 l / s. This will enable the commissioning of a water treatment plant, which provides microbiologically and physically and chemically correct water. The commissioning of the water treatment plant will provide the necessary quantities of water for the uninterrupted supply of quality water to Indija, Ljukovo, Jarkovac, Novi Karlovac, Stari and Novi Slankamen.

In the field of local regional and economic development, the following important projects stand out in the cities of Vojvodina: The project of arranging and urban equipment of the Great Park in Palić includes the area of the northern shore of the lake, Urban-architectural design of the ground floor arrangement and urban equipment of the Great Park in Palić with the coastal belt, as a unique ambient unit, which includes paving pedestrian paths through the park, promenades along the coast, plateaus, arranging paths for cyclists, as well as accompanying furniture and lighting contributes to the preservation of the park with all its values, as a protected natural good of the third level of protection. In Pančevo, with the construction of the missing road in Crepajski put Street, the works on the construction of the traffic infrastructure in the "Northern Industrial Zone" in Pančevo will be completed. The project envisages the construction of 620 m of road. By improving the conditions for the development of the industrial zone, conditions are created for attracting new investors in the zone which covers 75 hectares, and thus the development of small and medium enterprises and the overall future economic development of Pančevo.

In the municipality of Sid, the first phase of infrastructural equipment of the work zone in Adaševci is being realized, which includes the construction of service roads, the construction of a pedestrian-bicycle path, a water supply network and the installation of a substation. The infrastructural equipment provides the conditions for the intended use of the space provided for the work zone, that is, it will enable access and construction of infrastructure facilities and installations for future users of this work zone. Construction of the access road to the work zone 10.2 Šibac, preconditions are being created for the opening of a new work zone in the immediate vicinity of the E70 highway, with a total area of 204 hectares. The project envisages the construction of an industrial road in the length of 980 m. Infrastructural and communal equipment is the basic precondition for bringing and realization of new investments in the new work zone that is being formed on the territory of the municipality of Pećinci.

The construction of a sports center in Kula is the construction of a sports complex with swimming pools and accompanying sports and catering facilities, which will contribute to increasing sports and recreational facilities for citizens, as well as for the development of tourism. The project is planned to be carried out in three phases, and the first phase of the project covers about 33,472 m<sup>2</sup>. The industrial zone in Sombor is located along the southeastern border of the city on an area of about 500 hectares and it currently operates numerous companies such as "Mercury paints and varnishes", "Meteor commerce", "Fiorano" - Calzedonia member group... In the part of the zone where the old power plant is scheduled for demolition and in order to equip the plot for new investors, the project envisages the construction of a 20 kv cable line in the length of 433.50 m to the new MBTS "Industrial Zone 2", which will ensure the connection of new consumers to the network and improve supply of electricity to existing consumers. The project also envisages the construction of a sewage system from the "Trepča" battery factory to the existing collector in the industrial zone in the length of 3,021.39 meters, as well as the construction of a pumping station. In Pančevo, the completion of infrastructural equipment of the Northern Industrial Zone will enable access to all plots on the southern side of the zone, which will contribute to attracting new investment projects of potential domestic and foreign investors, and thus improve the investment climate. This project involves the construction of about 600 m of road with water supply and public lighting. With the construction of the third section, the construction of 7th Street will be completed, which was already built in the length of 1,070 meters in the previous period, with water supply and public lighting, and public lighting connections to the transformer station and water supply to the Pančevo-Kačarevo main water supply. The location of the future industrial park in Horgos is 4 km away from the state border of Serbia and Hungary, covering an area of 22 hectares. The project envisages asphaltting of the road in the length of 524 m.

Regarding projects in traffic infrastructure, projects in the municipality of Nova Crnja should be singled out, where the road in Aleksa Šantić, Karađorđeva and Nikola Tesla streets in the settlement of Vojvoda Stepa was repaired, which will contribute to safer traffic and improve the quality of life in the territory of municipality of Nova Crnja. The project envisages the rehabilitation of the road in the total length of about 5,000 meters. Reconstruction of roads in Sremski Karlovci includes reconstruction of the road in the part of Karlovačkih đaka Street from Braće Anđelić Street to the turntable for the city bus, including the turntable, as well as the reconstruction of the pedestrian path and car access to households. The total length of the section planned for reconstruction is about 580 m. The realization of the project will increase traffic safety in the part of the settlement of Dudara, but also provide better conditions for further development of tourism



in Sremski Karlovci. The construction of the road in Baštenska and Čuka Zoltana streets in Senta implies the construction of about 4.3 km of road in both streets, with the installation of vertical and horizontal traffic signaling. The construction of these roads improves the quality of life of the local population, and ensures safer traffic. With the rehabilitation of the road in the streets of Novi Sad and the part of the street Kolareva, the works on the intensified maintenance of the state road II and row number 111 in Bački Petrovac will be completed. The works will include the relocation of bus stops, the arrangement of curbs, the reconstruction of the intersection with Sladkovičeva, as well as the installation of horizontal and vertical signalization.

Rehabilitation and increased maintenance of the local road from Vrdnik to the settlement of Jazak will improve the traffic infrastructure on the territory of the municipality of Irig. Better roads, safe traffic are just some of the advantages that contribute to better connections between Vrdnik, as a center of spa tourism on Fruška Gora with neighboring municipalities, and thus further development of not only tourism, but also economy and agriculture in the local community. Increased maintenance of the part of Želežnička and Iva Lola Ribara Streets in Odžaci implies the continuation of the project of reconstruction of roads with high traffic intensity. The realization of the project, which includes the reconstruction of roads, pedestrian paths and drains, will increase the safety of all traffic participants. Improving the traffic infrastructure by reconstructing Golubinačka Street in Šimanovci, a local road that is currently in very poor condition, will increase traffic safety, but also enable higher throughput due to increased frequency of trucks and passenger vehicles on the road section connecting Šimanovci and the work zone "North" The project envisages the reconstruction of a 1.2 km long road. In Subotica, the construction of 1,485 meters of road in Anke Butorac Street is planned, as well as the reconstruction and extension of 431 meters of Petrinjska Street. Both streets are located in the MZ "Poppy Week", and the realization of this project will improve the living conditions of the citizens of Subotica because the flow of traffic and traffic conditions of all participants will be easier and safer.

In Sremska Mitrovica, the reconstruction of Vuk Karadžić Street represents the final phase of the pedestrian zone reconstruction project in the city center. The pedestrian zone was reconstructed in 2008, and Vuk Karadžić Street, which passes next to the pedestrian zone, connects all important tourist sites in Sremska Mitrovica. The reconstruction of the street will include works on the reconstruction of the road and atmospheric sewerage, which will enable better traffic flow on this section, and an unobstructed tour of Žitni trg, the Museum of Srem, the Imperial Palace, the Basilica of St. Demetrius, the gallery "Lazar Vozarević". The third and fourth phases of the construction of the local road

Opovo-Debeljača will include the construction of 2,300 meters of road. The construction of the road between Opovo and Debeljača will contribute to better connections between the municipalities of Opovo and Kovačica, but also enable easier attraction of potential investors, because there is a newly formed industrial zone on this section of the road. In Bečej, the intersection of Miloš Crnjanski, Žilinski Endrea, Zoltan Čuka and Dositej Street is 297 meters long in total. The access to the intersection is congested, and due to the lack of appropriate traffic signals, the safety of all traffic participants is endangered. Reconstruction of the intersection envisages the construction of a roundabout, which will enable greater traffic flow and increase the level of traffic safety.

In the field of energetic and energetic efficiency energy efficient lamps have been installed on public lighting poles in the industrial zone in Bela Crkva, which completes the previously started infrastructural equipment of the industrial zone, thus providing better conditions for companies in the industrial zone and also for attracting new investors. A similar project was realized in Beočin, where the project of adaptation of the electric power infrastructure was adopted, which will include the replacement of public lighting on the access road and in the vicinity of the Beočin monastery. By improving the infrastructure that contributes to the improvement of energy efficiency, electricity will be used more rationally, but also provide safer access to the monastery, and provide better conditions for traffic in this part of the municipality. Energy rehabilitation of the building of PU "Plavi čuperak" in Titel will include reconstruction of the roof and thermal insulation of external walls in order to move the building to energy class C and achieve the ultimate goal, which is to reduce consumption of all types of energy by about 30%. In the previous period, the local self-government has already started working on improving the living conditions of children and employees by replacing the external carpentry. The construction of the hot water pipeline represents the fourth, last phase of replacing the dilapidated and energy inefficient hot water pipeline along Braće Tatić Street, which is located in the central part of Kikinda, and which is jointly financed by the Capital Investment Administration of AP Vojvodina and the City of Kikinda. In previous years, two high schools were connected to the central heating system, as well as about 1,000 consumers, who received reliable and safe heating. By installing pre-insulated pipes, the consumption of natural gas will be reduced, which will contribute to the reduction of carbon dioxide emissions, but also to the reduction of costs. Ensuring safe and continuous transport of thermal energy will provide the basic precondition for the construction of a biomass heating plant, which is planned in the coming period. By installing new windows, the temperature in the classrooms will be uniform, in accordance with the regulations, and the stay in the school will be more pleasant for students and teachers, regardless of the weather conditions.

In the field of social and health care, projects are planned and implemented in Bela Crkva in the Special Hospital for Lung Diseases "Dr Budislav Babić", which began construction of a new hospital facility, in order to enable expansion of hospital capacity in 2008, when most construction works. However, since 2013, work has stalled. With the funds provided at the competition of the Capital Investment Administration of AP Vojvodina, the necessary construction and craft works will be performed, which will contribute to the completion of the facility, and to provide optimal hospital capacity with 200 hospital beds, and thus adequate and quality medical care to health care users. Reconstruction and rehabilitation of the Clinical Center of Vojvodina envisages reconstruction of the Clinic for Orthopedic Surgery, rehabilitation of the Clinic for Ear, Throat and Nose Diseases, rehabilitation of the boiler room and laundry of the Clinic of Neurology, and rehabilitation of electrical installations and power lines of the Clinic of the Neurology Clinic. . The realization of the project will provide better conditions for the work of employees and the stay of patients, and meet the standards of energy efficiency of facilities, which will ensure the smooth operation of the Clinical Center of Vojvodina. The Institute for Health Protection of Children and Youth of Vojvodina is in the process of purchasing an MRI machine, which will be housed in the newly built facility of the MRI Department. Within the phases of the first construction of the building, the construction of the ground floor of the building is planned, the final number of floors of which will be  $P + 1 + M$ . Providing space for storing MR devices will enable its smooth operation, and better conditions for providing the necessary health services.

In the special hospital for psychiatric diseases "Kovin" in Kovin, the Department of Psychogeriatrics - Pavilion A, built 50 years ago, is located in a ruined building, and the safety of patients and staff working in the department is endangered. The project of reconstruction, adaptation and energy rehabilitation of the building envisages the repair of dilapidated structural elements such as parts of the facade and interior walls, floor, construction of a new sloping roof, replacement of installations and other necessary works. After the reconstruction and adaptation of the facility, the hospital will be able to provide care services to patients with disabilities and patients with reduced mobility in better conditions, which will have a positive impact on the psychophysical condition of patients. The building of the Institute for Emergency Medical Aid will be built on the corner of Patriarch Pavle Boulevard, Jastrebačka and Ilarion Ruvarac Street in Novi Sad, on a plot of over 3,000 square meters. The construction of the Ambulance will improve the quality of health services on the territory of the City of Novi Sad.

Regarding investments in the protection of the cultural heritage of Vojvodina, investments were made in Šid to rehabilitate the stage in the Cultural

and Educational Center of Šid, in Senta as well as in Novi Kneževac and Senta, while the birth house of Borislav Mihajlović Mihiz was reconstructed in Irig.

### **Novi Sad as the capital of culture**

Novi Sad is one of the three largest cities in Serbia. The citizens of Novi Sad also make it beautiful and noble, who, even in the age of fast communication, have preserved the peace of the plains and natural kindness. Beneath the "Petrovaradin rock", a small Pannonian mountain Fruška Gora, a city on the Danube - Novi Sad was created, which has existed for just over 300 years. It was first mentioned in 1694, two years after the construction of the Petrovaradin Fortress began. There, on the "Danube Gibraltar", on the 1255th kilometer of this river, the Central European Habsburg monarchy rose from the ashes with the erection of the Fortress, after the Turkish siege of Vienna in 1683. Numerous ethnic and religious groups have found refuge in the three rivers of the Danube, the Tisza and the Sava, where three civilizations clashed - the Christian East, the Christian West and Islam. The Serbs called it Vojvodina, and others accepted it. This area, both before and after the Romans, has an interesting history of long life, numerous peoples and cultures. The Gepids, Huns, Avars, Slavs, Germans, Hungarians, Byzantines and Turks lived there. On the "Petrovaradin rock", the Romans built a fortification, which was later rebuilt by the Hungarians and maintained by the Turks. Opposite the Fortress, on the left, swampy bank of the Danube, after the expulsion of the Turks, at the end of the 17th century, the Austrians built a bridgehead, and a settlement of soldiers, craftsmen and merchants sprang up around it. At first, the settlement was called Račko (Serbian) village, and later Petrovaradinski šanac. When the settlement grew, after the end of the wars at the beginning of the 18th century, the citizens eager for freedom managed, with a financial ransom of 80,000 forints, to obtain the status of a free royal city from Empress Maria Theresa, under the name of Novi Sad and it happened February 1, 1748.

Novi Sad has always been a multinational city. Most of them were always Serbs, and there were others: Hungarians, Croats, Slovaks, Ruthenians, Greeks, Cincars, Jews, Romanians, Roma and who knows when. Who has preserved his multicultural image to this day? One traveler wrote about Novi Sad in the 18th century: "This free city is a famous example of what tolerance and trade can do." Since 1748, the city has been governed by a freely elected Magistrate. Novi Sad then had 4,620 inhabitants. According to the agreement between the Serb and non-Serb populations, Orthodox and Catholics took turns in the city's leadership positions. At the beginning of the 19th century, the Serbian educator, spelling

reformer Vuk Stefanović Karadžić, said that Novi Sad was "the largest Serbian society in the world". In the Revolution of 1848/9, the city was bombed and destroyed. With some strange power, its citizens renewed it in a combination of baroque and other architectural styles, elevating it in the second half of the 19th century to "Serbian Athens". After the First World War and the collapse of the Austro-Hungarian monarchy in 1918, Novi Sad - like the whole of Vojvodina - with its historical decision became part of Serbia, i.e. the newly created Kingdom of Serbs, Croats and Slovenes, i.e. future Yugoslavia. In the 19th century, Novi Sad continued its quiet Danube path of cultural and economic development. It has developed into a modern European city with boulevards and large buildings, such as the magnificent Banovina building, one of the city's symbols. After the tragic events in the Second World War, when many Novi Sad residents died, the city suddenly increased and expanded in all directions.

From the very beginning of the city, its citizens took care of their spiritual life. They immediately built churches. The first Orthodox were: Nikolajevska, Saborna crkva Sv. Đorđe, Assumption and Alma. Their own believers were gathered by: the Roman Catholic, Greek Catholic, Reformed, Protestant, Armenian churches, and later the Jewish synagogue. In addition to church buildings, schools were also built. In the 19th century, the people of Novi Sad had two high schools: Serbian and Hungarian, and other schools. The cultural life of the citizens is nurtured. Book publishing, journalism and printing were developed. Among the cultural institutions, the Serbian Reading Room (1845) and the Serbian National Theater (1861) were founded. But the most important event was the relocation of Matica Srpska to Novi Sad in 1864. Matica Srpska, the oldest Serbian cultural institution of the new century, was founded in Budapest in 1826.<sup>5</sup> Other institutions have developed in her lap, such as the Matica Srpska Library, the Matica Srpska Gallery, the Museum of Vojvodina, the Museum of the City of Novi Sad and others. In the era of national romanticism and civic liberalism, the political life in Novi Sad and Vojvodina was awakened by Svetozar Miletić, the greatest politician among the Serbs of the 19th century. His monument is located on the very center of the city on Freedom Square, where the building of the former Magistrate is, today the City Hall. The epithet "Serbian Athens" actually marked the golden age of Novi Sad culture. Along with the institutions, important personalities contributed the most to that. This is a city with a short history, but interesting people and events. Albert Einstein and his wife Mileva Marić-Einstein also lived here at the beginning of the 20th century.

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<sup>5</sup> Franyo, Zs, David, Cs, Dorman, L, Grujić, G, Ifju, G, Nemeth, M, Otos, A, Vojnović, B. *Az Újvidéki Színház húsz éve*, Újvidék : Dániel print, 1994, 29-38

At this crossroads of river, road and trade routes, meetings of cultures and civilizations, European and oriental economies, fairs were created in the 19th century. In the tradition of fairs, the Novi Sad Fair was created, which holds exhibitions of European significance throughout the year. The civic life of Novi Sad would be uninteresting without cafes and parties, but also without traditional cultural events. The atmosphere of the famous old cafes in Novi Sad is still felt. But modern life has brought new traditions. In Novi Sad are held: theater festival "Sterijino pozorište", children's festival "Dragon's Children's Games", poetry meetings "Brankovo kolo", Novi Sad music festivities, "Tamburica fest", and recently the summer international rock festival "Exit" "At the Petrovaradin Fortress, as well as the Festival of Street Musicians. The people of Novi Sad have been playing sports since the beginning of the city.<sup>6</sup> The oldest sports are equestrian, archery and rowing. The modern age has brought new, Olympic sports, in which the people of Novi Sad have won numerous medals. The "Novi Sad Marathon" is run in Novi Sad every autumn, and there is also the "Fruškogorski maraton" on the other side of the Danube. Novi Sad residents spend the summer on their river, mostly on the famous beach "Strand".

When he crosses the right bank of the Danube, the man finds himself in Petrovaradin. This pearl of Baroque architecture, SUBURBIUM, was built at the same time as the Petrovaradin Fortress, from 1692 to 1780. The fortress, a fortification according to the system of Marshal Vauban, on 112 hectares, is adorned with numerous bastions and barracks. Today it is a civil, cultural treasury which houses the hotel "Leopold I", restaurants and an exceptional terrace-lookout. There is also the Museum of the City of Novi Sad with a guide service that allows you to tour the underground military galleries. The Fortress also houses the Historical Archive of the City of Novi Sad, part of the Academy of Arts of the University of Novi Sad, "Atelier 61" (art tapestry workshop) and numerous art galleries, workshops and cafes. The fortress is dominated by the famous clock tower, one of the visual symbols of Novi Sad.<sup>7</sup>

### **Infrastructure projects in Novi Sad**

In the field of local and regional development, pedestrian paths with accompanying furniture and public lighting have been arranged in Novi Sad, which has increased the accessibility of tourist infrastructure facilities in Kamenica Park, as the central city park. The total length of the paths planned for

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<sup>6</sup> Radovanov, Z. *Petrovaradinska tvrđava*, Muzej grada Novog Sada, 2006, str. 110-111.

<sup>7</sup> Kapor, P. "Međunarodno finansiranje infrastrukturnih projekata", *Pravni informator*, 2004, str. 491

the rehabilitation is about 1892 meters, and the installation of new solar lighting is also planned, as well as the installation of 49 benches, 12 tables and 28 waste bins. A new children's playground made of natural materials was built in the park, where the youngest citizens of Novi Sad got their own training ground for developing motor skills, balance and coordination of movements.

In the field of traffic infrastructure, in Novi Sad, in order to ensure stable, safe and long-term traffic, ie improve the safety of all traffic participants, the project envisages the construction of a roundabout on road Doktor Goldman, Moše Pijade and Branislav Bukurov streets in Sremska Kamenica. In addition to the construction of the roundabout, the project also envisages the construction of bicycle and pedestrian paths, the relocation of the water supply and sewerage network from the road zone, as well as the construction of a drain. Also, a project is being implemented that includes rehabilitation of Partizanska Street, reconstruction of the road and construction of public lighting in Svetozara Miletića Street and intensified maintenance of the embankment of the first defense line along the Danube, from Heroja Pinkija Street to Sime Šolaja Street in order to increase traffic quality in Novi Sad. The maintenance of traffic areas within the University Campus in Novi Sad has been intensified, which includes the reconstruction of roads, parking lots and pedestrian paths in the streets of Sime Milošević, Ilija Đuričić, Dr. Zoran Đinđić, Vladimir Perić Valter and Vase Savić. The project also includes the reconstruction of the intersection at the intersection of Sima Milošević and Ilija Đuričić Streets, by forming a roundabout.

In the field of energetic and energetic efficiency, in Novi Sad, the electrical installations of the sports (large) hall of JP SOC Vojvodina in Novi Sad were repaired, which included the installation of new lighting equipment in the hall where most trainings of professional and recreational athletes are held. Which annually visits about 100,000 citizens at various sports and cultural events. By installing new lighting equipment, the installed power and consumption are reduced, which also means reduced electricity consumption and lower maintenance costs.

In the field of health and social protection, the Clinical Center of Vojvodina has been reconstructed and rehabilitated in Novi Sad and power lines of the Neurology Clinic, Eye Clinic and Administration Building. Reconstruction of the clinic for orthopedic surgery rehabilitation on the clinic for ear, throat and woes disease, rehabilitation of the boiler room and laundry. The realization of the project will provide better conditions for the work of employees and the stay of patients, and meet the standards of energy efficiency of facilities, which will ensure the smooth operation of the Clinical Center of Vojvodina. Then, the Institute for Health Protection of Children and Youth of Vojvodina was

reconstructed, and the purchase of an MRI machine is in progress, which will be placed in the newly built facility of the MRI Department. Within the phases of the first construction of the building, the construction of the ground floor of the building is planned, the final number of floors of which will be  $P + 1 + M$ . Providing space for storing MR devices will enable its smooth operation, and better conditions for providing the necessary health services. Finally, the construction of a Shelter for the care of all categories of users from the territory of the City of Novi Sad is planned, which is located in a facility in Futog that is inadequate and does not meet the needs for temporary accommodation. The project envisages the upgrade of a new floor on the existing size of the building, the upgrade of the annex for the living room of the user with the expansion of the dining room, as well as the upgrade of the appropriate elevator that can accommodate a hospital bed. After the reconstruction of the facility, the necessary accommodation capacity of the facility will be increased to 55 people.

In the field of education and protection of pupils 'and students' standards, the completion of the central kitchen of the preschool institution in Novi Sad, the construction of which began in 2011, is planned in Novi Sad. It includes the execution of all craft works on the adaptation of the facility, the completion of which will enable the installation of technological equipment and thus the commissioning of the facility. The construction of the central kitchen will improve the working conditions and the educational process of the preschool institution, and will improve the conditions for food preparation for 16,000 children enrolled in the preschool institution. Also, the addition of two floors over the part of the school building of the elementary school "Vasa Stajić" was done, which means the construction of the necessary additional space of 400 m<sup>2</sup> for teaching and work of teaching staff. The addition of two floors will provide space for new classrooms, a assembly hall, an office for the principal, a pedagogue and a reception hall for parents.

## **Conclusion**

The Republic of Serbia is in the process of rapid economic, economic and infrastructural development. In the past period, Serbia has been an underdeveloped country, while in the previous decade many significant projects have been done that have contributed to infrastructural, economic and economic progress. The paper lists many important projects that have greatly improved the quality of life of Serbian citizens, especially in the construction of traffic infrastructure, and especially the construction of the Miloš Veliki highway, as well as the construction of numerous road and highways.



As the paper deals specifically with the autonomous province of Vojvodina, all capital projects in Vojvodina's cities and municipalities are listed, with special emphasis on the city of Novi Sad as the capital of Vojvodina. The paper presents the historical development of Vojvodina, as well as Novi Sad. For the source of information, the official data available at the presentation of the Agency for Capital Investments in the Autonomous Province of Vojvodina, as well as the annual reports of this Agency were used. The data available for 2020 and 2021 were taken into account.

The results of the research are very positive, because it is evident that many important infrastructure projects have been done in the Autonomous Province of Vojvodina and that the quality of life of citizens has been significantly raised in various areas of life, such as water management and water supply, electric power industry and energy development. Education and upbringing and raising student standards, infrastructure development, transport infrastructure, raising culture and improving cultural content, as well as the development of sports, both professional and amateur.

Although much has been done positively in the development of Vojvodina, unfortunately there are still many infrastructural and other problems that need to be solved in the coming period. For example, in Zrenjanin there is a big problem with water supply. Based on samples taken in the period from November 14 to December 14, 2020, purified drinking water paid by the City of Zrenjanin to the "water factory" repeatedly did not comply with regulations due to increased arsenic concentration, higher color intensity, increased consumption of potassium permanganate. Which indicates an increased content of total organic matter in the water, and due to the increased concentration of ammonia and orthophosphate. Also, in Vojvodina, there is a big problem of garbage disposal and processing, which would mean the construction of recycling centers.

Positive examples of construction of recycling centers have already been noted in the previous period. In Omoljica, Pančevo municipality, in the presence of Shan McLeod, the British Ambassador to Serbia, an agreement was signed between the BIS recycling center and the English company "Donasonik" on the procurement of a recycling plant in March 2020. The plant is worth half a million pounds and the money was provided from the Green Fund of the Republic of Serbia. The new plant in the BIS recycling center in Omoljica will be used for the treatment of electrical and electronic waste, mainly stoves, water heaters, washing machines, small household appliances. The investment is worth half a million pounds. The money was provided from the Green Fund of the Republic from the collection of environmental taxes. Delivery is expected in the second half of the year, and by then the facility where it will be located will be reconstructed.

As a general conclusion of this paper, the fact is that Vojvodina is at the zenith of its economic and infrastructural development. Deficiencies are being worked on intensively and the problem is being resolved relatively quickly and efficiently, such as the example of opening recycling centers. If this progressive curve does not stop, it is very likely that the remaining problems on the territory of the Autonomous Province will be solved in the coming period. Thus, Vojvodina justifies its name of the Euro region and the center of culture and development of the Republic of Serbia with good reason.

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**DETERMINATION OF POLYSACCHARIDE  
CONTENT OF *AGARICUS MACROSPORUS* AND  
*RUSSULA VESCA* MUSHROOM EXTRACTS**

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**ABSTRACT**

*The aim of this research was to determine the polysaccharide content of aqueous and ethanolic extracts from Agaricus macrosporus and Russula vesca mushrooms. Generally, aqueous extracts were characterized with higher ( $p < 0.05$ ) glucan content compared to the ethanolic extracts. From the point of the same extract from different mushrooms, can be seen that the extract from R. vesca was characterized with higher values ( $p < 0.05$ ) for most of the analysed parameters, compared to the extract from A. macrosporus. In accordance, both  $\alpha$ - and  $\beta$ -glucan had higher ( $p < 0.05$ ) values in the aqueous (3.79%; 15.19%, respectively) as well as in the ethanolic extract (1.61%; 13.23%, respectively) from R. vesca compared to aqueous and ethanolic extract from A. macrosorporus. On the other hand, in the aqueous extract from R. vesca was determined higher ( $p < 0.05$ ) monosaccharide content (18.46%) compared to the aqueous extract from A. macsorporus (16.27%). Therefore, it can be concluded that water extraction of mushrooms, especially of Russula vesca, is a successful method by which the most important bioactive polysaccharides (total,  $\alpha$ - and  $\beta$ -glucan) can be extracted, as well as a high percentage of monosaccharides. This opens up new possibilities for further use of the extracts in various industries.*

**Keywords:** wild mushrooms, extracts, polysaccharide.

## INTRODUCTION

Mushrooms have long been considered to have medicinal value. The early herbalists were more interested in the medicinal properties of mushrooms than in their basic value as a source of food (Chang and Miles, 2004).

Several important compounds including bioactive polysaccharides (lentinan), dietary fiber, ergosterol, vitamins B<sub>1</sub>, B<sub>2</sub>, C, phenols, flavonoids and minerals have been isolated from the fruiting body, mycelia, culture medium of the mushrooms, as well as their extracts (Gursoy et al., 2010).

The study of biologically active compounds (phenolic compounds, flavonoids, carbohydrates) that are part of the mushroom or plants composition occupies an increasingly important place in terms of the medical effect these compounds have on consumers' health (Stojanova et al. 2021; Stojanova et al., 2022).

Among the bioactive compounds in mushrooms, polysaccharides are those that show most antitumoral, antiviral and immunomodulatory activity. In particular the polysaccharides that are found on the cellular wall are those that show most bioactivity. These polysaccharides are: chitin, cellulose and  $\beta$ -glucans (Mizuno and Nishitani, 2013).

*Agaricus macrosporus* (F.H. Muller and Jul. Schäff.), commonly known as the white button mushroom, is one of the most economically important edible mushrooms. It is considered as a valuable health food with high contents of polyphenols, ergothioneine, vitamins, minerals and polysaccharides (Dubost et al., 2007; Tian et al., 2012). Moreover, this mushroom has been demonstrated to possess various valuable biological properties including antitumor, anti-aromatase, antimicrobial, immunomodulatory, anti-inflammatory as well as antioxidant activities.

*Russula vesca* (Fr.) is a common and widespread edible mushroom on mainland Europe and North America. This mushroom appears in summer or autumn and grows primarily in deciduous forests. *Russula vesca* is considered edible and good, with a mild nutty flavour. In some countries, including Russia, Ukraine and Finland it is considered entirely edible even in the raw state (Dahlberg, 2019).

Numerous studies have shown that regular consumption of certain mushroom species as either a regular food or as extracted compounds is effective in both preventing and treating specific diseases, mainly through immunopotential and antioxidant activity. Thus, the intake of mushrooms and their extractable bioactive compounds appears to be effective in cancer

prevention and growth inhibition. Another important fact is the certainty that mushroom extracts, compared with other drugs, show a very low toxicity when regularly consumed, even in high dosages (Reis et al., 2014).

The aim of this research was to determinate the polysaccharide content of aqueous and ethanolic extracts of the wild mushroom species *Agaricus macrosporus* and *Russula vesca*.

## MATERIAL AND METHOD

In this research, as a work material two types of mushrooms collected from the territory of the Republic of North Macedonia were used: *Agaricus macrosporus* and *Russula vesca*. The collected fresh mushrooms were chopped into thin slices. The mushroom pieces were dried in a chamber dryer with hot air at a temperature of 40 °C for 6–7 h. Dried mushrooms were first ground to a fine powder and then, extracted in two ways, with water and ethyl alcohol as extragens.

### Preparation of aqueous extract

Aqueous extract was prepared by Sławińska et al. (2013) and Ribeiro et al. (2015) method. The measured mass of dried and finely powdered mushroom sample (10 g) was poured with about 200 mL of distilled water, and after that was extracted on a boiling water bath for 1 h. To determine the yield of the extract, the mass of empty evaporation flask while it is empty was measured, and then with the evaporated sample. From the difference of these two values, the extract yield was obtained.

### Preparation of ethanolic extract

Ethanol extract was prepared by Vidović et al. (2011) method. The measured mass of dried and finely powdered mushroom sample (10 g) was poured with 100 mL of 50% ethanol and extract was covered for 40 minutes on an ultrasonic bath at 45 °C. To determine the yield of the extract, the mass of the evaporation flask while it is empty was measured, and then with the evaporated sample. From the difference of these two values, the extract yield was obtained.

## **Determination of total, $\alpha$ and $\beta$ -glucan content**

The content of total glucan and  $\alpha$ -glucan in aqueous and ethanolic extracts was determined using specific kits Mushroom and Yeast Beta-glucan Assay Procedure, K-YBGL 11, 2019 (Megazyme Co. Wicklow, Ireland) according to the manufacturer's instructions.

The  $\beta$ -glucan content was calculated as the difference between the total glucan content and the  $\alpha$ -glucan content.

## **HPLC analysis of the monosaccharide composition of the extracts**

Solutions of sugar standard 0.1g/10mL for fructose, glucose, rhamnose, ribose, mannose and galactose were prepared in water and stored at -20 °C. HPLC analysis equipment included an integrated system with "Wellchrom HPLC pump" K-10-01, detector RI-71, Shodek-RI detector. Efficient chromatographic separation was achieved by isocratic elution with a Lichrospher column (4.6 mm x 250 mm x 5 mm, MERCK) at 35 °C (Column thermostat 5–85 °C, injection value A 1365 included). The mobile phase contained a mixture of acetonitrile and deionized water in a ratio of 75:25 (v/v), the volume of the injected sample was 20  $\mu$ L, and the flow rate was 1 mL/min. Sugars were identified by comparing the retention time of RT samples with standards, and the obtained values were expressed in % dry weight of the extract.

## **Statistical analysis**

The obtained results were statistically processed using the software package SPSS 20. To determine the statistical significant differences of the obtained results was used the Independent Sample t-test ( $p = 0.05$ ).

## **RESULTS AND DISCUSSION**

Mushrooms are known to be a good source of various types of carbohydrates. They are divided into two groups, digestible and indigestible, ie. carbohydrates that the human body cannot digest. Of the digestible carbohydrates, the most common in mushrooms are mannitol with 0.30–5.50% (Vaz et al., 2011), glucose with 0.50–3.60% (Kim et al., 2009) and glycogen with 1 –1.60% (Díez and Álvarez, 2001). The most indigestible carbohydrates are oligosaccharides, chitin,  $\beta$ -glucan and mannan.

**Table 1. Glucan content in aqueous extract (% dry matter extract)**

| Aqueous extracts            | n | Total glucan                   | $\alpha$ -glucan              | $\beta$ -glucan                |
|-----------------------------|---|--------------------------------|-------------------------------|--------------------------------|
|                             |   | $\bar{x} \pm \text{SD}$        | $\bar{x} \pm \text{SD}$       | $\bar{x} \pm \text{SD}$        |
| <i>Agaricus macrosporus</i> | 3 | 15.83 $\pm$ 0.05 <sup>aA</sup> | 2.57 $\pm$ 0.07 <sup>aA</sup> | 13.26 $\pm$ 0.01 <sup>aA</sup> |
| <i>Russula vesca</i>        | 3 | 18.98 $\pm$ 0.01 <sup>bA</sup> | 3.79 $\pm$ 0.11 <sup>bA</sup> | 15.19 $\pm$ 0.02 <sup>bA</sup> |

<sup>a, b</sup> - values of the same extract of different fungal species marked with different letters, have a statistically significant difference ( $p < 0.05$ ), T-test.

<sup>A, B</sup> - values of different extract of the same fungus, marked with different letters, have a statistically significant difference ( $p < 0.05$ ), T-test.

**Table 2. Glucan content in ethanolic extract (% dry matter extract)**

| Ethanolic extracts          | n | Total glucan                   | $\alpha$ -glucan              | $\beta$ -glucan                |
|-----------------------------|---|--------------------------------|-------------------------------|--------------------------------|
|                             |   | $\bar{x} \pm \text{SD}$        | $\bar{x} \pm \text{SD}$       | $\bar{x} \pm \text{SD}$        |
| <i>Agaricus macrosporus</i> | 3 | 12.20 $\pm$ 0.01 <sup>aB</sup> | 1.03 $\pm$ 0.06 <sup>aB</sup> | 11.17 $\pm$ 0.09 <sup>aB</sup> |
| <i>Russula vesca</i>        | 3 | 14.84 $\pm$ 0.02 <sup>bB</sup> | 1.61 $\pm$ 0.02 <sup>aB</sup> | 13.23 $\pm$ 0.07 <sup>bB</sup> |

<sup>a, b</sup> - values of the same extract of different fungal species marked with different letters, have a statistically significant difference ( $p < 0.05$ ), T-test.

<sup>A, B</sup> - values of different extract of the same fungus, marked with different letters, have a statistically significant difference ( $p < 0.05$ ), T-test.

According to data presented in Table 1 and Table 2, can be seen that, generally, aqueous extracts were characterized with higher ( $p < 0.05$ ) glucan content compared to the ethanolic extracts. From the point of the same extract from different mushrooms, can be seen that the extract from *R. vesca* was characterized with higher values ( $p < 0.05$ ) for most of the analysed parameters, compared to the extract from *A. macrosporus*. Moreover, the higher total glucan content ( $p < 0.05$ ) was determined in aqueous extract from *R. vesca* (18.98%), while in the ethanolic extract this value was found to be significantly ( $p < 0.05$ )

lower 14.84%. Furthermore, both  $\alpha$ -glucan and  $\beta$ -glucan had higher ( $p<0.05$ ) values in the aqueous (3.79%; 15.19%, respectively) as well as in the ethanolic extract (1.61%; 13.23%, respectively) from *R. vesca* compared to aqueous and ethanolic extract from *A. macrosporus*.

In their study, Özcan and Ertan (2018) reported that highest  $\beta$ -glucan content from wild mushrooms was found in *B. edulis* (13.93 %), while *A. bisporus* (cultivated mushroom), which was cultivated on compost and used for commercial purposes, had the highest overall  $\beta$ -glucan content at 14.57 %. Bulam et al. (2018) pointed out that the  $\beta$ -glucan contents of the mushrooms vary between 0.22 and 0.53 g/100 g on dry weight basis. According to Manzi and Pizzoferrato (2000), *Pleurotus pulmonarius* seemed to be the richest source of fungal  $\beta$ -glucans and it has been reported that *L. edodes* contains high levels of  $\beta$ -glucans in the soluble fraction. Camelini et al. (2005) found that *Agaricus brasiliensis* had higher (1 $\rightarrow$ 6)- $\beta$ -glucan ratio and (1 $\rightarrow$ 3)- $\beta$ -glucan increased with the maturation of fruiting bodies.

**Table 3. HPLC analysis of the monosaccharide content from tested mushroom extracts (%)**

| Monosaccharide content | n | Aqueous extract                |                                | Ethanolic extract              |                                |
|------------------------|---|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
|                        |   | <i>A. macrosporus</i>          | <i>R. vesca</i>                | <i>A. macrosporus</i>          | <i>R. vesca</i>                |
|                        |   | $\bar{x} \pm SD$               | $\bar{x} \pm SD$               | $\bar{x} \pm SD$               | $\bar{x} \pm SD$               |
| Fructose               | 3 | 2.51                           | 2.99                           | 1.73                           | 2.26                           |
| Glucose                | 3 | 3.67                           | 10.02                          | 2.61                           | 8.17                           |
| Galactose              | 3 | 5.34                           | /                              | 4.15                           | 1.09                           |
| Ramnose                | 3 | 0.78                           | 1.01                           | /                              | 0.30                           |
| Ribose                 | 3 | /                              | 3.89                           | 0.99                           | /                              |
| Mannose                | 3 | 3.97                           | 0.56                           | 2.14                           | 0.79                           |
| Total                  | 3 | 16.27 $\pm$ 0.09 <sup>aA</sup> | 18.46 $\pm$ 0.15 <sup>bA</sup> | 11.62 $\pm$ 0.10 <sup>aB</sup> | 12.61 $\pm$ 0.07 <sup>bB</sup> |

<sup>a, b</sup> - values of the same extract of different fungal species marked with different letters, have a statistically significant difference ( $p<0.05$ ), T-test.

<sup>A, B</sup> - values of different extract of the same fungus, marked with different letters, have a statistically significant difference ( $p<0.05$ ), T-test.



According to data presented in Table 3, can be seen that once again aqueous extracts had higher ( $p<0.05$ ) monosaccharide content compared to both ethanolic extracts. On the other hand, extracts from *R. vesca* are characterised with better ( $p<0.05$ ) monosaccharide content compared to both *A. macrosporus* extracts. Thus, the in the aqueous extract from *R. vesca* was determined statistically significant ( $p<0.05$ ) higher monosaccharide content (18.46%) compared to the aqueous extract from *A. macrosporus* (16.27%). However, ethanolic extract from *R. vesca* had higher ( $p<0.05$ ) total monosaccharide content (12.61%) compared to the ethanolic extract from *A. macrosporus* (11.62%).

In addition, water as a polar extraction agent probably proved to be better due to the higher value of most of the polysaccharide components that are thought to be responsible for biological activity (Stojanova et al., 2021).

The difference in the composition of monosaccharides mainly depends not only on the type of fungus, but also on the conditions in which they grow (Kalogeropoulos et al., 2013). In this case, since these are species from natural habitats, their composition is conditioned only by the natural presence of moisture, sunlight, favorable air temperature and the like (Giri et al., 2012).

From the aspect of immunomodulatory and anticancer effects of mushrooms, the presence of glucans, especially  $\beta$ -glucans, is of the great importance. This component is thought to have the power to regulate the immune system, lower total cholesterol levels and LDL levels, and exhibit a number of other immunomodulatory effects (Ko and Lin, 2004).

Anticancer polysaccharides from the mushrooms are most often soluble in water, such as  $\beta$ -D-glucan,  $\beta$ -D-glucan linked to the heterosaccharide chain of xylose, mannose, galactose or  $\beta$ -D-glucan protein complexes, which is in accordance with this research (Mizuno, 1999).

## CONCLUSION

According to the presented data can be conclude that both aqueous and ethanolic extracts from *Agaricus macrosporus* and *Russula vesca* are characterized with good polysaccharide content. Nevertheless, both aqueous extracts are characterized with statistically significant higher content of total,  $\alpha$ - and  $\beta$ -glucan compared to ethanolic extracts. Even that, both extracts from *Russula vesca* showed significantly higher glucan content compared to those from *Agaricus macrosporus*. On the other hand, based on the HPLC analysis, can be pointed out that hot water extraction showed better results expressed through tested monosaccharide compared to the ethanol as less polar extragen.

Therefore, it can be concluded that water extraction of mushrooms, especially of *Russula vesca*, is a successful method by which the most important bioactive polysaccharides (total,  $\alpha$ - and  $\beta$ -glucan) can be extracted, as well as a high percentage of monosaccharides. This opens up new possibilities for further use of the extracts in various industries, such as food, cosmetics and pharmaceuticals.

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# **FARMING, HORTICULTURE AND FORAGE PLANTS**



## MAIZE YIELD DEPENDING ON FERTILIZATION AND SOIL COMPACTION

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**Abstract:** Soil fertility is a combination of mineral and biological properties of soil and the circulation of plant nutrients in the soil-plant system is constantly happening within it. In order for the root system to develop and function normally, it is necessary that there is enough oxygen in the soil. Only well-drained soils provide enough oxygen and good activity of microorganisms. Since most of the operations, from sowing to harvest, are performed with the help of heavy mechanization, soil compaction occurs and its structure deteriorates. Within compacted soil, there is a weaker development of the root system, weaker microbiological activity, slowing down the absorption of water and nutrients, thus slowing down the growth of plants. Growing plants on such soils result in reduced yields and increased production costs. The aim of this study was to determine the impact of manure and mineral fertilizers on soil compaction and maize yield. The experiment was performed on the territory of the municipality of Leskovac on smonica soil type. The experiment included four variants of fertilization with organic and mineral fertilizers. Compaction was measured after sowing and after maize harvest, by penetrometer Eijkelkamp hardware version 6.0, software version 6.03. The application of manure in combination with mineral fertilizers significantly reduced soil compaction. The greatest compaction was recorded at a depth of 40-50 cm, after which it stagnated and slightly decreased to a depth of 80 cm. The average compaction measured after harvest was 24.10% higher than that measured after sowing. The soil moisture content was higher on plots with manure and mineral fertilizers than on non-fertilized plots. Maize yield was significantly higher in variants where manure



*was used together with mineral fertilizers compared to variants with the only use of mineral fertilizers and variants without fertilizers. Variants with the lowest soil compaction achieved the highest yields. The recommendation to maize producers is to apply more organic matter on heavy and compacted soils, primarily manure, but also mineral fertilizers, in order to have high and stable yields.*

**Key words:** soil compaction, manure, mineral fertilizers, maize, yield.

## INTRODUCTION

Soil is one of the most important elements of plant production and is the basic substrate for plant roots. It is a very dynamic environment in which the growth of the root depends on its depth, plant species, root characteristics, compaction, moisture, etc. (Navaz et al., 2013). Soil composition and its properties are the basic preconditions for high yields of cultivated plants. Thus, Živanović (2012) points out that soil type has a very significant impact on maize yield, sometimes higher than hybrids and fertilizers. Since most of the operations from sowing to harvest are performed with the help of heavy mechanization, it results in soil compaction and deterioration of its structure. In such soils, unfavorable conditions for the development of the root system prevail, microbiological activity is weaker, which results in a yield reduction and an increase in production costs by 20-40%. The soil is exposed to various forms of degradation. Thus, Lynden (2000) points out that soil compaction participates with about 11% in the total extent of degradation. The usage of heavy mechanization causes deterioration of the structure, both in the upper and lower layers of the soil. This reduces land productivity and increases energy consumption (Mueller et al., 2010). Hamza and Anderson (2005) emphasize the importance of water content in the soil, especially in the upper layers, where fewer mechanization passes lead to less evapotranspiration, which preserves soil moisture. Soil compaction has a detrimental effect on crop production and is one of the greatest problems that modern agriculture has to face (Trükmann et al., 2008).

In general, compacted soil has a weaker development of the root system, its length and penetration into deeper layers, slowing down the absorption of water and nutrients, slower plant growth, which in turn results in poorer plant development and reduced yields (Nosalevicz and Lipiec, 2014; Prakash et al., 2014; Dimitri and Destain, 2016).

How compact the soil will be will also depend on the crop being grown. By growing maize, the soil is compacted more than when wheat is grown (Milošev et al., 2007).

The usage of organic fertilizers, especially in combination with mineral fertilizers, significantly increases the better use of water in the soil, even in profiles of 1-1.5 m. This creates better conditions for the growth of maize root, its biomass, and total yield. In addition, the use of organic fertilizers promotes sustainable soil productivity and better environmental management (Wang et al., 2020). Githongo et al. (2021) also emphasize the importance of the use of organic fertilizers, primarily manure, and minimal tillage in increasing soil fertility and organic carbon content in the soil. The application of manure, at least two to three seasons earlier, significantly increases the content of phosphorus, potassium, and organic carbon in the soil, and thus the yield of maize (Njoroge, et al., 2019). Motavalliet al. (2003) have examined the influence of manure on the reduction of soil compaction with a sandy texture, and have concluded that the application of manure reduced soil compaction, increased nitrogen intake, and led to a significant increase in maize yield. Savin et al. (2011) point out that manure application is the best agro-technical measure that will reduce soil compaction. Increased soil compaction has the following consequence - the biomass of the roots increases in the uncompressed part. The use of organic fertilizers increases the biomass of maize roots in the uncompressed part of the soil and decreases in the compacted part (Bawa et al., 2019).

The aim of this study was to determine the effect of manure and mineral fertilization on soil compaction and maize yield.

## MATERIAL AND METHODS

The experiment was set up during 2021 on the territory of the municipality of Leskovac (village Todorovci), on smonica soil type. Samples of soil for chemical analysis were taken from the plots before the experiment was set up. The experiment was set up according to the plan of the block system with three repetitions. The previous crop was winter wheat. The experiment included four variants of fertilization:

- I. 20 t ha<sup>-1</sup> manure;
- II. 20 t ha<sup>-1</sup> manure + 300 kg ha<sup>-1</sup> NPK (16:16:16) + 200 kg ha<sup>-1</sup> KAN in top dressing;
- III. 300 kg ha<sup>-1</sup> NPK (16:16:16) + 200 kg ha<sup>-1</sup> KAN in top dressing and
- IV. No fertilization.

Soil preparation included autumn plowing to a depth of 30 cm, where manure and mineral fertilizer were applied. Pre-sowing preparation was performed immediately before sowing with a seed drill. Sowing was done in the second half of April. Treatment against seed weeds was performed the day after

sowing, with *Basar* and *Rezon* preparations. Treatment against broadleaf and narrow-leaf weeds was performed with *Siran* and *Maton* preparations. Fertilization with KAN was done in the phase of 3-5 leaves, after the first inter-row cultivation. No diseases or pests were present during the vegetation. Maize was harvested at technological maturity. The yield was calculated on each plot and reduced to 14% grain moisture. Maize yield and soil compaction depending on fertilization were statistically analyzed by analysis of variance using WASP 1.0 software.

Compaction was measured after sowing and after maize harvest, by the penetrometer Eijkelkamp, hardware version 6.0, software version 6.03. Compression measurements were performed in accordance with the NEN 5140 standard, with a penetration speed of 2 cm sec<sup>-1</sup>, where the deviation was not greater than 0.5 cm s<sup>-1</sup>, all according to the standard (ASAE S313.1). Before the beginning of the measurement, a reference plate, a certain position of the plot (GPS), and soil moisture were set. Soil moisture at the time of compaction measurement was determined by the Theta probe and is expressed in % vol. Measurements were performed on the inner part of the plot at a depth of 0 to 80 cm in 5 repetitions. Compression results are presented as average and are shown graphically.

### Climatic and soil characteristics

Table 1 shows the total monthly precipitation and average monthly temperatures during the maize vegetation period. The total amount of precipitation during the vegetation period, in 2021, was 270 mm. During the same year, in June, July, and August, 103 mm of precipitation fell, so this year can be considered less favorable for corn production. Average temperatures during maize vegetation were 17.7 °C and can be considered favorable for maize production.

Table 1. Precipitation (mm) and mean temperatures (°C) in Leskovac

|                                     | Apr. | May  | June | July | Avg. | Sep. | Oct. | Apr./Oct. |
|-------------------------------------|------|------|------|------|------|------|------|-----------|
| <i>The 2021 growing season</i>      |      |      |      |      |      |      |      |           |
| mm                                  | 45   | 47   | 55   | 44   | 4    | 24.0 | 51   | 270       |
| °C                                  | 10.3 | 17.4 | 20.7 | 24.5 | 23.7 | 16.8 | 10.8 | 17.7      |
| <i>Multi-year average 1985-2014</i> |      |      |      |      |      |      |      |           |
| mm                                  | 48   | 46   | 37   | 25   | 24   | 30   | 36   | 246       |
| °C                                  | 12.5 | 16.5 | 19.5 | 22.0 | 22.5 | 18.0 | 14.0 | 17.8      |

Compared to the multi-year average, the average monthly temperatures in June, July, and August were higher, while the average monthly temperatures in October were lower. Compared to the multi-year average (246 mm), this year had a higher amount of precipitation. Total precipitation during the vegetation was 46 mm higher than the multi-year average. This is especially true for precipitation in critical months, such as June, July, and August.

Table 2. Chemical properties of the soil

| Type of soil     | pH               |      | Humus | Nitrogen | Available (mg/100g of soil)   |                  |
|------------------|------------------|------|-------|----------|-------------------------------|------------------|
|                  | H <sub>2</sub> O | KCl  | (%)   | (%)      | P <sub>2</sub> O <sub>5</sub> | K <sub>2</sub> O |
| Smonica-Vertisol | 6.77             | 5.89 | 2.18  | 0.15     | 20.5                          | 27.3             |

Soil acidity was determined by the Kapen method, humus was determined by the Kotzman method, total nitrogen by the Kjeldahl method, and available phosphorus and potassium by the Engner-Riehm Al method.

According to the pH values in KCl (5.89), the soil belongs to the group of moderately acidic soils. According to the content of humus in the arable layer (2.18), the soils belong to the group of poorly humus soils (Škorić, 1991). Based on the content of total nitrogen (0.15), the soil is moderately provided with this element. The phosphorus content of 20.5 mg/100 g shows that the soil is optimally provided with this element. Also, the potassium content of 27.3 mg /100 g indicates the optimal provision with this element. Although these soils belong to the group of potentially fertile lands, their intensive use mainly requires the application of reclamation measures.

## RESEARCH RESULTS AND DISCUSSIONS

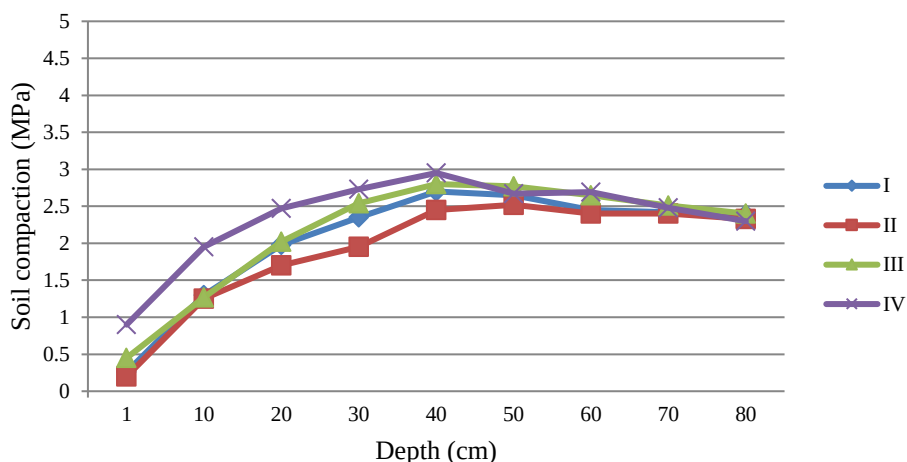
### Soil compaction

Maize is a highly productive plant species that has pronounced requirements when soil is in question (Bekavac, 2012).

The soil is an extremely dynamic environment and the substrate where the plant makes roots. Root growth depends on the plant species, layer depth, root characteristics, soil compaction, moisture, etc. (Nawaz et al. 2013). Soil compaction, for the most part, is caused by heavy mechanization during various agro-technical operations applied in plant cultivation. In such soils, a difficult absorption of water and nutrients, poorer development of the root system, and

slower growth of plants appear which altogether results in poorer plant development and reduced yields. Graf. 1 and 2 shows the compaction of soil depending on fertilization, measured after maize sowing and harvesting.

Graf. 1. Soil compaction after sowing (MPa)



I-manure; II-manure + NPK + KAN; III- NPK + KAN i IV- without fertilizers

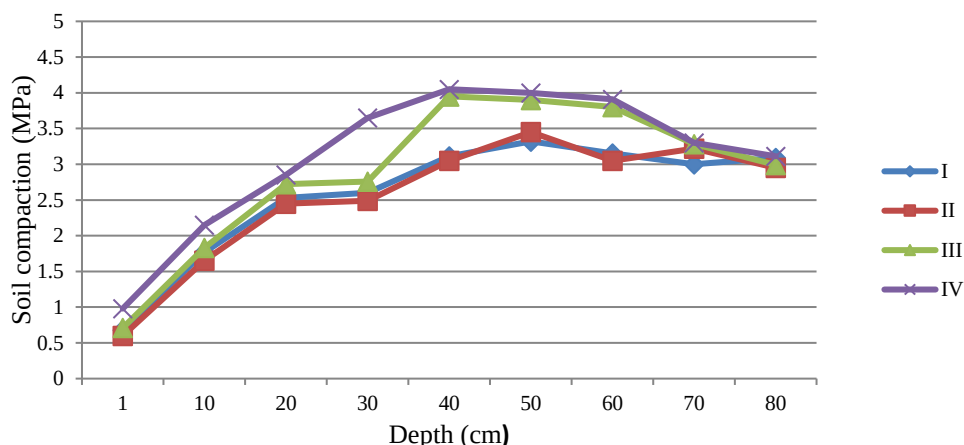
LSD (fertilizations): 0.05-0.16; 0.01-0.21; (depth): 0.05-0.26; 0.01-0.30

Soil compaction, depending on the method of fertilization, was measured at a depth of up to 80 cm, immediately after sowing maize. The average compaction at depths up to 80 cm ranged from 1.91 MPa on variant II to 2.35 MPa on variant IV. The highest (2.35 MP) was on variant IV (without the use of fertilizers) and the lowest (1.91 MPa) on variant II (manure + NPK). Statistically, the use of fertilizers significantly reduced soil compaction compared to the variant without the use of fertilizers. There were no statistically significant differences in soil compaction between variants I and II. Statistically significant differences in soil compaction were found between variants II and III, as well as between variants II and IV.

The average soil compaction for all fertilizer variants ranged from 0.45 MP at a depth of 1 cm to 2.70 MP at a depth of 40 cm. As the depth increased from 40 to 80 cm, the compaction slightly decreased, to 2.35 MP at a depth of 80 cm. A statistically very significant increase in soil compaction was recorded in the profile from 1 to 40 cm, after which the compaction decreased without statistically significant differences.

The average moisture content ranged from 19% when variant IV was in question (without the use of fertilizers) to 22.1% on variant I (where manure was used). The highest moisture content (22.1 and 21.4%) was on variants I and II, where the lowest soil compaction was recorded. These results are in agreement with the results obtained by Savin et al. (2011) who point out that soil moisture reduces compaction.

Graf.2. Soil compaction after harvesting (MPa)



I-manure; II-manure + NPK + KAN; III- NPK + KANi IV- without fertilizers

LSD (fertilizations): 0.05-0.28; 0.01- 0.32; (depth): 0.05-0.31; 0.01-0.42

Soil compaction, depending on the method of fertilization, was measured immediately after harvesting maize (graf. 2). It was found that the average compaction, for all variants of fertilization at a depth of up to 80 cm measured after harvest, was higher by 24.10% compared to that measured after sowing. The average compaction at depths up to 80 cm ranged from 2.54 MP on variant II to 3.11 MP on variant IV. The highest (3.11 MP) was on variant IV (without the use of fertilizers) and the lowest (2.54 MPa) on variant II (manure + NPK). Statistically, the use of fertilizers significantly reduced soil compaction compared to the variant without the use of fertilizers. There were no statistically significant differences in soil compaction between variants I and II, while statistically significant differences in soil compaction were found between variants II and III, as well as between variants II and IV. The variant without the use of fertilizer had significantly higher soil compaction than the variant on which fertilizer was used.

The average soil compaction for all fertilizer variants ranged from 0.74 MP at a depth of 1 cm to 3.67 MP at a depth of 50 cm. With increasing depth

from 50 to 80 cm, the compaction decreased slightly, and at a depth of 80 cm, it was 3.03 MP. Statistically very significant increase in soil compaction was recorded in the profile from 1 to 40 cm, and after a depth of 50 cm compaction stagnated and then decreased slightly without statistically significant differences. Our results are consistent with the results of other authors such as Alakuku and Pavo (1994), who point out that soil compaction is not the same in all profiles, and that it is most pronounced up to a depth of 50 cm. Furthermore, many authors (Aliev, 2001; Yavuzcan et al., 2005; Manuwa et al., 2011; Jerzy and Leszek, 2012) emphasize the pronounced compaction of the soil at a depth of up to 50 cm, which is accompanied by more difficult uptake of water and nutrients. The average soil compaction measured after harvest was higher than that measured after sowing by 0.67 MP, which indicates that maize in the second part of the vegetation had poorer soil conditions. Results obtained by Nikolić et al. (2006) and Simikić et al. (2005) show that the resistance measured in the spring is lower than that measured in the fall, which is the result of multiple mechanizations passes during the season. Our results agree with these allegations.

The average moisture content, for all variants of fertilization measured after harvest, was lower by 14.15% compared to that measured after sowing, while compaction in the same period was increased by 24.10%. The average moisture content ranged from 17.5% on variant IV (without the use of fertilizers) to 19.1% on variant II (manure + NPK). The highest moisture content (19.1 and 18.4%) was on variants II and I, where the lowest soil compaction was recorded. These results are in agreement with the results obtained by Savin et al. (2011), who point out that soil moisture affects the reduction of compaction. The data of Ćirić et al. (2008) also shows that the current humidity is a key factor which soil compaction depends on.

### **Grain yield**

In order for maize to achieve high and stable yields, it is necessary to choose the right hybrids, appropriate agricultural techniques, and favorable climatic conditions. Of all the agro-technical measures, special attention is drawn to fertilization. When it comes to heavy soils with poor chemical and mechanical properties, the intake of organic fertilizers, especially manure, is extremely important. Only fertile, well-drained, and aerated soils provide enough oxygen and good activity of microorganisms, thus creating preconditions for high plant yields (ATA 2013). Table 3 shows the yield of maize depending on fertilization.

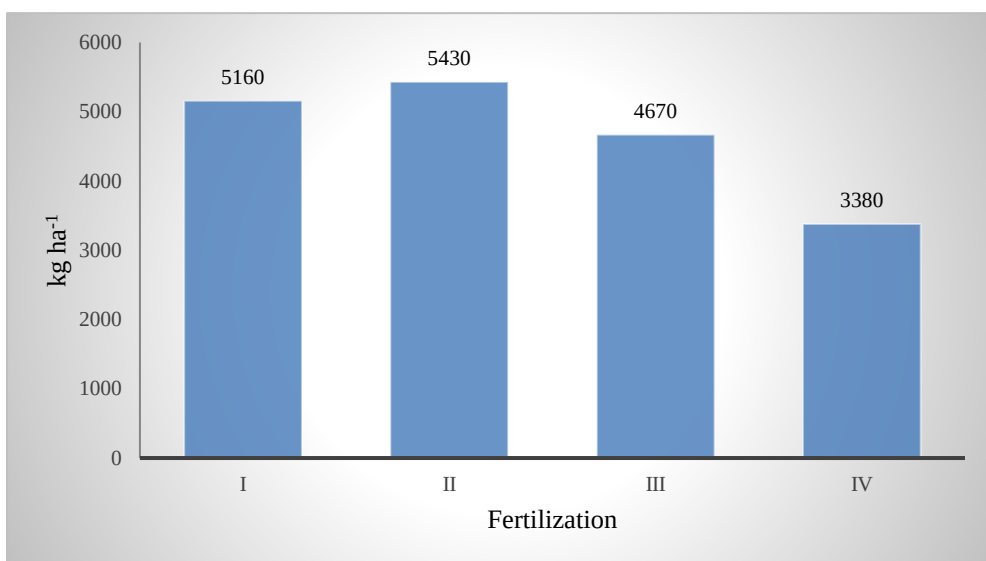
Table 3. Maize grain yield ( $\text{kg ha}^{-1}$ ) depending on fertilization

| Yield        | Fertilization |      |       |       | Average |
|--------------|---------------|------|-------|-------|---------|
|              | I             | II   | III   | IV    |         |
| 2021         | 5160          | 5430 | 4670  | 3380  | 4660    |
| Reducing (%) | 4.42          | 100  | 13.99 | 37.75 |         |

I-manure; II-manure + NPK + KAN; III- NPK + KAN i IV- without fertilizers

The average maize yield for all variants of fertilization was 4660 kg ha<sup>-1</sup>. The highest yield of 5430 kg ha<sup>-1</sup> was achieved on variant II, where manure was used in combination with NPK fertilizers. A slightly lower yield of 5160 kg ha<sup>-1</sup> was achieved on variant I where only manure was applied, and this yield reduction is 4.42%. The yield of 4670 kg ha<sup>-1</sup> was achieved on variant III, where only NPK fertilizers were applied. This yield reduction, compared to variant II is 13.99%.

Graf. 3. Maize grain yield (kg ha<sup>-1</sup>) depending on fertilization



I-manure; II-manure + NPK + KAN; III- NPK + KAN; IV- without fertilizers

Wanga et al (2020) says that the combination of organic and mineral fertilizers increases the yield of maize and the use of soil moisture compared to the use of



only mineral fertilizers, which is evident in our research. Also, the data of Njoroge et al. (2019) indicate a significant increase in maize yield using manure compared to the usage of mineral fertilizers. The lowest yield of 3380 kg ha<sup>-1</sup> was achieved on the variant without the use of fertilizers and the yield reduction compared to variant II is 37.75%. When analyzing the results on soil compaction according to fertilization variants, it can be concluded that fertilizers, both manure and NPK fertilizers, influenced the reduction of soil compaction and thus the yield of corn. Results obtained by Riedella et al. (2005) show that there is a negative correlation between soil compaction and maize yield, which is consistent with our results. The data cited by Marinković et al (1999) show that soil compaction directly affects the reduction of yield and this reduction when maize is in question is from 4.7 to 21.3%, which is confirmed by our results. Nevens et al. (2003) point out that maize plants are lower on compacted soil, flowering is late and the yield is lower by 13.2%. Thus, other authors also point to the negative consequences of soil compaction, which are reflected in multiple yield reductions (Friton, 2001; Nikolić et al., 2003; Ramazan et al., 2012).

Based on our results, as well as the results of other researchers, maize producers are advised to apply more organic matter on heavy and compacted soils, primarily manure, but also mineral fertilizers, in order to improve conditions for better growth of the root system, thus achieving higher yields.

## CONCLUSION

Based on the results on the impact of fertilization on soil compaction and maize yield, the following can be concluded:

Soil compaction significantly depended on fertilization and soil depth. The application of manure in combination with mineral fertilizers significantly affected the reduction of soil compaction. The average compaction measured after harvest was 24.10% higher than that measured after sowing. The highest compaction was recorded to a depth of 40-50 cm, after which it stagnated and slightly decreased to a depth of 80 cm. The moisture content in the soil was higher on plots with manure and mineral fertilizers than on non-fertilized plots. The yield of maize was significantly higher on the variants where manure was used together with mineral fertilizers in relation to the variants with the use of only mineral fertilizers and the variant without fertilizers. Variants with the lowest soil compaction achieved the highest yields.

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## INVESTIGATION OF THE IMPACT OF THE SYSTEM FOR DIRECT SOWING AND CONSERVATION TILLAGE ON ENERGY CONSUMPTION AND WINTER WHEAT YIELD

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**Abstract:** *Wheat is one of the most important plant species in the world, and in addition to rice, it is mostly used in human nutrition. Wheat production can take place using various technological and technical systems of processing and sowing. In the case of inadequate choice of the technological and technical system of land cultivation and sowing of wheat, the unfavorable impact on moisture conservation, yield, increased energy consumption, and increase in production costs come to the fore. These tests aimed to determine the effects of work and the impact of different technological and technical systems for tillage and sowing on energy consumption and the amount of winter wheat yields, as well as which of the applied systems allows the lowest energy consumption and maximum yields in given test conditions. Based on the obtained research results, it was concluded that both tested systems were applied in similar production conditions, where they achieved different results. The highest average fuel consumption was measured in the variant of conservation tillage (RT) of seeders type D and was 22.71 [L ha<sup>-1</sup>], and the lowest in the variant of direct sowing NT type B and was 6.91 [L ha<sup>-1</sup>]. The lowest energy consumption was recorded in the direct sowing system NT-type B, 267.42 [MJ ha<sup>-1</sup>], and the highest in the conservation tillage and sowing RT, seeder type D, which amounted to 878.88 [MJ ha<sup>-1</sup>]. The highest average yield was achieved in the variant of conservation treatment - RT, seeder type C and was 7.12 [MJ ha<sup>-1</sup>], and the lowest in the variant of direct sowing - NT seeder type B, 6.61 [MJ ha<sup>-1</sup>].*

**Keywords:** direct sowing, conservation tillage, yield, energy, wheat.

## INTRODUCTION

Successful wheat production is reflected in terms of the volume of yields achieved, the quality of the harvested grain, and the production costs. Wheat is one of the most important plant species in the world, and in addition to rice, it is mostly used in human nutrition, for making bread and pasta, which feeds over 70% of the world's population. In the Republic of Serbia in 2020, wheat production was represented at 581,128 ha, with an average yield of  $4.9 \text{ t ha}^{-1}$  (Stat. Yearb. Serb. 2021). Wheat production can take place using different technological and technical systems of processing and sowing (Rusu et al., 2013; Büchi et al., 2018; Omara et al., 2019; Wozniak et al., 2020; Gaweda, et al., 2021). Generally, the benefits of NT originate from the three main principles: reduced soil disturbance improved soil cover from crop residues, and increased species diversity through crop rotation (Hobbs et al., 2007; Kassam et al., 2019). In order to limit the environmental impact of agriculture, alternatives to traditional systems have been proposed. Conservation agriculture is one of these alternatives, which is more and more adopted worldwide (Holland, 2004). It is based on three fundamental principles: 1. diversification of crop rotation, 2. reduction of soil tillage, and 3. permanent soil cover (FAO, 2017). Compared to classical plowing tillage, reduced tillage has several advantages, such as reduction of fuel costs, reduced disturbance for soil organisms, preservation of soil fertility, higher soil macroporosity, and better water retention (Lienhard et al., 2013; Mazzoncini et al., 2011; Palm et al., 2014; Soane et al., 2012). Tillage systems determine soil properties (Aziz et al., 2013), water-air balance, and consequently crop yield and its quality (Rachon et al., 2015; Wozniak et al, 2017). In the case of inadequate choice of the technological and technical system of tillage and wheat sowing, there is an adverse effect on moisture conservation, yield, increased energy consumption, and increased production costs (Boydas et al., 2007; Sarauskis et al., 2009; Taner et al., 2015). The evaluation of the quality of work of seeders is reflected through the fulfillment of requirements related to the realization of the desired sowing norm and uniform distance of sown seeds in a row (Marković et al., 2007). In the case of conventional tillage, good soil structure is often not achieved, so the quality of wheat sowing is inadequate. Zero and minimal tillage reduce soil load and mineralization and preserve the surface layer so that they occupy a significant place in plant production in many countries (Saljnikov et al., 2014; Honsdorf et al., 2020). Minimizing cultivation reduces land cultivation to the required minimum, with a significant reduction in costs. The results have shown that in dry and hot climate conditions with a deficit moisture regime, direct sowing of rainfed winter wheat can be successfully applied provided an

appropriate combination, dosage, and timing of fertilizers and crop protection chemicals are applied (Turebayeva et al., 2022 ). Direct sowing omits cultivation as the most difficult and at the same time the most expensive procedure in agrotechnics of crops, and better preserves humus and soil structure. Several researchers have dealt with the problems of testing technological and technical systems for tillage and sowing. Successful sowing in precision agriculture is difficult to achieve without the use of appropriate sowing aggregates while knowing their characteristics (Auernhammer, 2004; Wiesenhoff and Koller, 2004; Šumanovac et al., 2006). The conservation system of tillage and sowing enables fuel savings of 27.4% compared to the conventional production system, while in the variant of direct sowing and zero cultivation-NT savings are much higher and amount to 82.7% (Filipović et al., 2004). The same authors conclude that in the variant of conservation tillage the highest yield of wheat was  $5.73 \text{ t ha}^{-1}$ , while in the variant of conventional tillage the yield was lower and amounted to  $5.22 \text{ t ha}^{-1}$ , and in the variant of direct sowing  $5.62 \text{ t ha}^{-1}$ . The application of conservation tillage and sowing (RT) in wheat production achieved energy savings of 32.5% compared to the conventional tillage system (CT), while direct sowing (NT) achieved energy savings of 90.4% (Košutić et al., 2006). The same authors state that in the variant of conventional cultivation and sowing the wheat yield was  $6.49 \text{ Mg ha}^{-1}$ , in the variant of conservation tillage (RT)  $6.16 \text{ Mg ha}^{-1}$ , and the variant without cultivation (NT)  $6.79 \text{ Mg ha}^{-1}$ . Winter wheat grain yield achieved by reduced tillage systems was on average either higher  $\text{CH} = 5.59 \text{ t ha}^{-1}$  or not different  $5.38$  and  $5.23 \text{ t ha}^{-1}$  for DS and NT, respectively than CT  $5.28 \text{ t ha}^{-1}$  (Jug et al., 2006). Analyzing the impact of different processing and sowing systems in wheat production (Košutić et al., 2008), we conclude that in the conventional system (CT) fuel consumption was  $67.34 \text{ L ha}^{-1}$ , in conservation (RT)  $52.84 \text{ L ha}^{-1}$ , while in the variant of direct sowing-without cultivation (NT) the consumption was  $6.59 \text{ L ha}^{-1}$ . The yield performance of wheat in conventional tillage was 8.06 tons per hectare whereas for reduced tillage and No-tillage were 7.9 and in No-till 6.3 tons respectively. As a result, a good replacement for conventional tillage is the No-till system with disk furrow openers in dryland fields and fields that are rain irrigated (Akbarnia et al., 2010). A higher barley grain yield was determined in the CT system compared to the RT system (by 24.8%) and NT (by 54.2%) systems (Wozniak, 2020).

The aim of these tests was to determine the effects of different technological and technical systems for tillage and sowing, as well as which of the applied systems allows the lowest energy consumption achieves the highest yields in the given test conditions.

## MATERIAL AND METHODS

The impact of the technological and technical system of direct sowing and conservation treatment on energy consumption and winter wheat yield was examined in the vicinity of Pancevo during 2020/21. years. The tests included measuring fuel consumption, energy, performance, and the amount of winter wheat yields depending on the applied system of tillage and sowing. The system of direct sowing NT and conservation treatment and sowing RT with two types of seeders were represented in the tests. Great Plains MD 1500 - type A and SK-HMA-24- type B seeders were tested in the NT – No-till planter direct sowing system, while HE-VA Kulti seeder - type C and Amazone seeders were tested in the conservation tillage and RT sowing system D9 4000 Super-type D. In the RT conservation tilling variant, a plate was applied in two passes, at an angle of  $10^\circ$  in relation to the direction of the preculture rows, after which sowing was performed. According to all tested variants of the seeder, the pre-crop was maize, and Pesma wheat (variant A) and Solehio (variant B, C and D) were used for sowing. The seeders worked in the unit with tractors of 92 kW, 60 kW, 85 kW, and 145 kW.



Typ A



Typ D

Figure 1. Tested systems for direct sowing and sowing in conservation tillage  
(Photo by S. Barać)

Seeder type-D for sowing in the system of conservation treatment and sowing is a combined type with several groups of working bodies, a working width of 4 m. The first working body is a rotary harrow with wedges, the next working body is a steel-toothed roller, and behind the feather seedbed, there are pressure wheels. The values of fuel consumption, speed of movement, and achieved effects were read in the tractor cab from the display for seed drill types A, C, and D because the tractors were equipped with ISOBUS technology for advanced operation of agricultural machinery. Fuel consumption in variant D was measured by the volumetric "top-up" method (Moitzi et al., 2013), and the effects were achieved by timing.



Table 1. Technical characteristics of the tested technological and technical systems and testing conditions

| Parameters                |                        | Type of sowing aggregate |            |            |            |
|---------------------------|------------------------|--------------------------|------------|------------|------------|
|                           |                        | Type A                   | Type B     | Type C     | Type D     |
| Number of rows            | /                      | 30                       | 24         | 24         | 33         |
| Distance between the rows | [cm]                   | 15.20                    | 15.00      | 12.50      | 12.00      |
| Working width             | [m]                    | 4.56                     | 3.60       | 3.00       | 4.00       |
| Weight                    | [ kg]                  | 4800                     | 3100       | 1030       | 1150       |
| Acquired power            | [kW]                   | 85+                      | 55+        | 80+        | 55+        |
| Working speed             | [km h <sup>-1</sup> ]  | do 10                    | do 10      | do 10      | 6 - 10     |
| Sowing date               | /                      | 4.11.2020.               | 4.11.2020. | 4.11.2020. | 4.11.2020. |
| Cultivar                  | /                      | Pesma                    | Solehio    | Solehio    | Solehio    |
| Seedbox volume            | [l]                    | 1269                     | 940        | 760        | 1380       |
| Pre-crop                  | /                      | maize                    | maize      | maize      | maize      |
| Transport width           | [m]                    | 4.85                     | 3.80       | 3.40       | 4.25       |
| Length of plot            | [m]                    | 390                      | 900        | 380        | 390        |
| Sowing qty                | [kg ha <sup>-1</sup> ] | 303                      | 220        | 200        | 250        |
| Sprouting                 | [%]                    | 96                       | 93         | 94         | 93         |

Energy consumption was obtained by calculation, while the realized yields were determined by the diagonal of the plot at the time of harvest and were calculated for the entire experiment. All values were read in 5 replicates, and the experiment was performed in a completely randomized variant.

The obtained results were processed using Microsoft Office Excel 2007.

## RESULTS AND DISCUSSION

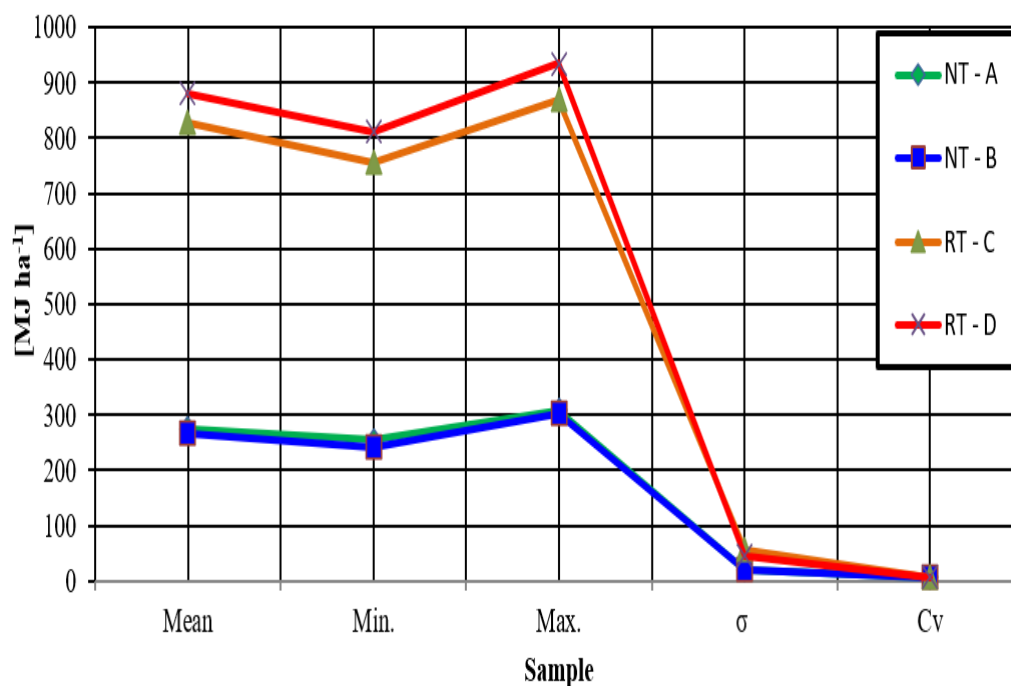
Tables 2 - 4 and graphs 1 and 2 show the results of testing the impact of direct sowing and conservation tillage systems on energy consumption, yields, and effects on winter wheat production.

Based on the results presented in Table 2, it can be observed that significant differences in fuel and energy consumption were achieved between the examined systems of direct sowing of NT and conservation treatment and sowing of RT. The highest average fuel consumption was achieved in the variant of conservation treatment and sowing RT- type D, namely 22.71 [L ha<sup>-1</sup>], while the lowest average fuel consumption of 6.91 [L ha<sup>-1</sup>] was achieved in the variant of direct sowing in winter wheat NT- No till planter type B. In the variant of conservation treatment and sowing RT- Type C, high average fuel consumption was also measured and it amounted to 21.38 [L ha<sup>-1</sup>], while in the variant of direct sowing NT- Type A the average fuel consumption was significantly lower and amounted to 7.11 [L ha<sup>-1</sup>]. The differences in terms of average fuel consumption between the applied systems of direct treatment and sowing of NT and conservation treatment and sowing of RT were very significant. Within the tested systems of direct sowing NT between the tested types of seeders A and B, the average fuel consumption was not significantly different, as well as within the system of conservation of processing and sowing RT between seeders type D and C (Table 2).

Table 2. Energy consumption depends on the tillage and sowing system

| Parameters                                                            | Tillage System |        |         |         |
|-----------------------------------------------------------------------|----------------|--------|---------|---------|
|                                                                       | NT - A         | NT - B | RT - C  | RT - D  |
| Average fuel consumption [L ha <sup>-1</sup> ]                        |                |        |         |         |
| <i>Mean</i>                                                           | 7.11           | 6.91   | 21.38*  | 22.71*  |
| <i>σ</i>                                                              | 0.54           | 0.57   | 1.48    | 1.15    |
| <i>Cv</i>                                                             | 7.53           | 8.32   | 6.94    | 5.19    |
| <i>Min</i>                                                            | 6.56           | 6.24   | 19.56   | 20.96   |
| <i>Max</i>                                                            | 7.98           | 7.82   | 23.16   | 24.15   |
| Average energy requirement [MJ ha <sup>-1</sup> ]                     |                |        |         |         |
| <i>Mean</i>                                                           | 275.16         | 267.42 | 827.41* | 878.88* |
| <i>σ</i>                                                              | 20.68          | 19.83  | 57.06   | 44.52   |
| <i>Cv</i>                                                             | 7.54           | 7.48   | 6.97    | 5.18    |
| <i>Min</i>                                                            | 253.87         | 241.49 | 756.97  | 811.15  |
| <i>Max</i>                                                            | 308.83         | 302.63 | 869.29  | 934.61  |
| *Different letters indicate significant ( $p \leq 0.01$ ) differences |                |        |         |         |

Assessing the impact of the direct sowing system - NT and conservation tillage - RT on energy consumption in winter wheat production (Table 2), it can be seen that the applied system had a very significant impact on energy consumption. By applying the system of conservation tillage and sowing of winter wheat, higher values of average energy consumption were measured and they amounted to 878.88 [MJ ha<sup>-1</sup>] RT-type D, which is the highest measured value of consumption, i.e. 827.41 [MJ ha<sup>-1</sup>] in RT-type C variant.



Graph 1. Statistical indicators of energy consumption of tested systems

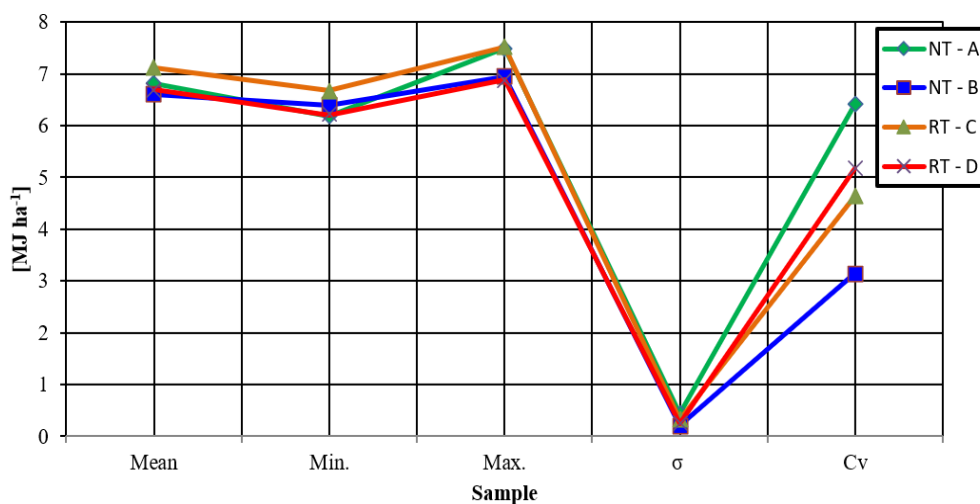
In the direct sowing variant, energy consumption was lower, with the lowest average values measured in the NT-type B variant being 267.42 [MJ ha<sup>-1</sup>], while the average energy consumption in the NT-type A variant was 275.16 [MJ ha<sup>-1</sup>]. Energy consumption within the tested systems of direct sowing NT between types of seeders A and B was not significantly different as well as within the system of conservation tillage and sowing RT - seeders type D and C.

Similar results have been obtained in other studies (Filipović et al., 2004; Košutić et al., 2006; Boydas et al., 2007; Košutić et al., 2008; Sarauskis et al., 2009; Lienhard et al., 2013; Moitzi et al., 2013; Taner et al., 2015).

Table 3. Influence of tillage system on the yield of winter wheat – basic descriptive statistics

| Parameters  | Tillage System                             |       |        |        |
|-------------|--------------------------------------------|-------|--------|--------|
|             | Winter wheat yield [ Mg ha <sup>-1</sup> ] |       |        |        |
|             | NT -A                                      | NT -B | RT - C | RT - D |
| <i>Mean</i> | 6.81                                       | 6.61  | 7.12*  | 6.69   |
| <i>σ</i>    | 0.44                                       | 0.21  | 0.33   | 0.25   |
| <i>Cv</i>   | 6.41                                       | 3.14  | 4.63   | 3.74   |
| <i>Min</i>  | 6.19                                       | 6.39  | 6.67   | 6.21   |
| <i>Max</i>  | 7.49                                       | 6.95  | 7.52   | 6.88   |
| lsd         | 5%                                         | 0.419 |        |        |
|             | 1                                          | 0.558 |        |        |
|             | %                                          |       |        |        |

Analyzing the results shown in Table 3, it can be concluded that the system of direct sowing and conservation tillage had a significant impact on the level of realized yields of winter wheat. The highest average yield of winter wheat was achieved in the variant of conservation tillage - RT, seeder type C and was 7.12 [Mg ha<sup>-1</sup>], and the lowest in the variant of direct sowing - NT seeder type B, 6.61 [Mg ha<sup>-1</sup>]. In the variant of direct sowing - NT seeder type A, average yields of 6.81 [Mg ha<sup>-1</sup>] were achieved, while in the variant of conservation tillage - RT, seeder type D, the average yield of 6.69 [Mg ha<sup>-1</sup>] was achieved. (Table 3).



Graph 2. Statistical indicators of realized yields according to the examined systems

Assessing the level of differences between the tested systems of direct sowing and conservation tillage in terms of the amount of winter wheat yields, it can be stated that the conservation tillage system RT-type C achieved statistically significantly higher yields compared to other variants, while between types of seeders A, B and D there were no statistically significant differences in the amount of realized yields.

Similar results have been reported by other authors (Filipović et al., 2004; Košutić et al., 2006; Jug et al., 2006; Boydas et al., 2007; Sarauskis et al., 2009; Taner et al., 2015; Wozniak, 2020).

Important exploitation indicators of the effects of direct system operation – No-till planter sowing and conservation tillage RT represent the achieved effects. Based on the results shown in Table 4, it can be noticed that different values of the achieved effects were measured according to the examined variants.

Table 4. Achieved effects of the tested systems

| Parameters                                                    | Tillage System                           |       |        |        |
|---------------------------------------------------------------|------------------------------------------|-------|--------|--------|
|                                                               | NT -A                                    | NT -B | RT - C | RT - D |
|                                                               | Average efficiency [ha h <sup>-1</sup> ] |       |        |        |
| <i>Mean</i>                                                   | 1.99                                     | 1.89  | 2.11*  | 1.81   |
| <i>σ</i>                                                      | 0.19                                     | 0.12  | 0.10   | 0.11   |
| <i>Cv</i>                                                     | 9.55                                     | 5.27  | 4.83   | 5.69   |
| <i>Min</i>                                                    | 1.69                                     | 1.75  | 1.94   | 1.68   |
| <i>Max</i>                                                    | 2.27                                     | 2.02  | 2.25   | 1.96   |
| *Different letters indicate significant (p≤ 0.05) differences |                                          |       |        |        |
| Average working speed [km h <sup>-1</sup> ]                   | 6.52                                     | 5.31  | 6.05   | 7.11   |

The highest average achieved effects were obtained by the RT conservation treatment system with seeder type C and amounted to 2.11 [ha h<sup>-1</sup>], and the lowest also in this applied system - seeder type D and amounted to 1.81 [ha h<sup>-1</sup>]. Within the direct sowing system NT- No-till planter, the achieved effects varied from 1.89 [ha h<sup>-1</sup>] seeders type B to 1.99 [ha h<sup>-1</sup>] seeders type A. Effects achieved by

the system of conservation treatment and sowing RT - type C were significantly higher in relation to seeder types A, B, and D. No significant differences were achieved between seeder types A, B and D. in terms of performance.

## CONCLUSION

Based on the results of the research, it can be concluded that both tested systems were applied in similar production conditions, where they achieved different results. The highest average fuel consumption was measured in the variant of conservation tillage (RT) of seeders type D and was  $22.71 \text{ [L ha}^{-1}\text{]}$ , and the lowest in the variant of direct sowing of winter wheat NT type B and was  $6.91 \text{ [L ha}^{-1}\text{]}$ . The differences in terms of average fuel consumption between the applied direct tillage and sowing systems No-till planter and conservation tillage and RT sowing were very significant. By applying direct sowing and conservation tillage, different values of energy consumption in wheat production were measured. The highest energy consumption was recorded in the system of conservation tillage and sowing RT, seeder type D and amounted to  $878.88 \text{ [MJ ha}^{-1}\text{]}$ , and the lowest in the system of direct sowing NT-type B,  $267.42 \text{ [MJ ha}^{-1}\text{]}$ . The impact of the processing and sowing system on energy consumption was significant, with very significantly higher values of energy consumption compared to the direct sowing system of NT in the variant of conservation tillage and sowing of winter wheat. The applied systems had a significant impact on the amount of realized yields of winter wheat. The highest average yield was achieved in the variant of conservation tillage - RT, seeder type C and was  $7.12 \text{ [Mg ha}^{-1}\text{]}$ , and the lowest in the variant of direct sowing - NT seeder type B,  $6.61 \text{ [Mg ha}^{-1}\text{]}$ . Assessing the level of differences between the examined systems in terms of the amount of winter wheat yields, it can be concluded that the conservation tillage system RT-type C achieved statistically significantly higher yields compared to other variants, while between types A, B, and D there were no statistically significant the difference in terms of the amount of realized yields. The highest average achieved effects were measured in the RT conservation tillage system with seeder type C and were  $2.11 \text{ [ha h}^{-1}\text{]}$ , and the lowest also in this applied system - seeder type D and were  $1.81 \text{ [ha h}^{-1}\text{]}$ .

The general conclusion of our research is that different tillage and sowing systems can be successfully used in the test area. With the system of conservation tillage and sowing, higher yields were achieved compared to direct sowing. However, the differences in terms of time savings and energy consumption are very large, with the NT direct sowing system consuming significantly less energy compared to conservation tillage and sowing, which fully justifies the application of this

system in winter wheat production in addition to conservation tillage and sowing in the study area.

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## **POSSIBILITY OF GROWING TRITICALE AS A MULTIPURPOSE CEREAL DEPENDING ON THE VARIETY, SOIL, FERTILIZER AND WEATHER CONDITIONS**

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### **ABSTRACT**

*Triticale, a wheat-rye interspecies hybrid created by human labor, is one of the species that from the agronomic and economic aspect belongs to the most important group of field plants - cereals. Since it was created by crossing two species, it inherited a high tolerance from its parents for less favorable abiotic and biotic factors. Much work has been done on the selection of triticale, both in Europe (Poland, France, Germany, Sweden, Belarus, Russia) and in our country, where the creation of genotypes interesting for commercial production has a decades-long tradition. The results of this work are varieties in which the expression of most positive properties of rye is achieved, such as early maturity, increased tolerance to lodging, but also higher grain yield and increased tolerance to some diseases inherited from wheat. The grain thus obtained has characteristics that make it suitable for use in human nutrition, domestic animals as well as industrial processing.*

*How triticale will be used depends primarily on the characteristics of the variety, i.e. whether a variety that looks more like wheat or rye was created in the process of breeding. Varieties of uniform large grain in chemical composition similar to wheat are intended for human consumption, while those that form a large biomass are intended for the preparation of voluminous animal feed. Also, grain yield and its chemical composition are largely, apart from the variety, conditioned by soil characteristics, type and amount of fertilizers used, as well as weather conditions during triticale cultivation. The aim of this paper is to emphasize the importance of triticale as a multipurpose cereal, especially for producers in less developed regions, but also in areas where limiting factors limit the production of more demanding types of cereals.*

**Key words:** triticale, yield, chemical composition, nutritional value, rural area

## INTRODUCTION

Triticale, a hybrid created by crossing wheat and rye, was created with the idea of combining the positive characteristics of the parent species. Accordingly, it is characterized by a high genetic potential for yield with a pronounced high tolerance to biotic and abiotic stresses (Dumbravă et al., 2016; Liu et al., 2017). Although initially used primarily as a grain for livestock (grazing or silage preparation), due to the high content of protein and essential amino acid - lysine, it is increasingly recommended in the fattening of pigs and chickens (Đekić et al, 2012), but also as a substitute wheat or corn in mixtures for the consumption of consumer fish - carp (Marković et al., 2016). Due to its pleasant taste and smell, it is often recommended for human nutrition by nutritionists. Also, Obuchowski et al. (2010) emphasize the importance of triticale in bioethanol production, and Kučerová (2007) ranks it among the best species for bioethanol production, referring to high amyolytic activity and, consequently, a low falling number. Thanks to the above, in the technological process itself, there is no need to add enzymes that hydrolyze starch to glucose, so the fermentation takes place in a relatively short time, with the economic factor coming to the fore. Triticale is also of great agro-technical importance, since after the harvest during June-July, the soil remains unweeded, less trampled and has excellent physical and chemical properties (Glamočlija et al., 2017). The same authors state that triticale is an excellent precondition for the largest number of arable plants, primarily unrelated, and that it is an important member of crop rotation in the system of organic production due to the possibility of cultivation with alternative protection, enriching the soil with crop residues and increasing its fertility. In any case, better triticale performance, even in poorer growing conditions, gives it an advantage over other small grains, other alternative fodder and cover crops (Blum, 2014; Kavanagh and Hall, 2015).

Triticale can also be grown at altitudes up to 3000 m with a rainfall of 500-600 mm that is properly distributed during the growing season (Bezabih, et al. 2019). The results of many studies indicate the possibility of growing triticale on less fertile, shallow, degraded and acidic soils, also Dumbravă et al. 2016 underlined that triticale reacts more favorably with higher yields than those obtained with wheat and corn where intensive technologies are applied in limited soil and climatic conditions.

Compared to the parent species, this species can have higher yields, especially in areas characterized by less favorable agroecological conditions and poorer land, thus opening the possibility of its cultivation in a wider geographical area, on recultivated lands and in sustainable agricultural production systems

(Glamočlija et al., 2018). It is also important to point out that the level of agro-technical investments in production is lower than other real grains, which increases its importance, especially in areas of poorer agro-ecological and soil conditions, thanks to which Erekul and Kohn (2006) can be an alternative to bread wheat.

Fertilization with mineral fertilizers, with a special emphasis on nitrogen nutrition, is a key factor on grain yield as well as the ability of plants to fully realize the genetic potential for yield (Dumbravă et al. 2016). In intensive agricultural production, as Janušauskaitė (2013) points out, the amount of nitrogen fertilizers as well as the time of their application have the greatest impact on achieving high yields, which are accompanied by good grain quality. Experiments examining the impact, importance and optimal amounts of fertilizers to achieve optimal yields exist around the world and this indicates the importance and enduring relevance of this type of research.

### **Influence of cultivar on triticale yield**

For intensive agricultural production, in addition to ecological conditions and levels of applied agricultural techniques, varieties of high genetic potential for grain yield are of great importance, which should be accompanied by appropriate characteristics of technological quality. However, in addition to varieties with high genetic potential for yield, breeders pay special attention to the creation of varieties intended for different production conditions. Taking into account that different areas are characterized by different climatic and soil conditions, it is of special importance to create varieties of high biological plasticity and stability with pronounced adaptability to different growing conditions. Frequent variation of climatic factors by years in certain regions requires the simultaneous cultivation of a large number of varieties of different genetic origin, approximately the same genetic potential for yield, which differ in the most important agronomic traits (Mladenov et al., 2005).

Selection of the variety that best suits certain growing conditions with appropriate production technology according to Serafin et al. (2013) and Đurić et al. (2015) is of great importance in obtaining high yields. In addition to yield, the characteristics of the variety greatly affect the quality of grain, which is confirmed by the results of Kara (2009). Based on research conducted at Lakes region, Isparta in Turkey, this author pointed out that differences in grain yield found in varieties included in the study are the result of genetic differences between varieties that in certain meteorological conditions using certain agricultural techniques lead to the manifestation of these differences.

Bezabih et al. (2019) studied the productive traits of seven national and regionally recognized varieties in three localities in the marginal highlands of the Wag-Lata region (Ethiopia). They concluded that the biological and production characteristics of the variety are influenced by environmental conditions and applied agrotechnics. Significant variations in grain yield and quality are the consequences of different adaptability of the variety as well as the interaction of agroecological and agrotechnical factors. The obtained results are important for the correct choice of the variety which, through the achieved height and quality of yield, shows that it best suits the mentioned area.

Research by Đekić et al. (2010), Lalević et al. (2016, 2019) showed that the choice of variety, which best suits the conditions of a habitat and planned agricultural techniques, has a significant role in achieving stable production that allows high yields of grain and biomass of good quality.

Đurić et al. (2015) in comparative studies of two varieties of winter triticale: PKB Vožd and PKB Cardinal (Institute PKB Agroekonomik, Padinska Skela) with standard varieties: Favorit (Center for small grains, Kragujevac) and variety Odyssey (Institute of Field and Vegetable Crops, Novi Sad ) came to the conclusion that in the first year of testing the most productive variety was PKB Cardinal ( $9\,437.33\text{ kg ha}^{-1}$ ). The same variety had a significantly higher yield ( $\text{LSD} < 0.01$ ) compared to the yield recorded in other tested varieties. The lowest average yield was achieved by the variety Favorit ( $6\,379.67\text{ kg ha}^{-1}$ ). In the second year of testing, the cultivar PKB Cardinal and the cultivar Odyssey achieved a significantly higher value of grain yield ( $8\,400\text{ kg ha}^{-1}$ ) compared to the other two analyzed cultivars. Compared to the analyzed varieties, the variety PKB Cardinal reached the highest yield in the second year and it amounted to  $10\,475\text{ kg ha}^{-1}$ . Lalević et al. (2012), also point out that the cultivar Favorit proved to be a cultivar of significantly lower yield compared to the cultivar Kg-20, while Đekić et al. (2010) cite the Favorit variety as the most productive after the research in which the Kragujevac varieties Kg-20, Favorit and Triumph were used as research material. A couple of years later, the results of a study by Đekić et al. (2014a), as the most productive variety, they put in the foreground the variety Triumph, which with a yield of  $5.01\text{ t ha}^{-1}$  was ahead of the varieties Favorit and Kg-20. Based on the examination of four varieties of winter triticale during three years, Đekić et al. (2014b) state that all observed varieties have a high degree of adaptation to environmental conditions, which is manifested by a satisfactory yield, weight of 1000 grains and hectoliter weight, which recommends them for cultivation in agroecological conditions of Serbia.

## **Influence of soil on triticale yield**

Soil fertility, its physical, chemical and biological properties, reaction of soil solution are some of the properties that greatly affect the growth, development and fruiting of triticale as well as grain quality. All cereals achieve the best results on deep, fertile and loose soils where there is no need to perform any reclamation measures. Such soils are most types of chernozem, fertile groves, neutral reaction ponds, as well as alluvial soils if they are not endangered by groundwater and surface water during the winter period. The success of production on poor quality land depends on the system and intensity of previously applied reclamation repair measures. Thanks to the strong root system of extremely high suction power and genetic characteristics, triticale can be grown on lands that were not suitable for growing other real grains, such as marginal soils that can be acidic, saline, sandy, compacted, poor in trace elements (copper, manganese, zinc) or soils with high concentrations of toxic elements, such as boron and aluminum (Zhang et al. 1998). Studying 55 varieties of winter triticale across 61 locations and 13 spring triticale genotypes tested across 31 locations during 8 growing seasons (years 2009–2017), and Wójcik-Gront and Studnicki (2021) concluded that higher grain yield is achieved on better quality soils.

The same authors found that on lands of poorer quality in dry conditions, high yields can be achieved with the use of herbicides in quantities greater than  $3.5 \text{ kg ha}^{-1}$ , while the production of triticale on fertile lands achieves higher yields with less herbicides. Triticale has the ability to grow higher on podzolic, sandy, marshy, newly conquered lands, as well as on dried ponds and clearings, i.e. on all lands whose acidity is not less than 5.3, compared to any other field plant. However, if they are recultivated by applying modern agro-technical measures, high yields of biomass or grain will be achieved (Glamočlija et al., 2017). Repair of neglected, degraded soils is most often performed by applying calcification in combination with enhanced mineral nutrition and manure (Biberdžić et al., 2012). Pivić et al. (2008) previously add drainage, drainage, irrigation and enhanced mineral nutrition of plants. Therefore, with smaller or larger investments, triticale can be grown on almost any type of soil if it is provided with plant assimilates, if it has a deeper arable layer, moderately moist and neutral, i.e. weakly acidic or weakly alkaline reactions (Glamočlija, Đ., 2017 ). Numerous researchers state that triticale has the possibility of growing in perennial plantations and on sloping terrains in order to prevent torrential and aeolian erosion, then on all lands where recultivation is carried out, for example around mines with high levels of cadmium, zinc, lead, aluminum and other harmful metals (Baier et al. 1998; Butnaru et al., 1998). A common problem in the cultivation of cereals is the lack



of zinc, which is especially pronounced on barren and calcareous soil. Studies have shown that triticale is relatively resistant to zinc deficiency, while resistance to copper deficiency mainly depends on the pH value of the soil, but also on the genotype itself (Blum, 2014).

### **Influence of fertilization on triticale yield**

Grain yield per unit area is one of the most important factors influencing the profitability and economy of production. In addition to the genotype, the yield of triticale grains is greatly influenced by fertilization, which should be harmonized with the characteristics of the soil and climate, but also with the requirements of the variety. According to (Zečević et al., 2010; Bielski, 2015), fertilization, and especially nitrogen nutrition, is one of the most important agrotechnical measures that affects grain yield and ensures the realization of high genetic potential for yield, while Nogalska et al. (2012) points out that fertilization is a crucial factor and indicator of the efficiency of agricultural production. Jaśkiewicz (2011) states that only by knowing and meeting the agrotechnical requirements of a particular variety it is possible to achieve yields that are close in height to its maximum genetic potential for yield. Experiments in which the influence of the quantity and type of fertilizers on the height and quality of yield are examined are current everywhere in the world, all with the aim of determining the optimal quantities of fertilizers that ensure optimal production. As Ivanova and Tsenov (2014 a, b) state, the decisive factors that determine the yield in new triticale varieties are meteorological conditions during vegetation and fertilization. The use of nitrogen in the diet without a clear diagnosis and precise determination of the amount can lead to the opposite effects than expected. Unnecessary increase in leaf area, caused by excess nitrogen, increases water consumption, reduces the resistance of plants to biotic and abiotic stresses, as well as resistance to lodging, etc. (Bogdanović et al., 2005; Glamočlija et al., 2015). Weber et al. (2008), Pecio (2010) and Wang et al. (2017) point out that the yield components, in addition to the dose of nitrogen used, are greatly influenced by the time of its application. Nitrogen fertilization of plants at the right time directly affects both the yield and the protein content in the grain (Kara and Uysal, 2009; Lestingi et al., 2010). Lalević et al. (2019) investigated the impact of increasing nitrogen doses (60-90 kg ha<sup>-1</sup>) on grain productivity and quality in five winter triticale cultivars (Odyssey, Kg-20, Triumph, Rtanj and Tango) and found that most cultivars are the largest grain yield was achieved on the variant that included the use of the largest amount of nitrogen with the exception of the variety Tango in the first and the variety Kg-20 in the second year of research which achieved the highest yields on the variant of fertilization in which 60 kg ha<sup>-1</sup> nitrogen was used in combination with 80 kg ha<sup>-1</sup> of phosphorus and potassium.



However, these differences were not statistically significant. Similar results were obtained by Wojtkowiak et al. (2013) and Bielski and Falkowski (2017), noting that grain yield varied depending on the dose of nitrogen applied, but also that the differences observed were not statistically significant. According to Lestingi et al. (2010) application of nitrogen in the amount of 50 kg ha<sup>-1</sup> compared to 100 kg ha<sup>-1</sup> is a good compromise because a relatively small amount of nitrogen contributes to the yield of grain, protein and other quality parameters, and is also environmentally friendly. What distinguishes triticale as a cereal compared to others, is the high content of protein in the grain, which is directly conditioned by fertilization with mineral fertilizers. Tosheva and Stoeva (1996) point out that the protein content of grain increases with the increasing amount of nitrogen used in fertilization, while the results presented by Kirchev and Popov (2010) show that triticale grain contains the most amino acids when using nitrogen in the amount of 120 kg ha<sup>-1</sup>. On the other hand, Lalević et al. (2019) indicate a decrease in grain protein content with an increase in the amount of nitrogen applied in fertilization. The same authors state that the increasing amount of nitrogen caused an increase in yield, and at the expense of reducing the concentration of nitrogen in the grain and thus reducing the protein content. There was a moderately strong negative correlation between grain yield and protein content in grain ( $r = -0.65$ ).

### **Influence of weather conditions on triticale yield**

Weather conditions during the vegetation are one of the most important factors determining the amount of yield (Biberdžić et al. 2012). Alaru et al. (2009) also, when we talk about the impact on the yield, put meteorological conditions in the foreground, ahead of the variety and nitrogen fertilization. Research by Lalević et al. (2016, 2019) showed the importance of meteorological conditions and their impact on the yield of winter triticale, emphasizing the importance of autumn precipitation (October, November) for timely germination, germination and initial growth of plants. The same authors note that moderate air temperatures are also very important in the period of fertilization, grain formation and maturation in order for these phases to take place gradually. The results obtained by Nožinić et al. (2009). During the ten-year research (2001-2010) in the area of Banja Luka, the mentioned authors noticed that in the mentioned period, the vegetation season 2002/03 was the most unfavorable for small grains. They state that according to the RS Statistical Office, the average yield of wheat was only 2.2 t ha<sup>-1</sup>, rye and oats 1.7 t ha<sup>-1</sup>, barley 2.0 t ha<sup>-1</sup>, while triticale with an average yield of 3.5 t ha<sup>-1</sup> was significantly ahead of other cereals. The results of micro-experiments conducted by these researchers show that all varieties of triticale with an average yield of 7.57 t ha<sup>-1</sup> withstood drought and "tropical temperatures" in

the generative phase of development. Similar observations regarding the possession of pronounced drought resistance in the study of a large number of lines and varieties of triticale were presented by Milovanović et al. (2006) and Mandić et al. (2009) which classifies triticale as a highly tolerant species under adverse weather conditions. However, it should be born in mind that in years with extremely high air temperatures in the mountain area, higher grain yields can be achieved compared to grain yields achieved in the plains because weather conditions affect triticale grain yields differently in each locality (Nožinić et al., 2009). This increases the importance of setting up experiments on several localities, in order to find the optimal varietal agrotechnics in different agroecological conditions. Also, the current problem of climate change indicates the importance of researching the stability of important properties of triticale in comparative experiments with other winter cereals.

## Conclusions

If we take into account all the advantages and disadvantages of triticale, the climate change we are facing, the expansion of agricultural production to marginal areas, triticale has yet to gain in importance as a cereal at the global level. Despite the fact that until now, as a grain that due to its nutritional properties and ability to produce significant biomass is considered mainly as animal feed, breeding that would go in the direction of improving the technological quality of grain for human consumption could contribute to its popularization for use in these purposes. Because it shows a high degree of adaptability to a large number of biotic and abiotic stresses, triticale has the potential to take a significant place in solving a global problem such as food production in difficult conditions.

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## **SURVIVAL OF YERSINIA PSEUDOTUBERCULOSIS IN SOIL**

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### **ABSTRACT**

*The dynamics of the pseudotuberculous microbes population number in the soil was monitored with the use of bacteriological method. The number of this microbe increased during the first week to  $10^6$ - $5 \times 10^6$  CFU/ml, after which it stabilized until the third week at level  $10^6$ , after which there is a continuous decline in the number of *Yersinia pseudotuberculosis* until the end of the second month, when their growth stops.*

**Key words:** *microbe, survival, soil*

### **INTRODUCTION**

The pseudotuberculous microbe belongs to the group of sapronose agents that are characterized by the ability to live in the external environment outside of any connection with the organism of warm-blooded animals and humans, because they are random parasites of these organisms (Đukić et al., 2011). The specific forms and ways of bacterial populations survival in soil or water have not been sufficiently studied, although Willcocks et al. (2018) stated that *Yersinia pseudotuberculosis* is well adapted to survival in the soil. Accordingly, Santos-Montañez et al. (2015) reported the ability of *Yersinia pseudotuberculosis*

persistence in soil and water and in association with fresh produce, but the mechanism by which it persists is unknown. They also quoted that it has been shown that *Yersinia pseudotuberculosis* co-occurs with protozoans in these environments. However, it is known that some of them can be maintained in the external environment in special forms that do not grow on the usual nutrient media.

Under the influence of a large number of factors, these pseudotuberculous microbes regain the ability to actively grow on nutrient substrates. Such forms, called “uncultivated ” (Đukić et al., 2007; 2015; 2020), are known today in legionella, vibrio cholera, salmonella and a number of other microorganisms (Đukić et al., 2011; Vesković and Đukić, 2017 ). The possibility of bacteria becoming uncultivated significantly complicates the study of their ecology in the saprophytic phase, especially the assessment of population dynamics using traditional microbiological methods.

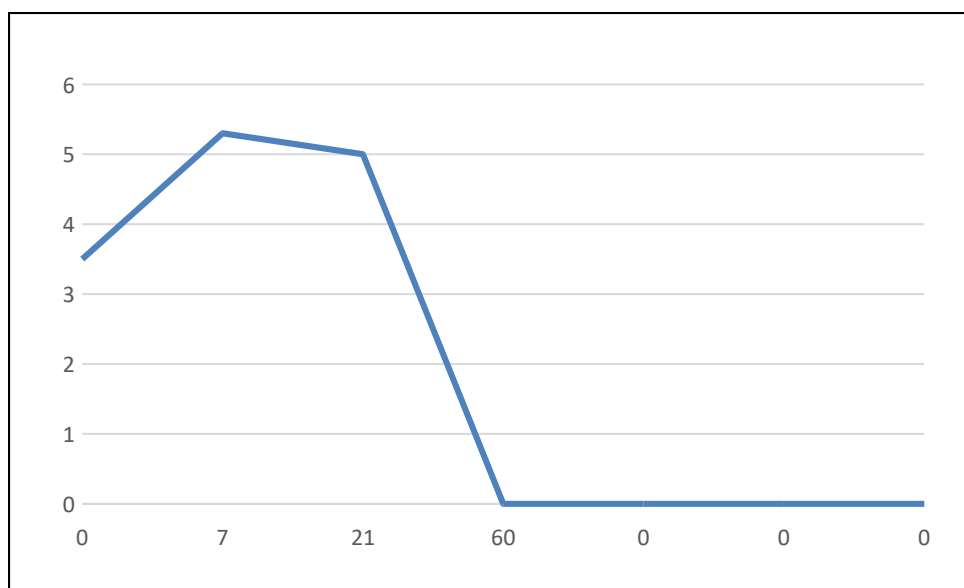
## MATERIALS AND METHODS

Pseudotuberculosis microbe (*Yersinia pseudotuberculosis*) from the collection of the Laboratory for Microbiology, Institute of Public Health in Čačak, was used in this paper. *Yersinia* colonies were grown on endo-medium. LB-broth and sterile aqueous soil extract (1.2 atm. for 40 minutes) were used as liquid culture media. Cultivation of *Yersinia* in soil extract (initial *Yersinia* concentration- $10^4$ /ml) was performed at room temperature (18-20°C). The number of *Yersinia* during long-term (2 months) presence in the soil was estimated on the basis of CFU.

## RESULTS AND DISCUSSION

During two-month research, it was determined that the number of *Yersinia* increased during the first week to  $10^6 - 5 \times 10^6$  CFU/ml, after which it stabilized at the level of  $10^6$  CFU/ml by the third week. After that period, the number of *Yersinia*, that give colonies on agar, decreased until the end of the second month, when the growth of *Yersinia* stopped (Graph 1). However, it is known that during a longer stay in the external environment there is an increase in the number of uncultivated forms of *Yersinia* at the expense of the cultivated part of the population (Đukić et al., 2009; Mandić et al., 2010). It is not excluded that because of that, *Yersinia* are rarely detected in a low percentage by bacteriological method in soil and other substrates of the external environment. In order to get a

true idea of the presence of yersinia in the environment, it is necessary to determine the quantitative assessment of uncultivated forms of yersinia, using PCR and other methods.



Graph 1. Dynamics of *Yersinia pseudotuberculosis* abundance in sterile soil extract, lg CFU/ml

Further research should clarify not only this, but also many other issues, which are related to the new form of adaptive variability of microorganisms in the environment, with the mechanisms of their transition to uncultivated state, as well as environmental and molecular genetic factors affecting that process.

## CONCLUSION

Based on the obtained results, it can be concluded that the number of *Yersinia pseudotuberculosis* increases until the end of the first week, then stabilizes by the end of the third week, and then follows a continuous decline until the end of the third month. In order to get a more realistic idea of the yersinia presence in the soil, it is necessary, with the use of PCR method (or some other), to assess the qualitative presence of uncultivating forms of these microorganisms.



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## THE IMPORTANCE OF FORAGE LEGUMES FOR ANIMAL FEED PRODUCTION

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### **Abstract**

*Legumes play a significant role in conventional agricultural production systems, especially in sustainable systems. They are grown in the world in large areas as arable crops, whether they are annual or perennial species. In the Republic of Serbia, forage legumes are grown on about 170,000 ha as pure crops, often as combined crops with perennial or annual grasses, other legumes, or plants from other groups. In addition, forage legumes are a component of natural, semi-natural and sown swards (long-term or short-term). In natural associations, the share of legumes depends mainly on the agro-ecological conditions of the area, the way of exploitation, and the applied agro-techniques. The most important advantage of growing forage legumes is the production of quality protein-rich food for domestic animals, as well as food for humans in an indirect way. The high nutritional value, digestibility as well as palatability of legume forage, make leguminous plants indispensable in fodder production systems. Thanks to their good adaptation to different edaphic and climatic conditions, as well as cultivation methods, there are many other advantages of growing forage legumes such as soil protection from erosion, green manuring, mulching, interspersed sowing in orchards and vineyards, as honey plants, beautification of the environment and for medical purposes.*

*In order to develop a system of sustainable agriculture, the increase of areas under forage leguminous plants, as well as their end products, should be supported by agricultural policy measures. In the first step, it is necessary to improve the technical support for their cultivation and spreading, in order to be comparable with the technical solutions in the cultivation of leading cultivated*

*plants. Then it is necessary to develop measures such as subsidies, premiums, or any other type of incentive, at least for the production of the most important legumes. This would ensure the production of quality food for animals and humans, as well as the accompanying positive effects of legumes on the entire ecosystem.*

**Key words:** *forage, legumes, sustainable agriculture systems*

## Introduction

Leguminous plants play a very important role in the agricultural production of every country. The most important advantages of growing forage legumes come from their basic purpose - the production of quality food for domestic animals, as well as food for humans in an indirect way.

Forage legumes are mainly represented as a component of natural, semi-natural and sown grasslands (long-term or short-term), or on arable land on which crops of perennial or annual legumes are purposely based as pure crops, or in a mixture with grasses or cereals. Regardless of the method of utilization, grass forage is realized mainly through meat and milk as the end product of ruminants. Extensive fodder production systems, which largely depend on natural, semi-natural and sown grasslands, as well as the production of basic grain nutrients in arable land, are exposed to overexploitation, and thus the risk of degradation and erosion of grasslands, lack of protein nutrients, especially from arable land, as well as the reduced, temporally and spatially unevenly distributed amount of precipitation, which is projected as a consequence of climate change (FAO 2010).

Increasing the production of legumes on farm areas has recently become more attractive, because it contributes to sustainable agricultural development, through less dependence of agriculture on fossil fuels, lower greenhouse gas emissions, increasing the diversity of cultivated plants, increasing biodiversity in soil and environment, increasing soil fertility, as well as the establishment of new cultivated plant-animal relationships (Bokan et al. 2016). The advantages of growing leguminous plants are largely the result of their most important and unique feature - the ability to fix atmospheric nitrogen through symbiosis with root bacteria, thus satisfying most of their needs for this nutrient and providing it to other organisms - soil microorganisms, non-leguminous plants in combined crops or cultivated plants that come after them.

Consumption of animal products worldwide is constantly increasing (Lassaletta et al. 2014). In Europe, meat consumption has quadrupled in the last 50 years, with pork consumption increasing by 80% over the same period (Westhoek et al.

2011). Consumption of meat and other products from monogastric animals has led to a change in the sowing structure, ie the way of using agricultural land (Pelletier and Tyedmers 2010). The breeding of monogastric animals has experienced expansion due to the favorable prices of the components of concentrated fodder. The intensification of agriculture, on the other hand, has led to the increasing use of stables instead of grazing, which in itself has led to greater use of concentrated nutrients (Vicenti et al. 2009). Technical solutions, especially in the preparation of concentrated food, as well as in indoor breeding systems, have led to increased concentrations of certain species of domestic animals in some regions, especially pigs and poultry, which inevitably leads to greater pollution of agroecosystems and the environment as a whole. Increased concentrations, especially of nitrogen, but also of phosphorus compounds, make agricultural areas less suitable for growing legumes. At the expense of reducing the area under legumes, as a rule, the area under cereals and somewhat oil plants is increasing, and in that way production systems are becoming more and more homogenous. Forage legumes are characterized by high variability in feed/grain yield potential, as well as a variety of uses. One forage legume can be grown for very different purposes, such as protection of soil from erosion, green manure, mulching, sowing in rows in orchards and vineyards, as a honey plant, beautification of the environment and medical purposes. This indicates their good adaptation to different edaphic and climatic conditions, as well as cultivation methods (Sánchez-Díaz 2001).

### **Forage production systems for leguminous plants**

Legumes can be grown for various purposes, such as grain, green forage, hay, silage, haylage (from arable land or swards), or green manure, where the choice of production system depends on climatic and edaphic conditions, as well as the purpose of the final product. Forage can be produced on permanent grasslands (natural or semi-natural), temporary (sown), which are based on arable land and used for two to several years, and on arable land on which crops of perennial forage legumes, such as alfalfa, red clover, white clover or birdsfoot trefoil are based. Forage legumes are grown on about 170,000 ha in the Republic of Serbia as pure crops ("Statistical Yearbook of the Republic of Serbia" 2020), often as combined crops with perennial or annual grasses, other legumes, or plants from other groups. Such a production system is present on sown grasslands, while their share in natural associations depends mainly on agro-ecological conditions of the area, methods of exploitation and applied agricultural techniques (Tomić et al. 2018a; Zornić et al. 2019; Stevović et al. 2019).

In terms of forage yield, agronomic quality, especially forage quality, grass and legumes mixtures have significant agronomic advantages, compared to pure grasses, sometimes with some of the leading silage plants (Peyraud et al. 2009). Several shortcomings of such systems include poor growth at the beginning of the growing season (Peyraud et al. 2009), reduced grazing durability compared to pure grasses, the risk of bloat, and some difficulty in ensiling or haymaking (Phelan et al. 2015). Grass-legume mixtures are quite common in semi-intensive production systems, mainly due to the reduced need for inorganic nitrogen (e.g., sustainable agriculture, organic grasslands). Another significant disadvantage of these systems is the reduction of the share of leguminous components in increasing the amount of inorganic nitrogen, intensive grazing, or their combination when complete elimination of legumes can occur (Stevović et al. 2011; Tomić et al. 2018b).

Legumes for grain production are grown almost without exception as pure crops, although today there is an increased interest in growing them as combined crops (Malézieux et al. 2009). Combined crops of legumes and cereals on arable land as annual crops can be established for the production of grain or silage (haylage), while the second method of cultivation is far more common, with the main purpose of producing feed with higher protein content (Anil et al. 1998). Under favorable agro-ecological conditions, such combined crops have higher and more stable forage production (sometimes protein yield) compared to pure crops of their components (Bedoussac et al. 2015).

The advantages of production systems that include legumes are numerous, but the most important are: the reduction or absence of synthetic nitrogen, increasing the value of agroecosystems by providing nitrogen to the next crop, improving soil structure, and increasing soil biodiversity and ecosystems as a whole.

### **Significance of perennial legumes in security fodder from the lawn**

The high nutritional value, digestibility as well as palatability (tastefulness) of legume forage, make it indispensable in animal feed production systems. In addition, there is an increased interest in secondary dietary characteristics of their feed, especially those that have a beneficial effect on the health of domestic animals (Mortimer et al. 2006). However, the significant contribution of leguminous plants to the diet of domestic animals is reflected in the possibilities of utilization of forage nutrients, as well as certain characteristics of the production system, especially perennial mixtures of forage legumes and grasses. Namely, in such mixtures, the share of leguminous plants in the total forage varies, both between and during the same growing season, which is reflected in

the nutritional value of the forage. In addition, certain types of legumes do not tolerate some of the methods of exploitation, especially grazing, so their share in the forage of pastures during the season can be significantly reduced.

Although forage legumes contain high levels of protein, a significant portion of them is lost during digestion. Production systems containing legumes are also characterized by significant nitrogen losses due to leaching into deeper layers of the soil, as well as through the excrement of grazing animals, especially urine. Despite these limitations, forage legumes, as high-protein plants, form the basis of sustainable forage production systems (Frame et al. 1998). In support of this is the fact that protein production can be increased by using larger amounts of synthetically produced nitrogen fertilizers, which would increase the production of grass components, as well as protein content in their feed, but it is economically less profitable and unacceptable from an environmental point of view (Wilkins and Jones 2000). The very widespread way of producing fodder in the past period, which included the use of large quantities of synthetic fertilizers, a larger share of conserved forage, reduced grazing, and a stable way of farming, should be gradually changed by using grassland grazing, wherewith such a system of exploitation, particularly with greater participation of leguminous plants, the interaction between domestic animals, pastures, and the environment in the broadest sense, would have great influence on productivity, animal health status, as well as the sustainability of the ecosystem as a whole (Rochon et al. 2004).

As a confirmation of the irreplaceable role of leguminous plants in providing quality forage, regardless of the method of utilization, is in used the model of analysis of productivity and profitability of the system of the utilization of grasses and leguminous plants as silage (Topp and Doyle 2004). Based on the model, these authors predicted that, for example, red clover, either as a pure crop or in a mixture with grasses, would far surpass all silage systems of pure grasses exploitation, although the amount of nitrogen fertilizers applied in the latter was 400 kg ha<sup>-1</sup> N per year. According to the predictions of Topp and Doyle (2004), alfalfa and white clover in this system of exploitation would also surpass the systems of pure grass fertilized with 200 kg ha<sup>-1</sup> N per year. In contrast, systems that included the birdsfoot trefoil and the galega (*Galega orientalis* Lam.) Were economically and productively less valuable. However, Collins et al. (2006), note that there are large gaps in current knowledge about the ecophysiology of birdsfoot trefoil and galega, which may affect this assessment of their value.

In the pasture systems of grassland exploitation, in the area of Europe with a temperate continental and continental climate, the irreplaceable legume is the white clover, which, as a species, is morphologically adapted to this way of exploitation. Types of white clover that form a larger number of stolons are more resistant to trampling, they usually have a smaller leaf, because these

characteristics are negatively correlated. For lawns that are used only by mowing or in combination (mowing - grazing), the types of white clover of medium or larger leaves, which form fewer stolons, are more acceptable. The share of white clover in systems used by mowing or combined, as well as grazing, provided that the paddocks must be organized, leaving enough time for regeneration, can be maintained for many years at a satisfactory level, or about 30% (Williams et al. 2000; Williams et al. 2003).

In contrast to white clover, alfalfa and red clover are not particularly suitable for grazing, due to slow growth and poor regenerative capacity of damaged parts of the plant (Rochon et al. 2004). Therefore, selection, especially in alfalfa, is aimed in part at creating grazing varieties (Smith et al. 2000; Pecetti et al. 2006; Humphries et al. 2006). In accordance with this goal, forms with a crown that is formed deeper and has a laid type of growth have already been selected (Pecetti et al. 2006). In order to improve the persistence of red clover on pastures, selection in this species is focused on the choice of germplasm, which is characterized by the formation of more adventitious roots because, in addition to greater regenerative ability, such forms are characterized by greater resistance to root rot (*Sclerotinia trifoliorum*), a disease that is the cause of its poor durability (Christie and Martin 1999).

Birdsfoot trefoil, as a species that is adapted to our agro-ecological conditions, is not characterized by good regenerative ability, whether it is used for grazing or mowing. Types of upright growth show particularly poor regenerative capacity after grazing (Collins et al. 2006). The results of experiments with prostrate growth-type cultivars indicated their better regenerative ability (Marley et al. 2006). Compared to other legumes, birdsfoot trefoil has several positive agronomic and nutritional characteristics, such as greater tolerance to lack of plant nutrients, especially phosphorus, and lack of water in the soil (Hopkins et al. 1996). Their most important nutritional property is the higher content of condensed tannins in all parts of the plant compared to other legumes, as well as high digestibility. Although the birdsfoot trefoil is characterized by insufficient persistence and poor competitiveness, this species could be included in grass-legume mixtures on soils less suitable for growing other legumes, even white clover (Collins et al. 2006).

## Conclusion

The advantages of growing legumes have long been known, i.e. their impact on the improvement of agroecosystems, as well as the entire environment. The introduction of legumes in production systems would limit the increasingly

pronounced degradation of soil functions necessary for the unobstructed growth and development of plants, as well as the deterioration of environmental quality in the broadest sense, and thus improve sustainable agriculture systems. When analyzing the economics of the production of legumes on the farm, the currently invisible advantages of growing legumes are often not taken into account. With the leading types of grain, the potential for yield is constantly increasing, however, their yields are stagnating or even declining in real terms. The reason for that, in addition to the possible consequences of climate change, is largely related to the increasingly pronounced problems caused by diseases and weeds in their crops, precisely because of the lack of legumes from such, often monotonous production systems. The benefits of introducing legumes into systems that include only the leading profitable cultivated plants are becoming more and more apparent. This approach presupposes increased investments in breeding, as well as in the improvement of the technology for growing leguminous plants.

The prices of raw materials, especially the prices of fertilizers on which intensive production systems are based, will certainly continue the growth trend, which will inevitably be reflected in the increase in the price of food obtained from leading cultivated plants. In the market of basic grain cultivated plants, the price of soybeans has recently been growing faster than the price of other species, so in the near future the cultivation of this plant will certainly be more economically viable than most other agricultural plants. Observed through the basic ingredients of food, the price of plant proteins is growing faster than the price of carbohydrates and oils. Leguminous plants, which contain relatively more carbohydrates in grain than proteins (beans, broad beans), will become more and more competitive with cereals. In the diet of domestic animals, legumes not only complete the grain as a basic nutrient, but complementarity also occurs between certain types of legumes. In this way, in addition to the amount of protein, the food chain is supplemented in terms of its quality.

The increase of areas under leguminous plants, as well as their end products, should be supported by agricultural policy measures. In the first step, it is necessary to improve the technical support in the cultivation of legumes, in order to be comparable with the technical solutions in the cultivation of leading cultivated plants. Direct measures, such as subsidies, premiums, or any other type of incentive, at least for the production of the most important legumes, would ensure the production of quality food for animals and humans, as well as the accompanying positive effects of legumes on the entire ecosystem. In the production of animal feed, incentives in the field of livestock production are certainly a necessary precondition for the implementation of agricultural policy measures, directly aimed at growing legumes.



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## INFLUENCE OF LEAF WRINKLE ON VITAMIN C CONTENT IN LETTUCE

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**ABSTRACT:** *In this paper, the influence of leaf wrinkle on the qualitative, quantitative and sensory characteristics of lettuce. The study was on two types of head lettuce: "Crisped" and "Buterhead Type". The analyzes were performed in the laboratory of the Faculty of Agriculture, University of East Sarajevo during the 2020 year. "Butterhead Type" head lettuce had a statistically significantly higher head weight (399,30g) compared to "Crisphead" head lettuce (259,02g). Statistically significantly higher content of vitamin C was in "Crisphead" head lettuce (43,56 mg/100g) compared to "Butterhead Type" (22,97mg/100g). Eating habits for "Crisped" head lettuce were not expressive.*

*Key words: salad, leaf consistency, vitamin C, consumer habits.*

### INTRODUCTION

Today, over 200 cultivated varieties of lettuce are grown in the world, and in our areas is dominated by lettuce, with its two cultivars "Crisped" and "Buterhead Type". It was estimated that the total area harvested, globally, was more than 1.27 million hectares in 2018, with a total production of approximately 27.3 million tonnes (Faostat, 2018).

Lettuce is consumed as a fresh vegetable, mostly as a salad or as a minimally processed vegetable. Lettuce is the most popular vegetable according to the highest consumption rate and economic importance throughout the world (Coelho et al., 2005). Lettuce is consumed as a fresh vegetable, most often as a salad or as a minimally processed vegetable. It is therefore important in the human diet due to its content of vitamins C, B1, B2, minerals and antioxidants. The advantage of lettuce, eaten raw, is that it maintains more nutrients than thermally processed food (Aćamović Đoković et al., 2011). Vitamin C is the most important compound that contributes to nutritional quality and has numerous biological activities in the human body and it is the primary source of dietary antioxidants (S.K.Lee and A.A.Kader, 2000).

Vitamin C strengthens the immune system, has a positive effect on digestion and stimulates appetite. It has a stimulating effect on liver function and helps with gout Juríková et al. (2012). According to research by Šlosár et al. (2008) vitamin C may be anticancer and reduce the risk of cardiovascular disease.

Starting from the knowledge that lettuce is of special importance in human nutrition because of high biological value and taking into account that the yield and chemical composition of lettuce vary depending on variety and environment, the aim of this study was to determine the influence of lettuce cultivars on quantitative and qualitative characteristics.

## **MATERIALS AND METHODS**

The tests were performed in the laboratory of the Faculty of Agriculture in East Sarajevo in 2022. The study was on two types of lettuce: "Crisped" and "Buterhead Type". "Crisped" type of lettuce it has a larger rosette of wrinkled leaves, the edges are serrated, the leaf nerve is pronounced. The leaves of the "Crisped" type are very fragile, dark green, medium green and yellow-green. The leaves of the "Buterhead Type" are leaves softness and nonhairy, delicate consistency.

From the morphometric characteristics we analyzed: head mass (g) and number of leaves in the head.

The mass of the heads was determined on a technical scale with a reading to two decimal places.

From the chemical characteristics, we analyzed the content of vitamin C.

Contents vit. C was determined by titration using iodide solution (iodometric method).

Sensory quality of lettuce was determined on the basis of regulations ISO / TS 34, Agricultural and food products, Sensory analysis. Data on sensory properties were obtained by testing 30 participants. From the sensory characteristics, we analyzed: gustation, leaf consistency, bitterness, central nerve expression and salad color.

The obtained results were processed by the method of analysis of variance of monofactorial examination (ANOVA), the significance of differences in individual environments was tested by LSD test.

## RESULTS AND DISCUSSION

The average weight of the head shows differences depending on the type. Analysis of variance showed that the mass of the lettuce "Buterhead Type" was statistically significantly higher compared to the type of "Crisped".

Table 1. Average weight of lettuce head (g)

|                                         |      |               |
|-----------------------------------------|------|---------------|
| <b>"Buterhead Type" of head lettuce</b> |      | <b>399,30</b> |
| <b>"Crisped" type of head lettuce</b>   |      | 259,02        |
| <b>LSD</b>                              | 0,05 | 79,63         |
|                                         | 0,01 | 183,71        |

The results obtained in our experiment are in accordance with the data of Govedarica-Lučić (2012), who cultivated three varieties of lettuce in her experiment. The tests were performed on the varieties "Archimed" and "Santoro", which belong to the "Buterhead type", and the third tested variety, "Kibou", belongs to the type of "Crisped". Throughout all three years of testing, it is continuously confirmed that the "Santoro" variety had the largest head.

Number of leaves in the rosette

The results on the influence of lettuce type on the average number of rosette leaves indicate a uniform number of formed leaves (Table 2). There was no statistically justified difference between the examined types

Table 2. Number of leaves in the rosette

|                                         |      |             |
|-----------------------------------------|------|-------------|
| <b>"Buterhead Type" of head lettuce</b> |      | <b>37,2</b> |
| <b>"Crisped" type of head lettuce</b>   |      | 29,86       |
| <b>LSD</b>                              | 0,05 | 12,21       |
|                                         | 0,01 | 28,17       |

### Vitamin C content

The results of the analysis of variance indicate that head lettuce in the "Crisped" type has a statistically significantly higher content of vitamin C (43.56 mg/100g) compared to the "Buterhead Type" (22.97 mg/100g).

Table 3. Vitamin C content (mg/100g)

|                                         |      |              |
|-----------------------------------------|------|--------------|
| <b>"Buterhead Type" of head lettuce</b> |      | <b>22,97</b> |
| <b>"Crisped" type of head lettuce</b>   |      | <b>43,56</b> |
| <b>LSD</b>                              | 0,05 | 11           |
|                                         | 0,01 | 25,39        |

Similar results are found in the paper (Goveradica-Lučić, 2012). According to their research, the lowest level of vitamin C has the variety "Santoro" which belongs to the head lettuce in the "Buterhead type" (22.73 mg/100g), while the tested variety "Kibou" which belongs to the head lettuce in the type of "Crisped" achieved the highest content of vitamin C (25.71 mg / 100g). The differences were statistically justified. Also, the tests of Lešić et al. (2002) confirm that during the growth of "Buterhead Type" salad there is an increase in the amount of reduced sugars, and a decrease in the amount of nitrate and vitamin C.

### Sensory quality of lettuce

Unique influence and formed judgment (sensory analyst), ie. subjective opinion (consumer-consumer), which are obtained during sensory analysis, are very important parameters for the assessed quality, ie. determining food safety (Popov-Ralić and Stojšin, 2007).

The 30 students participated in poll. The quality of the examined types of salad was evaluated using different visual techniques and taste. In the study, we wanted to get an answer which of the two examined types of salad ("Buterhead Type", "Crisped") as a more pronounced, more pleasant taste.

According to most of the evaluated parameters, the participants liked the "Buterhead Type" lettuce the most, where 90% of the respondents said that it very pleasant taste. Regarding the consistency of the leaves, 50% of the respondents said that the consistency of the leaves was soft and 50% crunchy.



For the expression of leaf bitterness, 60% of the respondents said that it was not expressed and 40% that the bitterness was weak.

However, when it word of "Crisped" type of lettuce we can state that 80% of the respondents said that it tasted bitter and only 20% said that it tasted pleasant. In terms of leaf consistency, 80% of respondents said that crunchiness was present. No significant differences were found in the expression of the central nerve and the color. According to these results, we can say that the "Crisped" type of lettuce proved to be less pleasant to consume.

## CONCLUSION

Based on the results of research on the influence of leaf creases on the content of vit. S, as well as measuring morphometric properties and testing the sensory properties of head lettuce in type "Crisped" and "Buterhead Type" we conclude:

- The weight of the head of "Buterhead Type" is statistically significantly higher compared to the type of "Crisped",
- Differences in the number of rosette leaves were not statistically justified,
- Head lettuce of the type "Crisped" has a statistically higher content of vitamin C compared to "Buterhead Type",
- Wrinkled leaves have a positive effect on the content of vitamin C,
- Sensory quality of "Buterhead Type" shows the best characteristics and occupies a significant place among consumers, while consumer habits in the consumption of head lettuce in the type "Crisped" were not manifested.

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## THE MYCOPOPULATION OF RADISH SEEDS

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### Summary

*Seeds are an important source of primary inoculum for the development of many diseases. Due to the very short vegetation period, controlling the causative agent of the disease on the radish is very difficult. Considering this fact, it is necessary to provide sufficient quantities of seeds of adequate quality and health. The aim of this study was to determine the mycopopulation of radish seeds during different years of production. Seeds used for testing were collected during the period between 2017-2021 from different varieties and localities. After conducting the study, the infection index ranged from 1 to 7%. Fungi of the following genus are represented on the seed in different intensities: Alternaria spp., Fusarium spp., Penicillium spp., Rhizopus spp. and the species Aspergillus niger.*

**Keywords:** radish, seeds, fungi, mycopopulation

### Introduction

The radish (*Raphanus* spp.L.) is an annual vegetable from the Cruciferae family that has been used as food all over the world. Radishes are grown on all continents of the world. The first records of radish cultivation were inscriptions on the walls of the pyramids dating back to 2000 BC, but Herodotus (c. 484–424 BC) believed that the radish was grown as the main plant species in Egypt some 5,000 years ago (Becker, 1962) and was cultivated around the 13th century BC (Banga, 1976). In ancient times, it was assumed that the radish was grown as an oil plant (Curtis, 2008). This plant species was cultivated in Europe during the 15th or 16th century and was introduced to America during the 19th century. Many varieties and hybrids were developed in Asia, according to data it has been grown in China for 2450 years and in Japan for 1300 years (Kitamura 1958 loc. to Kaneko et al., 2007). In the diet, the thickened root is used as a fresh, fermented and dried product. Radishes can potentially be used to treat various diseases. It contains alkaloids and various compounds that act as calmodulin antagonists, growth inhibitors, antihypertensive agents and inhibitors of platelet aggregation (Shin et

al., 2015). Radishes are categorized into two groups based on root size: a group with a small root of short vegetation (one month) and a group with a large root of longer vegetation (three months) (Curtis, 2008).

The seed is a suitable substrate for the development of microorganisms that cause diseases, especially phytopathogenic fungi (Milošević et al., 2008). According to Petrović et al. (2010) due to the presence of pathogens, the seeds most often display a decrease in energy and germination, and even complete decay of the seedlings. The importance of pathogens and types of pathogens on radish seeds are cited by many authors. Noble and Richardson (1968) noted several fungal pathogens of edible radish seeds (*Raphanus sativus* L.). The aforementioned authors cite several species of *Alternaria* spp. and other fungi commonly found on the seeds of Cruciferous vegetable species. McLean (1947) and Noble and Richardson (1968) reported that seeds treated with 50°C warm water for 10 to 40 minutes successfully controlled *Alternaria raphani* from seeds.

The aim of the study was to determine the mycopopulation of radish seeds and the level of infection of seeds originating from different years, in order to determine the health status of the tested samples.

### Material and methods

Seeds for analysis were collected in the phase of physiological maturity of the plant during the period 2017-2021. A total of 13 samples were collected from different localities (Tab. 1). Each sample consisted of 3 subsamples weighing 2 g. After the seeds were delivered to the laboratory, analysis was performed.

Tab.1: Seed sample data

| Number | Code    | Variety      | Year of sampling | Locality   |
|--------|---------|--------------|------------------|------------|
| 1.     | R1/2017 | Verica       | 2017             | R. šančevi |
| 2.     | R2/2017 | Saxa Treib   | 2017             | Kucura     |
| 3.     | R3/2017 | Ledena sveća | 2017             | Kovilj     |
| 4.     | R4/2018 | Verica       | 2018             | Kucura     |
| 5.     | R5/2018 | Saxa Treib   | 2018             | Kucura     |
| 6.     | R6/2018 | Crna zimska  | 2018             | Kulpin     |
| 7.     | R7/2019 | Saxa Treib   | 2019             | Žabalj     |

|     |         |              |      |            |
|-----|---------|--------------|------|------------|
| 8.  | R8/2020 | Ledena sveća | 2020 | Žabalj     |
| 9.  | R9/2020 | Saxa Treib   | 2020 | R. šančevi |
| 10. | 10/2020 | Verica       | 2020 | Srbobran   |
| 11. | 11/2020 | Crna zimska  | 2020 | Begeč      |
| 12. | 12/2021 | Verica       | 2021 | Srbobran   |
| 13. | 13/2021 | Saxa Treib   | 2021 | Kulpin     |

Seed incubation was performed on a PDA medium (potato-dextrose-agar) supplemented with 0.5 mg mL<sup>-1</sup> streptomycin sulfate at a temperature of 25°C. In order to determine the presence of phytopathogenic fungi, a method developed by Vannacci and Gambogi (1980) and Punja et al. (2001) was used.

The seeds were surface disinfected in 2% sodium hypochlorite (NaClO) for 3 min, after which they were washed with sterilized distilled water. The experiment was set up in three replicates of 100 seeds placed in a Petri dish on PDA agar. The seeds were incubated at 25°C with 12h light and 12h darkness regime. The number of contaminated seeds was counted after 7 days according to the development of the colony. Contaminated seeds were detected after 5 and 8 days. The level of infection was estimated according to the following formula:

$$\text{Infection level (\%)} = \frac{\text{Total number of infected seed}}{\text{Total number of tested seed}} \times 100$$

Identification of developed colonies: Incubated seeds were observed daily. The colonies that developed around the seeds were transferred to another Petri dish on a PDA substrate.

Determination of species of the genus *Fusarium* spp. was performed on the basis of macroscopic and microscopic properties on PDA and water-agar substrate (WA) according to the method of Nelson et al. (1983) and Burgess et al. (1994), while other genera were determined by the method of Champion (1997).

## Results and Discussion

After the seed incubation period on PDA on the fifth day, the formation of colonies of fungi of different colors began around the seeds. The percentage of sporulation of fungi on radish seeds in 2017 ranged from 1 - 7%, in 2018 from 2 - 4%, in 2019, 5% in 2020 from 1 - 5% and in 2021, 2 - 3% (Tab. 2).

Based on the macroscopic characteristics of the colonies, isolates were formed in order to identify them according to the species level. After the development of colonies on the PDA substrate, the formation of a pale rose mycelium color was noted, indicating the species of the genus *Fusarium* spp., which was confirmed by determination.

The intensity of the infection with species of the mentioned genus is 1% in 2017 and 2% in 2020 (Table 2). Species of the genus *Fusarium* spp. are known to be pathogens of many plant species and conidia are widespread in the external environment. *Fusarium oxysporum* has been described in literature as a vascular pathogen of radishes originating from soil (Yu et al., 2019). However, very little information is available regarding the species of this genus that appears on radish seeds.

The genus *Alternaria* spp. was determined in the intensity of 1% in 2020 to 5% in 2019 (Tab. 2). Based on one year of research, Holtzhausen et al. (1978) reported the presence of *Alternaria brassicae*, *A. brassicicola*, *A. cheiranthi*, *A. raphani* and *Phoma lingam* on 18 samples of commercial Japanese radish seeds. Species of the genus *Alternaria* spp. are a group of destructive pathogens on fam. Cruciferae worldwide. They lead to reduced yield quantity and quality (Verma and Saharan, 1994) causing reduced photosynthetic potential, accelerated aging, premature pod bursting, and shriveled seeds (Shrestha et al., 2000).

Fungi of the genus *Penicillium* spp. were determined in the intensity of 2% during 2017 in the variety Ledena sveća. Genus *Rhizopus* spp. was determined in an intensity from 2% in 2018 and 2021 to 7% in 2017. The appearance of black scattered mycelium indicated *Aspergillus niger*, which was confirmed by identification based on the morphology of the spores. The mentioned fungus was recorded during 2020 on the Saxa Treib radish with an infection intensity of 3% (Tab. 2).

Tab. 2: Level of infection in relation to varieties and years

| Variety    | Code    | <i>Alternaria</i> spp. | <i>Fusarium</i> spp. | <i>Penicillium</i> spp. | <i>Rhizopus</i> spp. | <i>Aspergillus</i> spp. |
|------------|---------|------------------------|----------------------|-------------------------|----------------------|-------------------------|
| Verica     | R1/2017 | 0                      | 0                    | 0                       | 7                    | 0                       |
| Saxa Treib | R2/2017 | 2                      | 0                    | 0                       | 0                    | 0                       |

|                              |          |   |   |   |   |   |
|------------------------------|----------|---|---|---|---|---|
| <b>Leden<br/>a<br/>sveća</b> | R3/2017  | 0 | 1 | 2 | 0 | 0 |
| <b>Verica</b>                | R4/2018  | 0 | 0 | 0 | 0 | 0 |
| <b>Saxa<br/>Treib</b>        | R5/2018  | 3 | 0 | 0 | 4 | 0 |
| <b>Crna<br/>zimsk<br/>a</b>  | R6/2018  | 3 | 0 | 0 | 2 | 0 |
| <b>Saxa<br/>Treib</b>        | R7/2019  | 5 | 0 | 0 | 0 | 0 |
| <b>Leden<br/>a<br/>sveća</b> | R8/2020  | 0 | 0 | 0 | 0 | 0 |
| <b>Saxa<br/>Treib</b>        | R9/2020  | 2 | 2 | 0 | 3 | 3 |
| <b>Verica</b>                | R10/2020 | 1 | 0 | 0 | 4 | 0 |
| <b>Crna<br/>zimsk<br/>a</b>  | R11/2020 | 2 | 0 | 0 | 5 | 0 |
| <b>Verica</b>                | R12/2021 | 0 | 0 | 0 | 3 | 0 |
| <b>Saxa<br/>Treib</b>        | R13/2021 | 3 | 0 | 0 | 2 | 0 |

### Conclusion

The analysis of 13 samples of radish seeds revealed the presence of fungi from the genus *Alternaria* spp., *Fusarium* spp., *Penicillium* spp., *Rhizopus* spp. and *Aspergillus niger* in different percentages of infection. It is necessary to continue monitoring the mycopopulation of radish seeds, especially because of the established genera *Alternaria* spp. and *Fusarium* spp. due to the fact that certain species of this genus are significant pathogens in agricultural production.

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## **FRUIT AND WINE GROWING**



## DETECTION OF PEACH LATENT MOSAIC VIROID BY RT-PCR AND REAL-TIME PCR

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### ABSTRACT

*Peach latent mosaic viroid (PLMVd) is economically the most important viroid infecting stone fruits. In sensitive peach cultivars PLMVd induces mosaic and calico on the leaves and deformed fruits with cracked suture. Early and accurate detection of PLMVd is one of the main measures for its control. In our study we have evaluated the efficiency of RT-PCR and quantitative Real-time PCR methods for the detection of PLMVd isolates collected from peach and nectarine orchards in Serbia. RT-PCR assay proved to be efficient for PLMVd detection, but some of the isolates were on the limit of detection. Real-time PCR was characterized by higher sensitivity in the detection of all tested isolates. Both assays can be recommended for use, but for testing the material within the certification of peach planting material Real-time PCR should be used.*

**Key words:** peach, PLMVd, reverse transcription, PCR, Real-time PCR

### INTRODUCTION

Peach latent mosaic viroid (PLMVd) is the causal agent of the economically important peach latent mosaic disease. The disease is known for decades, but PLMVd was isolated just in late 1980s (Flores and Llacer, 1988). It was reported as a pathogen of peach, nectarine, cherry, plum and apricot. In most commercial peach and nectarine cultivars it is in a latent phase. In susceptible cultivars, PLMVd can induce mosaic symptoms on leaves and calico (Figure 1) (Llacer, 1997). Sometimes it prolongs foliation, flowering and ripening. Developed buds may become necrotic, ripen fruits are deformed with cracked sutures and enlarged pits (Figure 2) (Loreti et al., 1999). There is little information on the epidemiological characteristics of PLMVd, but its natural spread within the orchards have been reported (Luigi and Faggioli, 2011). Several studies confirmed transmission of PLMVd with contaminated blades and by leaf aphids in the experimental conditions (Desvignes, 1986; Flores et al., 1991, Hadidi et al., 1997). PLMVd is a pathogen included in the OEPP/EPPO Certification scheme for almond, apricot, peach and plum for the production of healthy peach plants for planting (Anonymous, 2001).



Figure 1. PLMVd symptoms on GF305



Figure 2. Deformed fruit with cracked suture induced by PLMVd

The detection of PLMVd is based on biological and molecular tests. Woody indicator peach GF305 is widely used for biological testing in the glasshouse. Seedlings of GF305 are inoculated by chip-budding and the symptoms are followed in the next several months (Anonymous, 2001). Due to the latency, some isolates do not induce symptoms on indicator plants for more than a year. In order to improve the sensitivity of detection and obtain results in shorter time, a polymerase chain reaction (PCR) method has been developed (Shamloul and Hadidi, 1999). More sensitive, precise and quantification method that has been developed for PLMVd detection is Real-time PCR assay (qPCR) (Parisi et al., 2011). PLMVd was reported in Serbia on three peach cultivars in 2006 using RT-PCR assay (Jevremović and Paunović, 2006; Jevremović and Paunović, 2008).

The aim of our work was to evaluate the efficiency of RT-PCR and quantitative Real-time PCR methods for the detection of Serbian PLMVd isolates.

## MATERIALS AND METHODS

Plant material (fresh leaves) was collected from different geographical regions of Serbia. A number of 70 samples were collected from 2014 to 2020 from commercial orchards, mother blocks and nurseries. Samples were taken from trees showing leaf symptoms (mosaic patterns) and asymptomatic ones. All samples were tested for the PLMVd presence using reverse transcription (RT) followed by a polymerase chain reaction (PCR) and Real-Time quantitative PCR (qPCR).

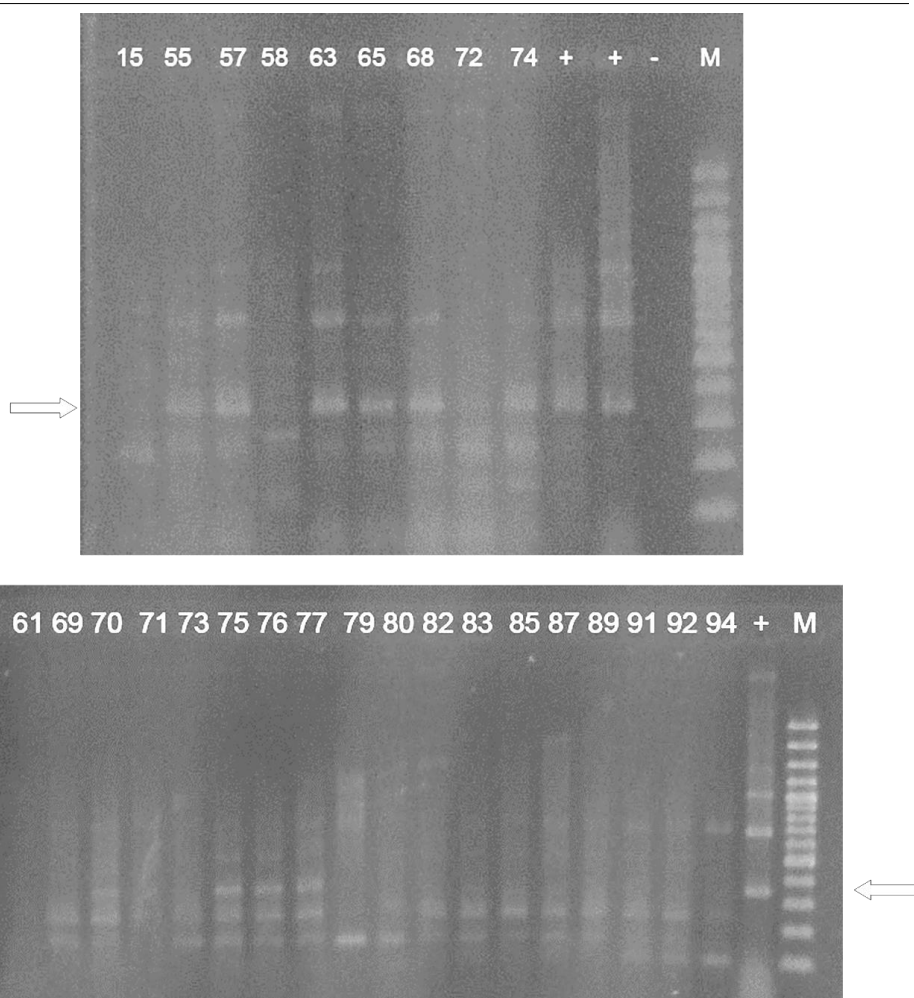
Total nucleic acids (TNA) were extracted using the CTAB extraction method of Li et al. (2008). The first strand cDNA synthesis was obtained by reverse transcription reactions using Maxima Reverse Transcriptase (Thermo

Fisher Scientific, USA) following manufacturer's protocol. The RT reaction was performed in two steps: denaturation at 65°C for 5 min, followed by incubation at 50°C for 30 min and 85°C for 5 min. PCR reaction was performed with primers PLMVd-c (5'-AACTGCAGTGCTCCGT-3') and PLMVd-h (5'-CCCGATAGAAAGGCTAAGCACCTCG-3') that amplify the complete PLMVd genome (Shamloul et al., 1995). The PCRs consisted of an initial denaturation at 94°C for 5 min; followed by 30 cycles at 94°C for 30 s, 62°C for 30 s and 72°C for 45 s; and a final extension at 72°C for 7 min (Jevremović and Paunović, 2008). Negative (sterile water) and positive control (isolate SD1, NCBI accn. JF416648) were used in all assays. RT-PCR reactions were performed in TPersonal thermal cycler (Biometra, Germany). PCR products were electrophoresed in a 1.5% agarose gel, stained with ethidium bromide and visualized under UV transilluminator (Hoefer, USA). The presence of an expected fragment of 339 bp was considered as a positive reaction.

Real-time PCR amplification of cDNAs was carried out in a StepOne® real-time PCR system (Applied Biosystems, USA) with Fast SYBR® Green Master Mix (Applied Biosystems, USA) according to recommended protocol. The F-PLMVd (5'-CCTCTCAGCCCCTCCACCTT-3') and R7-PLMVd (5'-CCTACCTTACGTCATTGCG-3') primer pair was used (Parisi et al., 2011). To optimize PLMVd detection, different concentrations of primers were used (150 nM and 300 nM). The qPCR included the following steps: 95°C for 20 s, followed by 40 cycles of 95°C for 3 s, and 55 and 60°C for 30 s. A melting curve was added to the end of the assay to check the specificity of the amplification. Two replicates were run for each sample. Obtained data was analyzed by StepOne® v2.3 software (Life technologies, USA).

## RESULTS AND DISCUSSION

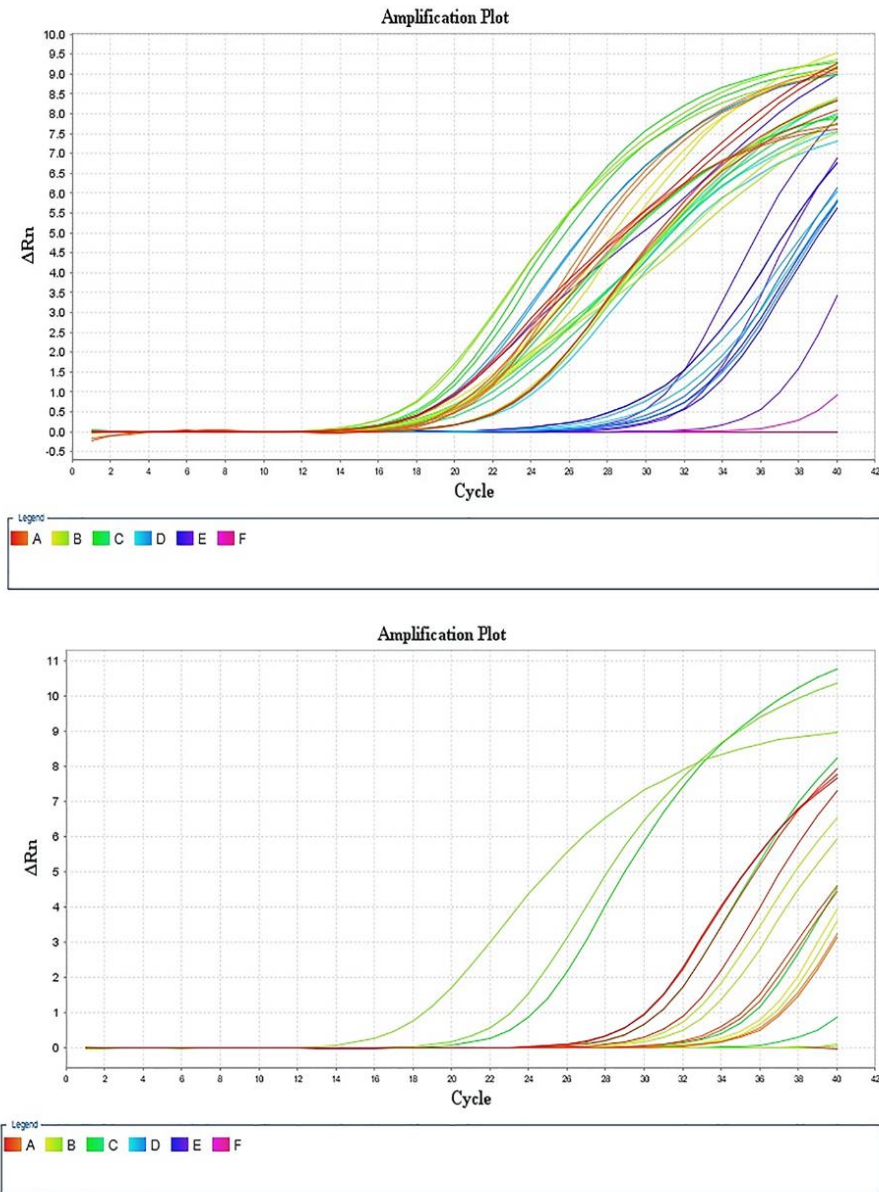
RT-PCR analysis revealed PLMVd presence in 10 samples (laboratory numbers: 55, 57, 63, 65, 68, 70, 74, 75, 76 and 77). All positive samples originated from commercial orchards from Rasina and Central Banat districts. Other 60 samples were proved to be PLMVd free. The intensity of the amplified 339 bp fragment varied in positive samples (Figures 3 and 4). In samples 55 and 70 very faint bands were obtained indicating that the test was at the level of sensitivity of detection (Figures 3 and 4). The presence of PLMVd was confirmed in the same districts as reported earlier (Jevremović and Paunović, 2014). RT-PCR assay proved to be efficient in PLMVd detection, but the detection limit for some isolates (55 and 70) was low. PCR method is routinely used in numerous laboratories for the detection of a large number of plant pathogens, including viroids. It is irreplaceable for fast and precise determination of the health status of plant material.



Figures 3 and 4. Agarose gel analysis of RT-PCR products for PLMVd. Line numbers 15–94 = tested samples; line += positive control; line – = negative control; line M = marker 100 bp ladder (Solis BioDyne, Estonia). Product size 339 bp (indicated by arrow).

All PCR positive samples were further tested by qPCR that confirmed PLMVd presence in all 10 samples (figures 5 and 6). Positive control was used in five different dilutions 1/5, 1/10, 1/100, 1/1,000 and 1/10,000. qPCR was efficient in PLMVd detection in all stated dilutions. Analyzing the obtained results with different primer concentrations and annealing temperatures we concluded that the optimal efficiency was achieved with primer concentration of 150 nM and annealing temperature of 55°C. Tests on negative control confirmed that both PLMVd and non-specific amplified products were detected before cycle 36. Cycle 36 was specified as the positive detection limit in the selected assay conditions. Moreover, in order to further test the efficiency and accuracy of the qPCR, 13 PLMVd-infected peach samples previously identified by RT-PCR (figures not shown) (Jevremović and Paunović, 2014) have been tested. As

expected, results obtained by qPCR confirmed presence of PLMVd in all samples (Figures 5 and 6). Our results are fully in line with the study of Parisi et al. (2011) confirming that chosen primer pair, concentrations and cycling conditions for real-time PCR with SYBR Green chemistry were well determined.



Figures 5 and 6. Detection of PLMVd isolates with Real-time PCR assay.



In a Real-time PCR assay a positive reaction was detected by accumulation of a fluorescent signal. The Ct (cycle threshold) is defined as the number of cycles required for the fluorescent signal to cross the threshold. Ct levels are inversely proportional to the amount of target nucleic acid in the sample (lower Ct – higher amount of PLMVd RNA). In our study, sample 18 had the lowest Ct value 14.94.

The melting curve analysis confirmed specific amplification of the PLMVd in the all samples. No peak corresponding to a nonspecific amplicon or primer dimer was observed (Figure 7).

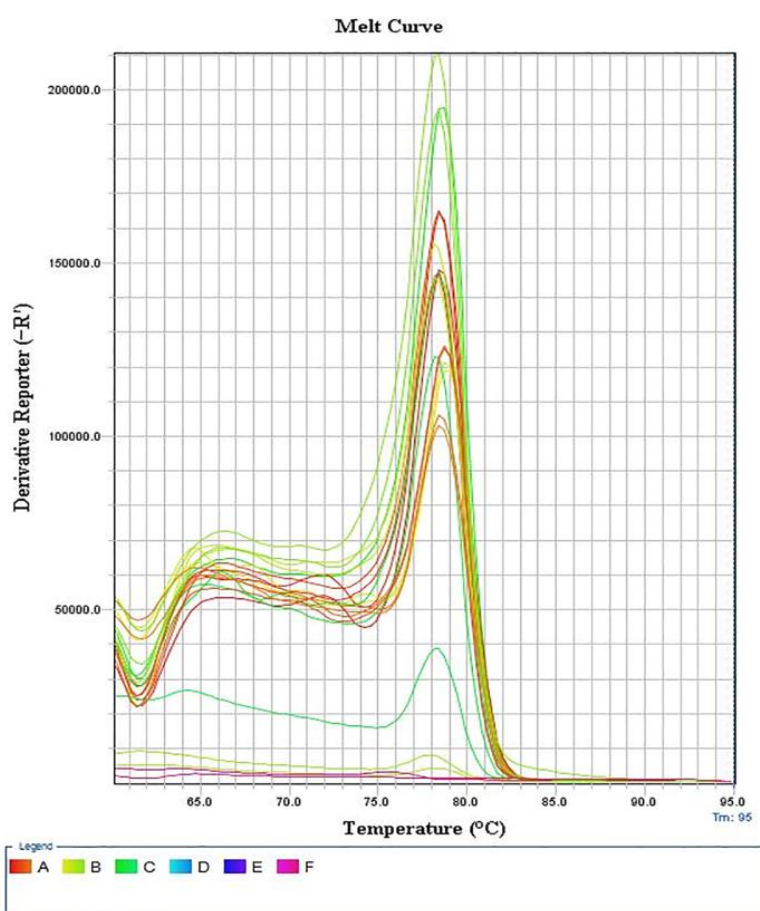


Figure 7. Melting curve analysis of the amplicons generated with PLMVd positive control. No signals of non-specific amplicons were present in standard amplifications and in the tested samples.

## CONCLUSION

The results obtained in our study confirmed high sensitivity and specificity of qPCR assay for the detection of PLMVd in peach and nectarine samples from Serbia. In comparison to conventional PCR, qPCR is characterized by higher sensitivity that was confirmed in the detection of all tested isolates. Both assays can be recommended for use, but for testing the material in the framework of the certification qPCR should be used.

## ACKNOWLEDGEMENTS

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## EFFECT OF ALTITUDE ON PRIMARY METABOLITES OF PLUM (*PRUNUS DOMESTICA* L.) FRUIT

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### ABSTRACT

*This research was conducted to examine the effect of altitude on the contents of primary metabolites (soluble solids, total sugars, invert sugars, proteins, total acids, pH and soluble solid/acid ratio) of the fruit of plum. Nine plum cultivars were involved, including 'Boranka', 'Čačanska Rana', 'Čačanska Lepotica', 'Timočanka', 'Krina', 'Mildora', 'Stanley', 'Čačanska Najbolja' and 'Čačanska Rodna'. The experiment was performed at two locations: Location 1 – (300 m altitude) and Location 2 – (550 m altitude). The contents of primary metabolites varied widely depending on altitude. The synthesis of soluble solids content was most intensive at the location situated at an altitude of 550 m. Also, the fruit of plums grown at 550 m had higher total sugars and invert sugars compared to those at 300 m altitude. In contrast, higher values of acids and proteins were determined at a lower altitude (300 m). During the three-year experimental period, significant differences in the tested parameters were observed among the cultivars. Regardless of the altitude, the highest values of soluble solids, sugars and pH, and the lowest levels of total acids were recorded in cultivar 'Mildora'. On average, the highest concentration of total acids was recorded in the fruits of 'Čačanska Rana', whereas 'Boranka' had the highest levels of proteins. The results suggest that plum fruits are rich in primary metabolites, and that altitude has an important effect on the synthesis of the tested parameters in the fruits.*

**Key words:** *Prunus domestica* L., altitude, cultivars, chemical properties

## INTRODUCTION

Plum (*Prunus domestica* L.) is a traditional fruit crop in Serbia, and the most important one in terms of the volume of production. The Republic of Serbia accounts for approximately 5% of the world's plum production, taking third place, behind China and Romania. The plum production is characterized by extensive growing technology, low unstable yields and low-quality fruit. In recent years, there has been an increase in the number of trees per unit of area in Serbia, which are an indicator of intensive production and the initiation of new plantations (Miletić et al., 2011). However, consumption of fresh plum is relatively low. The largest amount of fruits is processed into brandy (around 75-80%), whereas considerably lower quantities are used for fresh consumption, drying and processing into other products. Plum fruits are a rich source of carbohydrates and have a relatively high organic acid content (Gil et al., 2002; Walkowiak-Tomczak et al., 2008). The chemical properties of fruits may be affected by various factors, such as genotype, harvesting time, growth conditions including environmental factors and soil (Usenik et al., 2008; Zheng et al., 2009). Also, altitude strongly influences microclimate, including air temperature, precipitation and evaporation, influencing the accumulation and synthesis of metabolites, which result quality and commercial value of fruits (Dong et al., 2011; Zoratti et al., 2015). According to Lian et al. (2000), altitude showed effects on compounds in the fruits, and they confirmed correlations among total sugars, total acids and the sugar/acid ratio. In bilberry and strawberry at different altitudes, as found by Rieger et al. (2008) and Crespo et al. (2010), the chemical composition of fruits changes with altitude. For that reason, when growing plum, it is important to determine their adaptability to local climatic and soil conditions, including altitude (Blažek et al., 2004; Ionica et al., 2012).

Therefore, the objective of this study was to evaluate and compare the chemical composition of plum fruits growing at two different altitudes.

## MATERIAL AND METHODS

The research was conducted at the Fruit Research Institute, Čačak, Republic of Serbia, during 2017–2019, at two locations differing in altitude: Location 1 - Preljinsko brdo (43°54' 33" N latitude, 20°24'32" E longitude, 300 m altitude) and Location 2 - Jelica (43°47'34" N latitude, 20°21'36" E longitude, 550 m altitude). Plum cultivars are grafted on rootstock *Prunus cerasifera* L. The analysis involved 9 plum cultivars ('Boranka', 'Čačanska Rana', 'Čačanska Lepotica', 'Timočanka', 'Krina', 'Mildora', 'Stanley', 'Čačanska Najbolja' and 'Čačanska

Rodna'). All cultivars are represented by five trees. Orchard management was consistent with standard cultural practice (pruning, fertilization, pest and disease control, drip irrigation).

The chemical analysis of the fruit included the following: 1. The content of soluble solids (%) was determined by a digital refractometer (Kruss, Germany); 2. Total sugars (%) and invert sugars (%) were analyzed by the Loof-Schoorl method (Egan et al., 1981); 3. The total acids content (%) expressed in malic acid was determined by neutralisation of the fruit juice with 0.1 N NaOH, using phenolphthalein as indicator until pH 8.1; 4. Protein content was determined by Kjeldahl's method (Helrich, 1990); 5) Actual acidity (pH value) was measured by a pH Meter Iskra MA 5707, Slovenia.

The experimental data obtained in the research period were subjected to statistical analysis using Fisher's two-factor analysis of variance - ANOVA. Significant differences between the mean values of the tested factors and the interaction means were determined by LSD test at  $P \leq 0.05$  significance levels. The paper presents the average values of the tested parameters during the study period.

## RESULTS AND DISCUSSION

Various factors, such as genotype, location and climatic conditions, contribute to chemical composition of fruits (Mikulič-Petkovšek et al., 2012). One of these factors is altitude, which has not been sufficiently investigated so far. Fruit quality is determined by the content of primary metabolites (sugars, organic acids, etc.), which contribute to the flavor, aroma and appearance of the fruit (Veberič et al., 2012). The results on the effect of altitude on soluble solids content and sugars in plum fruits are presented in Figure 1 and Table 1. In this experiment, the synthesis of soluble solids and sugars in plum fruit was largely affected by altitude. The soluble solids content varied from 12.71% to 23.92%. Detected amounts of total sugars ranged from 6.91 to 14.94%, while the measured concentration of invert sugars varied from 4.27 to 10.18 %. As altitude increased, the accumulation of these primary metabolites increased. The difference in the content of soluble solids was 0.88% between altitudes, while the difference in contents of total and invert sugars was 1.15% and 0.99%, respectively.

In contrast, the accumulation of acids and pH value decreased with increasing altitude (Table 1). Detected amounts of total acids varied from 0.57 to 1.12% and pH value from 3.29 to 4.20%. Higher altitude reduced the plum fruit acidity with 0.12% compared to lower altitude.

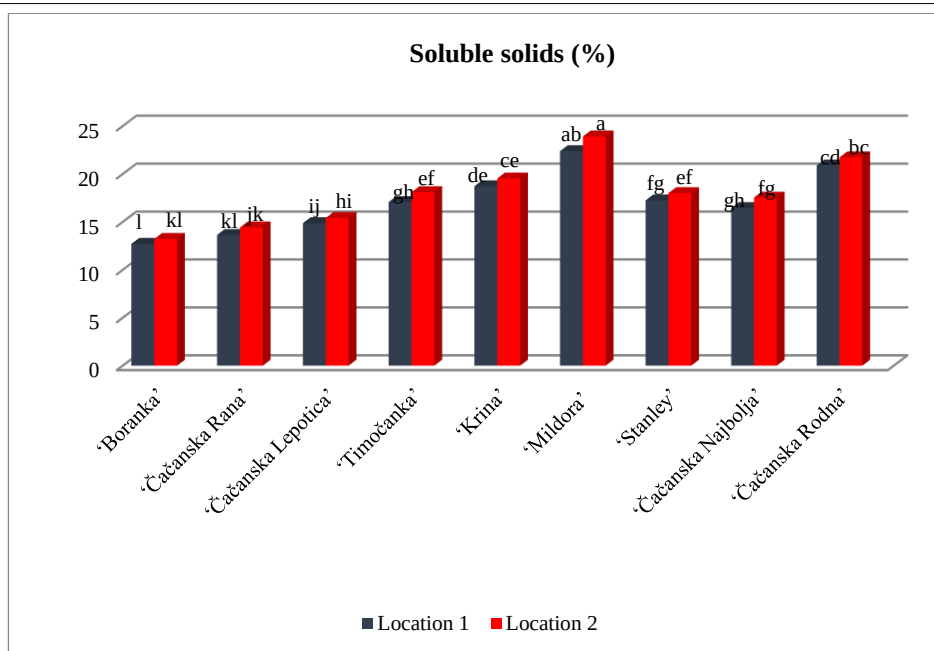


Figure 1. Effect of altitude on total soluble solids in plum fruits

The effect of altitude on the quality of plum fruits might be due to differences in temperature, humidity and precipitation between growing places at different altitudes, which directly affected the synthesis and accumulation of primary metabolites. These results can be supported by the findings of Rokaya et al. (2016), who observed that mandarins grown at 1300 m contained a higher amount of soluble solid and lower amount of acid compared to fruits grown at 1000 m and 700 m.

Table 1. Effect of altitude on sugars and acids in plum fruits

| Cultivars | Location   | Sugars          |                  | Acids            |                   | TSS/TA |
|-----------|------------|-----------------|------------------|------------------|-------------------|--------|
|           |            | Total sugar (%) | Invert sugar (%) | Total acid (%)   | pH                |        |
| 'Boranka' | Location 1 | 6.91±0.47 j     | 4.27±0.3<br>1 g  | 1.09±0.<br>11 ab | 3.63±0.<br>06 cde | 11.67  |
|           | Location 2 | 7.95±0.50 ij    | 4.89±0.3<br>4 fg | 0.95±0.<br>09 cd | 3.52±0.<br>05 efg | 13.91  |

|                     |             |                   |                  |                   |                   |       |
|---------------------|-------------|-------------------|------------------|-------------------|-------------------|-------|
| ‘Čačanska Rana’     | Locati on 1 | 8.45±0.59 ij      | 6.59±0.5<br>3 ef | 1.12±0.<br>16 a   | 3.47±0.<br>07 fgh | 12.18 |
|                     | Locati on 2 | 9.29±0.63<br>gh   | 7.39±0.6<br>2 de | 1.01±0.<br>10 bc  | 3.31±0.<br>01 hi  | 14.27 |
| ‘Čačanska Lepotica’ | Locati on 1 | 11.10±0.81<br>def | 7.54±0.6<br>4 cd | 1.10±0.<br>12 ab  | 3.41±0.<br>02 ghi | 13.51 |
|                     | Locati on 2 | 12.31±0.97<br>cd  | 8.35±0.7<br>5 bc | 0.91±0.<br>09 cde | 3.29±0.<br>01 i   | 16.93 |
| ‘Timočanka’         | Locati on 1 | 10.34±0.73<br>fg  | 6.70±0.5<br>8 ef | 0.74±0.<br>06 fgh | 3.70±0.<br>05 bcd | 23.07 |
|                     | Locati on 2 | 11.35±0.78<br>de  | 7.97±0.6<br>9 cd | 0.63±0.<br>04 hi  | 3.61±0.<br>04 def | 28.73 |
| ‘Krina’             | Locati on 1 | 11.57±0.83<br>de  | 7.75±0.6<br>8 cd | 0.72±0.<br>05 fgh | 3.84±0.<br>07 b   | 26.00 |
|                     | Locati on 2 | 12.17±0.91<br>cd  | 8.42±0.8<br>2 bc | 0.61±0.<br>04 hi  | 3.78±0.<br>05 bc  | 32.02 |
| ‘Mildora’           | Locati on 1 | 13.65±1.08<br>b   | 9.24±0.9<br>8 ab | 0.57±0.<br>03 ij  | 4.20±0.<br>09 a   | 39.28 |
|                     | Locati on 2 | 14.94±1.17<br>a   | 10.18±1.<br>07 a | 0.49±0.<br>02 j   | 4.06±0.<br>08 ab  | 48.82 |
| ‘Stanley’           | Locati on 1 | 12.49±0.95<br>cd  | 7.13±0.5<br>9 de | 0.78±0.<br>06 efg | 3.75±0.<br>05 bcd | 22.10 |
|                     | Locati on 2 | 13.33±1.02<br>bc  | 8.38±0.8<br>0 bc | 0.67±0.<br>05 ghi | 3.61±0.<br>04 def | 26.88 |
| ‘Čačanska Najbolja’ | Locati on 1 | 9.01±0.59<br>hi   | 5.34±0.4<br>9 fg | 0.84±0.<br>07 def | 3.64±0.<br>03 cde | 19.57 |
|                     | Locati on 2 | 10.94±0.80<br>ef  | 6.50±0.5<br>8 ef | 0.73±0.<br>05 fgh | 3.53±0.<br>02 efg | 24.01 |
| ‘Čačanska Rodna’    | Locati on 1 | 12.09±0.90<br>cd  | 7.17±0.6<br>2 de | 0.71±0.<br>04 fgh | 3.63±0.<br>04 cde | 29.42 |
|                     | Locati on 2 | 13.62±1.05<br>b   | 8.51±0.8<br>1 bc | 0.64±0.<br>03 hi  | 3.52±0.<br>02 efg | 33.97 |

Means followed by different letters within rows are significantly different at  $P \leq 0.05$  according to LSD test and ANOVA (F-test) results



Similarly, low altitude (229 m asl) persimmon fruits had higher soluble solids contents than those from a high elevation (770 m asl) region (Candir et al., 2009). For every 100 m increase in elevation, according to Büyüksolak et al. (2020), the values of soluble solids in fruits increase by 1.2°Brix. Also, the lower soluble solids content as the effect of the lower altitude was similar to previous studies by Parra-Coronado et al. (2018) and Musyarofa et al. (2020). Charles et al. (2018) also observed higher soluble solids contents at higher altitude, but no significant effect on total acids. Fischer et al. (2007) found that gooseberry had about two times more sugars at a higher altitude than at the lower site. According to Shulman et al. (1984), pomegranates cultivated in low altitude had more acids than fruits harvested from higher zones, which leads to delayed maturity. On the other hand, Lian et al. (2000) reported that the content of total sugar increased as altitude decreased, while the total acid content showed the opposite trend. Also, Gündüz and Özbay (2018) indicated that lower altitude significantly affected total soluble solids and total acidity. Moreover, Rieger et al. (2008) and Crespo et al. (2010) measured significantly lower content of sugars in bilberry and strawberry grown at a higher altitude compared with those grown at a lower altitude, but no significant difference was observed in the acid content. Besides, Trad et al. (2013) and Gatti et al. (2009) reported that altitude has no influence on soluble solids contents in sweet cherry and blueberry fruits.

To our knowledge, no previous studies aimed to determine the influence of the growing altitude on the protein content in plums. The high protein content (0.76%) was noted in plums from the lower growing altitude (300 m), whereas the low protein content (0.68%) was determined in plums from the higher growing altitude (550 m) (Figure 2). The present results are not in agreement with the findings of Büyüksolak et al. (2020), who observed that walnuts grown at 900 m contained a higher amount of protein compared to walnuts grown at 800 m and 650 m. Ratio of soluble solid/acid has been considered to be a reliable index of maturity of plum and provided a more precise definition of the fruit quality than the two parameters applied separately because it determines the taste of the fruits and their attractiveness for consumers (Crisosto et al., 2004). The findings of the study showed that the higher soluble solid/acid ratio was recorded in the fruits cultivated at the higher altitude location in comparison with lower altitude, which is consistent with the results of Büyüksolak et al. (2020) and Rokaya et al. (2016), who recorded that higher altitudes increased ratio of soluble solid/acid in walnuts and mandarins. The increased soluble solid/acid ratio can be due to further synthesis and accumulation of photosynthates in the plum fruits.

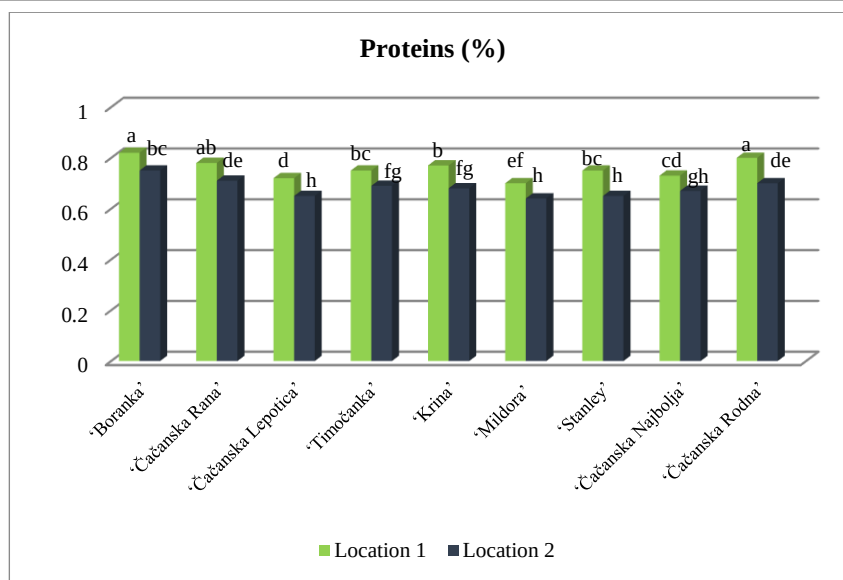


Figure 2. Effect of altitude on proteins in plum fruits

The presented results demonstrated significant differences among cultivars regardless of altitude. During the experimental period, the highest content of soluble solids was determined in cultivars 'Mildora' (23.15%) and 'Čačanska Rodna' (21.31%), and the lowest in cultivar 'Boranka' (12.96%). In terms of sugars, 'Mildora' had the highest content of total sugars (14.29%) and invert sugars (9.71%), as opposed to 'Boranka' (7.43% and 4.58%, respectively), which exhibited the lowest values. As regards acids, the highest values of total acids were obtained in 'Čačanska Rana' (1.06%), while the lowest levels were recorded in 'Mildora' (0.53%). 'Mildora' had the highest pH value (4.13), while 'Čačanska Lepotica' had the lowest levels (3.35) in the fruits. On average, the highest concentrations of proteins were found in 'Boranka' (0.78) and 'Čačanska Rana' (0.75), whereas 'Mildora' (0.67) and 'Čačanska Lepotica' (0.69) had the lowest levels of protein. In general, the contents of primary metabolites were in agreement with cultivar-specific traits. The results of the present experiment on the chemical composition of plum fruits are comparable to the results of Mitrović et al. (2006), Miletić et al. (2013) and Glišić et al. (2016). Popović et al. (2013) reported that fruits of 'Čačanska Rodna' had high content of total sugars, which is typical of this cultivar. The high total sugars of cultivar 'Mildora' identified in our samples, is in accordance with the report Miletić et al. (2014) and Milatović et al. (2016) who observed that very high soluble solids and low acid content of cultivar 'Mildora' confirms its suitability for drying.

## CONCLUSION

The results of the present research reveal that altitude has a significant effect on the synthesis of primary metabolites, which should be considered when establishing commercial plum orchards. The tested cultivars showed good results and could be the basis for modern intensive orchards and increase production of plum in Serbia. In general, plums are a rich source of sugars and acids, and can be used as a new source of natural foods.

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## INFLUENCE OF MICROELEMENT FERTILIZERS ON THE CONTENT OF VITAMIN C IN THE FRUIT OF DIFFERENT APPLE VARIETIES

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**Abstract:** *The research within this paper presents the results of the influence of fertilization (classical applications and foliar fertilization with micro and macroelements) on the content of vitamin C in apple fruit assortment: Jonagold Decosta; Red Idared and Gala Schniga. The two-year field experiment (2020–2021) was three-factorial (factors: variety, fertilization and year) placed according to a random block layout (five trees in three repetitions) at the site of the experimental range of the Federal Institute of Agriculture Sarajevo-Butmir. Fertilization treatments in the experiment included: control variant: (without fertilization), standard fertilization: NPK 6:18:36 - 300 kg/ha (spring) + top dressing 150 kg / ha KAN 27% (initially vegetation). Foliar fertilization it was done at the beginning vegetation with 0.3-0.8 % solution - FitoFert Crystal 10:40:10 + 1MgO + ME; then 0.3% solution Fitofert Kristal 20: 20: 20 + ME. In phase obstruction fruits fertilizer was applied Borax, 17, 5 % (two treatments). Aim of the study was to determine the effect of different types of fertilizers on the content of vitamin C in apple fruit. The results of this study show that the highest average content of vitamin C in apple fruit was achieved with Jonagold Decosta using standard fertilization NPK + KAN (66.69 mg/kg) in 2021, and the lowest with Gala Schniga in foliar fertilization 2020 (17.08).*

**Keywords:** *apple, variety, fertilizer, vitamin C.*

### INTRODUCTION

Apple (*Malus x domestica* Borkh.) Is the most widespread and one of the most important continental fruit species in the world, and therefore it is given great attention in improving cultivation and increasing production. Apple is a nutritious, hygienic, dietary prophylactic food necessary for the proper development and preservation of human health. Apple production is extremely

important, both for its fresh use and for the development of the food industry. The value of the apple has been known since ancient times, highly valued for its aroma, sweet taste, shape, color and aroma, which are the basic characteristics and characteristics of a particular type of apple fruit. Apple fruits have a high content of dietary fiber, sugar, vitamins and phenolic compounds. The quality of apple fruit is defined by physical, biochemical and organoleptic characteristics, while texture and taste are the most important elements of quality determined by consumers. Bosnia and Herzegovina provides very good conditions for the development and fruiting of apples with the application of modern agro-technical measures, which achieves high and stable yields, satisfactory fruit quality. The great ability to adapt apples to different agro-ecological conditions and the high quality of the fruits have secured it a very important place in fruit production. It is the only fruit from domestic production for which a continuous supply is provided throughout the year, since it can be easily and for a long time kept fresh. It is the most common fruit species in our diet. Good knowledge of apple varieties and their characteristics is the basis of modern fruit production (Gvozdenović, 1998). Many years of selection have created a large number of different varieties of apples that more or less differ in morphological, physico-chemical, organoleptic and other properties. Local population and old varieties are necessary genetic resource for providing selection progress and sustainable global productivity food good quality and sufficient amount for today and future needs of humanity (Kulina, M. 2010).

The quality of fruits, their color, taste, size, firmness, etc. are varietal characteristics. However, the mentioned characteristics are also influenced by agro-ecological factors, especially agro-technical measures, among which the impact of fertilization should be emphasized as the primary measure. Depending on the applied doses and the ratio of individual fertilizers, they can improve or, in case of lack, their unprofessional application, which can worsen the quality of fruits. Application fertilizers soluble in water foliar or irrigation it has become commonplace measure in the wider production practice. This type of fertilization is considered more efficient from conventional method fertilization cause of immediate contact between roots and liquid soil phases (Čmelik, Z. et al. 2006). Apple is one of the most important sources of vitamin C, and it is rich in other valuable ingredients. Vitamin C serves as an antioxidant in the human body, contributing to the body's resistance and treatment of diseases, and its intake in increased quantities is recommended as a countermeasure to daily stress. It is significant for his role in biochemical processes like transport electrons, synthesis collagen (Veršec, P. 2014). Aim of this research was to examine the impact of different ways of fertilization, on vitamin C content in the fruit of different apple varieties.



## MATERIAL AND METHOD OF WORK

A two-year field experiment was conducted in the period 2020–2021 at the site of the experimental range of the Federal Institute of Agriculture in Butmir, on an apple orchard erected in 2008 (with 35 apple varieties), at an altitude of 550 meters. Orchard is located at the very entrance to the Butmir settlement, surrounded by an iron stake and plastic-coated wire. As a support in the orchard, a wooden stake was placed, and a bamboo stick was placed next to each tree. The row space is grassed and maintained in the "grass mulch" system, while the row space in the row spacing zone is regularly mowed. Seedlings of examined the apple varieties were: B1-Jonagold Decosta; B2-Red Idared; B3-Gala Schniga, which are grafted on substrate M9, planted on intermediate distance from 3 m, and within rows distance from 0,8 m. Applied breeding the shape is a slender spindle. It is currently installed system for irrigation "cap per cap" and anti-hail network. Applied are modern agro and pomotechnical measures for the cultivation of apples on this one plantation, before all: summer and winter pruning, fertigation by applying mineral fertilizers NPK15:15:15, protection against pathogens diseases and pests, and regularly destruction of weeds (herbicides) and mechanical measures. Fertilization treatments in the experiment included:

**(A )** Mineral fertilizers NPK 6:18:36 - 300 kg / ha (spring) + top dressing 150 kg / ha KAN 27% - (beginning of vegetation).

**(B)** Foliar fertilizers - the beginning vegetation

- 0.6 solution Fitofert Crystal 10:40:10 + 1MgO + ME,
- 0.6 solution Fitofert Kristal 20:20:20 + ME,
- Sweeping fruits ( bormax, 20% - two treatments),

Foliar fertilizers - during the vegetation:

- Fitofert Kristal 20:20:20 + ME; 0.6% solution (two treatments)
- 0.5% Folifertil Ca (three treatments with 0.5% Folifertil Ca - growth period)
- Zn (0.3% ZnSO<sub>4</sub>) - two treatments.

**(C)** Control variant : (without fertilization )

A two-year field experiment was placed according to a random block system of five trees in three repetitions (15 trees) for each of the varieties according to the fertilization variant, which makes a total of 3 variants of fertilization x 15 trees (45) trees. The basic properties of the soil on which the experiment was set up were: pH in water 6.96, pH in KCl 5.90; N content 0.17%; CaCO<sub>3</sub> content 0.5%; humus content 2.26%; P<sub>2</sub>O<sub>5</sub> 7.65 mg/100 g; K<sub>2</sub>O 22.30 mg/100g. Soil analysis was performed at the Federal Institute for Agropedology according to standards:

BAS ISO 10390: 2005. Data obtained by measurement were processed by statistical analyzes: two-factor analysis of variance (Fisher test), standard deviation and testing of significance of factors using Tukey-Kramer test for significance level  $P < 0.05$ .

## RESEARCH RESULTS AND DISCUSSION

The application of various fertilizers and fertilizer treatments had a significant impact on the content of vitamin C in apple fruits. The average content of vitamin C in apple fruit was in the range of 17.08 mg/kg (2020) in variety Gala Schnig apples with application of foliar fertilizers solution FitoFert Crystal 10:40:10 + 1MgO + ME early vegetation and Fitofert Crystal 20:20:20 + ME at the beginning vegetation, at budding fruits (borax, 20%) and applying Fitofert Crystal 20:20:20 + ME; 0.6% solution (two treatments) 0.5% Folifertil Ca (three treatments with 0.5% Folifertil Ca - growth period) Zn (0.3% ZnSO<sub>4</sub>) - two treatments. The highest content of vitamin C was registered in the apple variety Jonogold Decosta (66.69) using mineral fertilizers NPK 6:18:36 - 300 kg/ha (spring) + top dressing 150 kg/ha CAN 27% - (beginning of vegetation in 2021)

*Table 1. Vitamin C content in apple fruits depending on variety, fertilization and year*

| FERTILIZATION VARIANTS |                  |      | B1 NPK + CAN                               | B2 Foliar | B3 Control |
|------------------------|------------------|------|--------------------------------------------|-----------|------------|
| SORT                   |                  | God. | Vitamin C mg / kg fresh juice fruit apples |           |            |
| A1                     | Jonogold Decosta | 2020 | 25.87                                      | 25.50     | 18.10      |
|                        |                  | 2021 | 66.69                                      | 69.21     | 67.78      |
| A2                     | Red Idared       | 2020 | 45.65                                      | 44.50     | 41.50      |
|                        |                  | 2021 | 63.33                                      | 60.09     | 53.34      |
| A3                     | Gala Schniga     | 2020 | 18.42                                      | 17.08     | 17.10      |
|                        |                  | 2021 | 55.52                                      | 51.16     | 51.10      |

Based on the presented data (Table 1) of the comparison of the average content of vitamin C in apple fruits, it can be concluded that there are differences between individual apple varieties, applied fertilization variants and year.

*Table 2. Variation of vitamin C content depending on variety, fertilization and year*

|                         | SS       | df | MS       | F        | p-value                |
|-------------------------|----------|----|----------|----------|------------------------|
| Year                    | 13490.04 | 1  | 13490.04 | 5662,311 | $1.05 \times 10^{-44}$ |
| Fertilizer              | 185.8636 | 2  | 92.9318  | 39,0072  | $4 \times 10^{-10}$    |
| Variety                 | 2465,537 | 2  | 1232,768 | 517.4423 | $2.59 \times 10^{-29}$ |
| Year x Fertilizer       | 1,585733 | 2  | 0.792867 | 0.332798 | 0.718881               |
| Year x Variety          | 2064,993 | 2  | 1032,496 | 433.3801 | $7.79 \times 10^{-28}$ |
| Fertilization x Variety | 67.6     | 4  | 16.9     | 7.093608 | 0.000205               |
| Between                 | 95.29707 | 40 | 2.382427 |          |                        |
| Total                   | 18370.92 | 53 | 346.6211 |          |                        |

\* statistical significance 0.05

Based on the presented results of the analysis of variance, it can be concluded that the applied combinations of mineral and foliar fertilization in the examined statistical characteristic of vitamin C content in apple fruit showed statistical significance ( $p < 0.05$ ). Year as a factor showed statistical significance, while the interaction of years and the combination of mineral and foliar fertilization concerning the examined apple varieties also showed statistical significance in vitamin C content. The highest content of vitamin C was achieved by foliar application of Fitofert Crystal 20:20:20 + ME in the values of 69.21 mg/kg. The lowest value of vitamin C is achieved in the control variants of apples Jonagold Decosta in 2020 (18.10 mg/kg). Obtained the results are something lower (2020) in variety Jonagold Decosta in a relationship to those who are obtained in research Czech, M 2013 (33.93 mg/kg), ail higher to obtained results in 2021. Similar to the results obtained are in research Veršec, P. 2014 with values of from 0.03 - 6.35 mg/100 g of sample. According to the results obtained for the total amount of vitamin C in the whole fruit, the varieties Granny Smith and Fuji contained the least (0.74 and 2.01 mg/100g, respectively), Gala the most (5.85 and 6.45 mg/100g) vitamin C.

### CONCLUSION

We can conclude that there are statistically significant differences in the content of vitamin C in apple fruit in relation to the examined varieties, fertilization and age ( $p < 0.05$ ). The content of vitamin C in the fruit of the Jonagold Decosta apple variety was significantly higher in 2021 compared to 2020. The highest content

of vitamin C (69.21 mg/kg) was achieved with the application Fitofert Crystal 20:20:20 + ME; 0.6% solution ( $p < 0.05$ ). The lowest value of vitamin C was recorded in the control variant of the Jonagold Decosta apple variety. In accordance with the obtained results, we can conclude that there is a statistically significant effect of fertilization, year and apple variety on the content of vitamin C ( $p > 0.05$ ).

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## INFLUENCE OF CULTIVARS ON THE PROPERTIES OF FRUITING TWIGS IN PLUM

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**ABSTRACT:** *The paper presents the results of research on the influence of five plums cultivars (Topper, Presenta, Elena, Hanita and Katinka) on the properties of fruiting twigs in agro-ecological conditions of Sarajevo. Morphological properties of fruiting twigs (long and short): length, thickness, number of flower buds, number of vegetative buds and density of flower buds were examined. The average length of long fruiting twigs was from 23.50 cm to 32.33 cm. The results of the study showed that cultivar had not a statistically significant influence on the length and thickness of long fruiting twigs. Cultivar Katinka had a statistically significant influence on the formation of the highest number of the flower buds per long twig. On the other hand, cultivars Presenta and Elena influence the smallest number of the vegetative buds per long twig. The average length of short fruiting twigs was from 2.80 cm to 8.19 cm. Number of flower buds per short fruiting twig ranged from 1.56 to 3.41, while the number of vegetative buds ranged from 2.09 to 4.09. Cultivar Katinka had the highest density of flower buds.*

**Key words:** *Prunus domestica L., cultivars, short fruiting twigs, long fruiting twigs, buds.*

### INTRODUCTION

Average plum production worldwide in the period 2017–2019. amounted to 12.2 million tons (FAOSTAT, 2021). Plum is the most important fruit species in Bosnia and Herzegovina. According to the Agency for Statistics of Bosnia and Herzegovina, the total area under plums in Bosnia and Herzegovina in the period 2017–2020. was 64,571 ha, while the realized production was 134,599 kg. Plum production in BiH is characterized some negative characteristics: big share of old orchards, out-of-date cultivars, and inadequate application of cultural practices.

Intensification of plum production is necessary for the achievement of a high and regular yields and good quality of fruits. It is necessary to plant new orchards with virus-free nursery trees, to introduce new quality cultivars, and to apply regularly cultural practices, with special emphasis on soil management, fertilization, irrigation, pruning and plant protection. The pruning is one of the most complex practices in fruit production. For the proper pruning performance it is necessary to know the basic physiological properties of species and cultivar and the morphology of bearing wood, e.g. properties of fruiting twigs.

*Veličković et al.* (1997) analyzed the fruiting twigs of plum in function of cultivars and combinations cultivar/rootstock. They found significant differences between plum cultivars in the share of different types of fruiting twigs. Significant differences were found in the flower buds density in different species and cultivars of stone fruits, which are directly related to yield (*Albuquerque et al.*, 2004; *Thurzó et al.*, 2006; *Nenadović-Mratinić et al.*, 2007; *Milatović et al.*, 2010). Cultivars which are characterized with higher flower bud density are adapted to the conditions of continental climate, considering that this increases the probability of survival of generative organs due to the frost appearance, thereby provides the higher yield (*Okie and Werner*, 1996).

The number of flower buds per fruiting twig is primarily caused by genetic factors and it is correlated with the yield (*Milatović et al.*, 2010). The intensity of the flower bud formation on the short fruiting twigs is significantly reduced with increasing of the bearing woodage. The density of flower buds is important for determination of the intensity of pruning, because the cultivars with higher number of flower buds require heavy pruning, while the cultivars with less number of flower buds require light pruning (*Milatović and Đurović*, 2010).

The aim of this study was to examine the influence of cultivars on the morphological properties of long and short fruiting twigs. On the basis of the results obtained, the recommendations will be done for pruning performance, which is the most important cultural practice for yield regulation.

## MATERIALS AND METHODS

The study was conducted in the plum orchard at the Experimental Station station „Butmir” on the Federal Institute of Agriculture Sarajevo (Bosnia and Herzegovina). During the one-year period (2022) the influence of five plum cultivars (Topper, Presenta, Elena, Hanita and Katinka) was studied on the properties of fruiting twigs. The orchard was planted in spring of 2007. The planting distance was 4.0 m between the rows and 2.0 m within the row. The

training system was the Spindle. Standard cultural practices were applied, including drip irrigation. Rootstock for each cultivar was clonal rootstock Fereley. Every cultivar was represented by six trees (three replications with two trees).

The fruiting twigs were taken in the spring, before flowering. From each cultivar was taken 20 long fruiting twigs (shoots) and five two-year old twigs, where all short fruiting twigs (spurs) were analyzed. Length of twigs was measured with a meter, and thickness with a caliper. Number of flower and vegetative buds was registered on every node. The density of flower buds was calculated as the number of flower buds per 1 m length of fruiting twig (*Lombard et al.*, 1988).

The results were processed statistically using the analysis of variance. The significance of differences between mean values was evaluated using Duncan's multiple range test for significance level of 0.05. Data analysis was performed using the statistical software package IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA).

## RESULTS AND DISCUSSION

The properties of long fruiting twigs of table cultivars are shown in table 1.

The largest values of length of long fruiting twigs were found in cultivar Hanita (32.33 cm), while the lowest values were found in cultivar Topper (23.50 cm). Differences in the length of long fruiting twigs between cultivars were not statistically significant.

Table 1. The properties of long fruiting twigs of plum cultivars (2022. year)

| Cultivar        | Length<br>(cm)               | Thickness<br>(mm) | No. of<br>FBs per<br>twig | No. of<br>VBs<br>per twig | Ratio<br>FB/VB | No. of FBs<br>per 1 m |
|-----------------|------------------------------|-------------------|---------------------------|---------------------------|----------------|-----------------------|
| <b>Topper</b>   | 23.50 ±<br>13.6 <sup>†</sup> | 4.37 ± 2.5        | 6.30 ±<br>3.6 b           | 15.0 ± 8.7<br>a           | 0.44 ±<br>0.2  | 26.76 ±<br>15.5.ab    |
| <b>Presenta</b> | 30.02 ±<br>17.4              | 4.22 ± 2.4        | 6.03 ±<br>3.5 b           | 8.67 ± 5.0<br>b           | 0.73 ±<br>0.4  | 19.96 ± 11.5<br>b     |
| <b>Elena</b>    | 25.17 ±<br>14.5              | 4.93 ± 2.9        | 5.01 ±<br>2.9 b           | 7.67 ± 4.4<br>b           | 0.68 ±<br>0.4  | 20.24 ± 11.7<br>b     |

|                |                 |            |                  |                   |               |                   |
|----------------|-----------------|------------|------------------|-------------------|---------------|-------------------|
| <b>Hanita</b>  | 32.33 ±<br>18.7 | 4.82 ± 2.8 | 7.67 ±<br>4.4 b  | 14.33 ±<br>8.3 a  | 0.54 ±<br>0.3 | 24.57 ± 14.2<br>b |
| <b>Katinka</b> | 30.67 ±<br>17.7 | 5.57 ± 3.2 | 11.33 ±<br>6.5 a | 17.33 ±<br>10,0 a | 0.66 ±<br>0.4 | 37.27 ± 21.5<br>a |

<sup>†</sup>Mean values followed by the same letter within a column do not differ significantly according to Duncan's multiple range test at  $P \leq 0.05$ ; FB-Flowering buds; VB-Vegetative buds.

Our results of length of long fruiting twigs were significantly lower than in the report of *Nenadović-Mratinić et al.* (2007), who studied 20 European plum cultivars grafted on the seedling rootstock Myrobalan in conditions of Belgrade. Also, in our study the length of long fruiting twigs of cultivars Topper and Katinka was lower than in results of *Milatović et al.* (2015). *Radović et al.* (2016) studied the influence of rootstocks on the properties of fruiting twigs in plum cultivar and they found that the clonal rootstock Fereley affected the average length of long fruiting twigs of 68.2 cm. The differences can be explained by the influence of different factors such as environmental conditions, rootstocks, age of trees, yield, and cultural practices. However, one of the main reasons is significantly lower of length of long fruiting twigs is age of trees, because the orchard in the year of research was 14 years old.

Among cultivars, the average thickness of the long fruiting twigs varied from 4.22 mm (Presenta) to 5.57 (Katinka). However, significant differences in thickness of the long fruiting twigs were not found between cultivars. Results of thickness of the long fruiting twigs in our research were slightly lower by comparison with the previous findings (*Nenadović-Mratinić et al.*, 2007; *Milatović et al.*, 2015; *Radović et al.*, 2016). Cultivar Katinka had a significantly highest number of the flower buds per twig in relation to compared to other cultivars. The number of vegetative buds ranged from 7.67 (Elena) to 17.33 (Katinka), and there were statistically significant differences between the cultivars.

According to many authors (*Nenadović-Mratinić et al.*, 2007; *Thurzó et al.*, 2008; *Milatović et al.*, 2014), the density of flower buds is an important parameter which indicates the potential yield of cultivars. The highest density of flower buds on one-year old twigs (37.27 per 1 m) was in the cultivar Katinka, while this value was lowest in the cultivar Presenta (19.96 per 1 m). Results of average density of flower buds in our research were lower than in the study of *Milatović et al.* (2015) and *Nenadović-Mratinić et al.* (2007). According *Radović et al.* (2016) the highest density of flower buds on one-year old twigs (27.9) was on the medium vigorous clonal rootstock Fereley.



Very important parameter for the yield and quality of fruit is a balanced relationship between flower buds and vegetative buds. Specifically, a certain number of leaves per fruit were necessary to achieve optimal quality of fruit. The lowest ratio of flower to vegetative buds per long fruiting twig was in the cultivar Topper (0.44) while the highest ratio was in the cultivar Presenta (0.73). Results of ratio of flower to vegetative buds per long fruiting twig in our research were lower than in the study of *Milatović et al.* (2015) and *Radović et al.* (2016). The lower value of the ratio of flower to vegetative buds points to lower yield, while the higher ratio indicates a potentially higher yield, but can have negative characteristics in terms of lower fruit quality because in that case a smaller number of leaves per fruit could be expected.

The short fruiting twigs were analyzed on the two-year old twigs and their characteristics are shown in table 2.

Table 2. The properties of short fruiting twigs of plum cultivars (2022. year)

| Cultivar        | Length<br>(cm)               | No. of<br>FBs<br>per twig | No. of<br>VBs<br>per twig | Ratio<br>FB/VB   | No. of FB per 1<br>m of<br>2-year old twig |
|-----------------|------------------------------|---------------------------|---------------------------|------------------|--------------------------------------------|
| <b>Topper</b>   | 2.80 ± 1.6<br>b <sup>†</sup> | 2.30 ±<br>1.3             | 2.09 ± 1.2<br>b           | 1.12 ± 0.6<br>a  | 63.7 ± 36.8 ab                             |
| <b>Presenta</b> | 8.19 ± 4.7<br>a              | 2.65 ±<br>1.5             | 3.45 ± 1.9<br>a           | 0.76 ± 0.4<br>ab | 63.2 ± 36.5 ab                             |
| <b>Elena</b>    | 6.61 ± 3.8<br>ab             | 2.85 ±<br>1.6             | 2.87 ± 1.6<br>ab          | 0.99 ± 0.6<br>a  | 55.3 ± 31.9 ab                             |
| <b>Hanita</b>   | 6.06 ± 3.5<br>ab             | 1.56 ±<br>0.9             | 4.09 ± 2.4<br>a           | 0.33 ± 0.2<br>b  | 36.2 ± 20.9 b                              |
| <b>Katinka</b>  | 4.34 ± 2.5<br>ab             | 3.41 ±<br>1.9             | 3.65 ± 2.1<br>a           | 0.93 ± 0.6<br>a  | 93.7 ± 54.2 a                              |

<sup>†</sup>Mean values followed by the same letter within a column do not differ significantly according to Duncan's multiple range test at  $P \leq 0.05$ ; FB-Flowering buds; VB-Vegetative buds.

The average length of short fruiting twigs varied from 2.8 cm (Topper) to 8.19 cm (Presenta). Among cultivars, the differences in the length of short fruiting

twigs were statistically significant. Compared to results of *Milatovic et al.* (2015) for cultivars Topper and Katinka in our study the higher length of short fruiting twigs was found. The results of our research of length of short fruiting twigs were higher than that reported by *Nenadović-Mratinić et al.* (2007) and *Radović et al.* (2016).

Number of flower buds per short fruiting twig ranged from 1.56 to 3.41. Cultivar had not a statistically significant influence on the formation of number of the flower buds per twig. Results of number of flower buds per short fruiting twig in our research were slightly lower by comparison with the previous findings (*Nenadović-Mratinić et al.*, 2007; *Milatović et al.*, 2015; *Radović et al.*, 2016).

The number of vegetative buds was in the interval from 2.09 (Topper) to 4.09 (Hanita), and there were statistically significant differences between the cultivars. In our study, the number of vegetative buds per twig for cultivars Topper and Katinka was the higher than previously established *Milatovic et al.* (2015). Also, the obtained results for the number of vegetative buds per twig were higher that reported by *Nenadović-Mratinić et al.* (2007) and *Radović et al.* (2016).

The average ratio of flower to vegetative buds was from 0.33 (Hanita) to 1.12 (Topper). Too high ratio can indicate lower fruit quality and also greater tendency to bare wood formation. The results of our research of average ratio of flower to vegetative buds were lower than that reported by *Nenadović-Mratinić et al.* (2007) and *Milatović et al.* (2015). *Radović et al.* (2016) established that, on average for all three plum table cultivars, clonal rootstock Fereley influenced the lower ratio of flower to vegetative buds (1.0).

The number of flower buds per 1 m of 2-year old twig was lowest in the cultivar Hanita (36.2), while the highest value was in cultivar Katinka (93.7). Among cultivars, the the differences of flower buds per 1 m of 2-year old twig were statistically significant. This parameter indicates the potential yield of the cultivar, as the low density of flower buds points to a lower yield potential. Results of flower bud density on 2-years old twig in our research were lower in cultivars Katinka and Topper compared with the previous findings *Milatović et al.* (2015). Our results of the number of flower buds per 1 m of 2-year old twig are not in agreement with *Nenadović-Mratinić et al.* (2007) and *Radović et al.* (2016) who obtained slightly higher values.

The differences between ours and results of others authors in properties of short fruiting twigs of plum cultivars can be explained by the influence of different factors such as environmental conditions, rootstocks, age of trees, yield, and cultural practices.

## CONCLUSIONS

Based on the one-year trial in the region of Sarajevo, it can be concluded that the cultivar had significant influence on the some properties of long and short fruiting twigs. Namely, cultivar had not a statistically significant influence on the length, thickness and ratio of flower to vegetative buds of long fruiting twigs. Cultivar had a statistically significant influence on the length, number of vegetative buds per twig, ratio of flower to vegetative buds of short fruiting twigs and number of flower buds per 1 m of 2-year old twig.

The cultivar Katinka had a statistically significant influence on the formation of the highest number of the flower buds per twig. On the other hand, the same cultivar had the highest number of flower buds per the short fruiting twig. There were no significant different in number of the vegetative buds per long twig between cultivars Katinka, Hanita and Topper. Based on the number of flower buds per 1 m, it can be concluded that in studied plums cultivars flower bud density was higher on short fruiting twigs than on long fruiting twigs. Therefore, the short fruiting twigs are the main holders of the yield. The cultivar Katinka had the highest number of flower buds per 1 m of long fruit twig, as well as well as the highest highest number of flower buds per 1 m of 2-year old twig.

Based on these results, we can make recommendations for pruning performance. Cultivars, which are characterized with higher density of flowers buds on short and long fruiting twigs, require more severe pruning for the better quality of fruit and preventing the bare wood formation. On the other hand, the cultivar that have lower density of flower buds require pruning of lower intensity.

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## INFLUENCE OF PRETREATMENT ON PLUM DRYING RATE

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**Abstract:** Plum drying is a slow and time-consuming process because whole fruits are being dried. As drying period is limited, since conditioned by technological properties of plum suitable for this type of processing, in order to increase production, drying period should be reduced which is achieved by different pretreatments such as dipping. Another possibility of production increase is to extend drying season using frozen fruit, considering its availability throughout the whole year. Consequently, freezing can be considered as a pretreatment in prune production. In this study, plum cultivars of the Fruit Research Institute recommended for drying (‘Čačanska Rodna’ and ‘Nada’) and cultivar ‘Stanley’, most commonly used for drying, have been tested. Drying was done at an experimental convective drier at the air temperature of 90 °C and the air streaming rate of 4 m/s until 75% of total dry matter was reached. The aim of the work was to examine the drying rate of frozen plum fruits compared to dipped ones (conventional pretreatment) and non-treated fruits (control). Drying rate differed depending on varietal characteristics and pretreatments applied. The highest drying rate was in frozen fruits of cultivars ‘Čačanska Rodna’. In cultivars ‘Nada’ and ‘Stanley’, pretreatments had insignificant effect on drying rate.

**Key words:** prune, pretreatments, dipping, freezing, drying time

## INTRODUCTION

Drying is the oldest method of food preservation that perhaps has been longest studied in food processing industry. It is defined as a process of water loss resulting from the simultaneous existence of phenomenon of mass (water) transfer and heat. The easiest and most common method of plum drying is a convective drying in which water evaporation is done by heated air. As drying season is limited, since conditioned by technological properties of plum suitable for this type of processing, in order to supply sufficient quantities of raw material

for drying, cultivars ripening at different times are used, on the one hand and fruits from different localities in which the same plum cultivar ripens at different times are harvested (Mitrović et al., 2006; Mitrović et al., 2007) on the other. Moreover, since plum drying is a slow and time-consuming process due to which fruits are dried whole while skin is covered with waxy bloom representing a hydrophobic layer affecting water evaporation rate from the fruit surface, modification of the process itself by different pretreatments needs to be involved. Dipping is the most commonly applied pretreatment characterized by a short-term immersion of fruits into hot or boiling water, base solutions or fatty acid ester solutions (Pangavhane et al., 1999; Doymaz, 2004; Tarhan et al., 2007; Mitrović et al., 2018a) with the aim to distort or remove the waxy bloom without skin damage. Disturbing that hydrophobic layer on the skin of the fruit leads to a more intensive evaporation of water during drying, which results in increased drying rate, i.e. reduction of drying time, in relation to non-dipped fruits. The way to increase prune production is to extend the drying season, which can be done by using frozen fruit. By nutritional composition, frozen fruit does not differ from fresh fruit (Radović et al., 2020), and is available throughout the year. Therefore, frozen fruit is used as much as fresh fruit in human consumption and as a raw material in food industry (for sweets and bakery products, production of juices, jams, fruit purees and other products). These are the reasons why we came up to an idea to use frozen fruit as a starting material for drying, that is, to define freezing as a pretreatment in production of prunes. Therefore, the aim of the work is to examine the drying rate of frozen plum fruit in comparison to dipped (conventional pretreatment) and non-treated fruit (control).

## MATERIALS AND METHODS

In this study, we have examined the fruits of plum cultivars of Fruit Research Institute that are recommended for drying ('Čačanska Rodna' and 'Nada') and cultivar 'Stanley' most commonly used for drying in Serbia, from the plantation of Fruit Research Institute, at Preljinsko brdo site, in which agro- and pomo-technical measures for this type of species are regularly applied. For the purposes of the experiment, fruits of approx. same average mass (37.80 g, 42.70 g and 37.50 g, respectively) are picked selectively, at the stage of technological maturity for processing by drying with the content of total dry matter of 19.45%, 19.50% i 19.50%, respectively. Such fruits were prepared immediately after harvest in three ways: as control (non-treated fruits), dipping (immersing fruits into boiling water for 20 seconds) and freezing (fruits were frozen at  $-20^{\circ}\text{C}$  and as such dried with no prior defrost).

Drying of fruits was done in an experimental drier for testing the convective drying process (Kandić et al., 2006). An air streaming drying procedure was applied at a constant air temperature of  $90^{\circ}\text{C}$ , and the air streaming rate in the drying chamber cross section was 4 m/s. Direction of vertical air streaming during drying was alternatively and periodically changed at 1 hour

intervals, and fruit mass on trays being measured at 1 hour intervals as well. On a stainless steel tray, plum fruits were placed in one layer (3500 g), and 6 trays were placed in drying chamber. Two variants of the experiment were dried at the same time, control and dipped fruits, on trays placed in drying chamber symmetrically so that the same drying conditions on all trays were enabled. The experiment with frozen plum was done separately, after two months of storage at  $-20^{\circ}\text{C}$ , in the same conditions applied for fresh fruit as well. Drying was finished once the fruits reached 75% total dry matter.

The initial dry matter of fresh plum fruits and final dry matter of prunes were determined by standard method, by drying at  $105^{\circ}\text{C}$  until constant mass was reached.

Nomenclature:

- DM [kg DM/kg] –dry matter content;
- W [kg W/kg] – moisture content on a wet base;
- U [kg W/kg DM] – moisture content on a dry base;
- DR – drying rate;

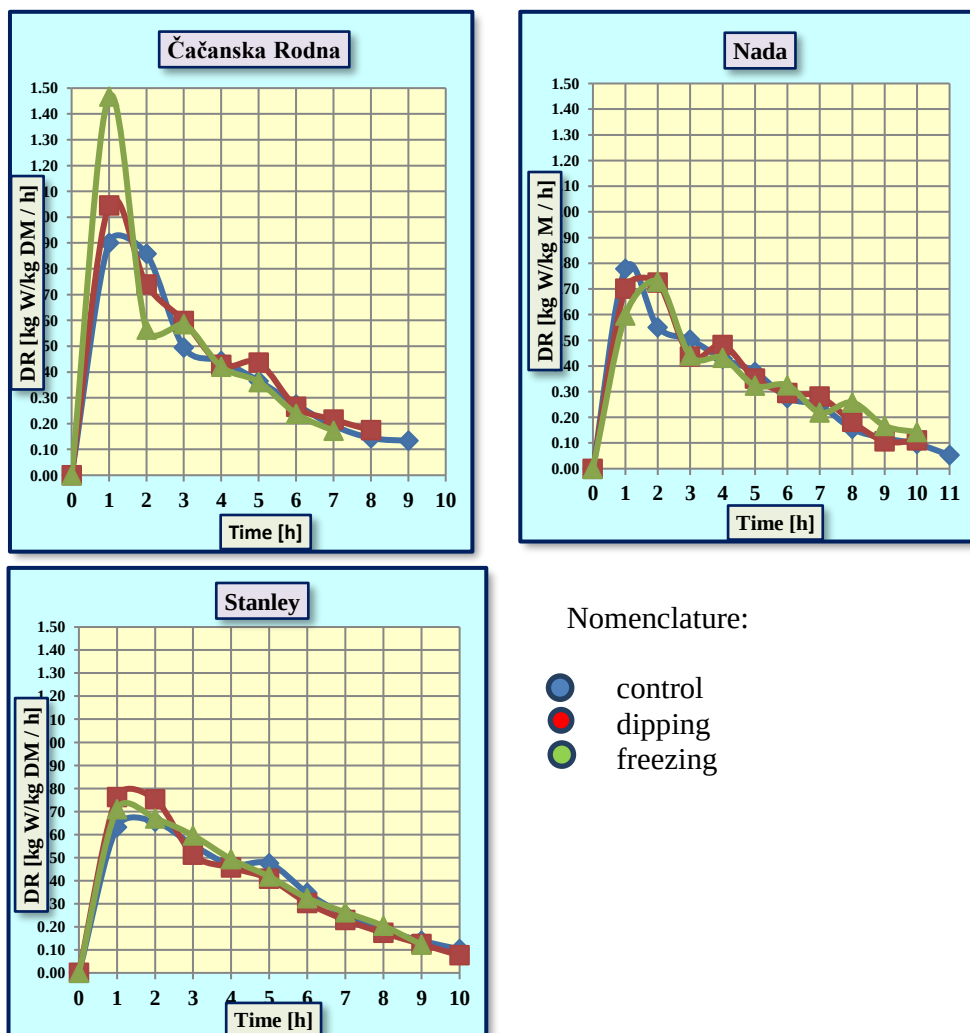
$$\text{DR} = \Delta U / \Delta \tau \text{ [kg W/kg DM / h]}$$

## RESULTS AND DISCUSSION

Drying kinetics can be presented by different curves showing time changes of different units of the condition of basic material drying, e.g. the units describing the condition of fruits of the tested plum cultivars. Since dry matter mass remains unchanged during drying, using the unit moisture content on a dry base (U) it is possible to much better observe different drying kinetics of the tested plum cultivars.

In Graphic 1 drying rate curves, e.g. curves of moisture content change based on the fruits of tested plum cultivars on a certain tray representing control, dipped and frozen fruits are shown. Since, at the beginning of drying process, drying rate equals zero, drying rate curves start from the origin of ordinates, i.e. from zero value. By analysing Graphic 1, it is concluded that fruits of different cultivars had different drying rates although of similar mass and the same ripeness degree. The highest drying rates at the beginning of drying were found in fruits of 'Čačanska Rodna' amounting/weighing 1.46 [kg W/kg DM / h] in frozen fruits, 1.04 [kg W/kg DM / h] in dipped fruits and 0.90 [kg W/kg DM / h] in control. In other cultivars, drying rates at the beginning had far lower values from 0.8–0.6 [kg W/kg DM / h]. In fruits of cultivar 'Čačanska Rodna' drying rate of frozen fruits at the beginning of drying was highest compared to other two treatments

(control and dipping). In cultivar 'Stanley', the highest drying rate was in dipped fruits while in cultivar 'Nada', drying rates were of similar values in all three applied variants of the experiment (control and pretreatments). The highest drying rates were at the beginning of drying, that is, after 1h of drying, in all examined cultivars and in all three variants of the experiment. High drying rate achieved at the beginning of drying was enabled by evaporation of a large quantity of free water from plum fruits. Testing the drying kinetics of green pepper, Darvishi et al. (2013) have concluded that high drying rate at the beginning of the drying process is reached due to the fact that material dried at the beginning of drying contains the highest moisture and that a diffusion is a predominant physical mechanism of steam flow inside green pepper.



Graphic 1. Drying rate of tested plum cultivars depending on different pretreatments



As drying continues, drying rate decreases, i.e. drying process enters the drying rate descending phase (Data, 2007), though water evaporation from fruits is still intense. Table 1 shows the loss of free water in relation to the total evaporated water for the fruits of the tested plum cultivars in 2h intervals in relation to the drying start, expressed in percentages, for the fruits representing control, dipped and frozen fruits. Since in our study, during drying, we have changed vertical air streaming at 1h intervals, after 2h of drying all trays in the drying chamber were under identical drying conditions. Therefore, in Table 1, values of free water loss at every 2h were shown in order to better observe differences between cultivars and variants of the experiment (control and pretreatments).

After 2h of drying, the loss of free water in relation to the total evaporated water differed in tested cultivars while in control, the lowest values compared to pretreatments applied were found. In cultivar 'Čačanska Rodna', the loss of free water after 2 h of drying was 53.32% in frozen fruits compared to 46.21% in control and 46.31% in dipped fruit.

Table 1. The loss of free water in relation to the total evaporated water for the fruits of the tested plum cultivars in intervals of 2h in relation to the drying start, expressed in percentages

| Cultivar       | Intervals of time from the drying start | Loss of free water in relation to the total evaporated water (%) |         |          |
|----------------|-----------------------------------------|------------------------------------------------------------------|---------|----------|
|                |                                         | control                                                          | dipping | freezing |
| Čačanska Rodna | 0 – 2 h                                 | 46.21                                                            | 46.31   | 53.32    |
|                | 0 – 4 h                                 | 70.86                                                            | 74.29   | 79.72    |
|                | 0 – 6 h                                 | 87.65                                                            | 89.74   | 95.47    |
|                | 0 – 8 h                                 | 96.49                                                            | 100     | –        |
| Nada           | 0 – 2 h                                 | 36.50                                                            | 40.00   | 36.50    |
|                | 0 – 4 h                                 | 62.26                                                            | 65.78   | 60.51    |
|                | 0 – 6 h                                 | 80.23                                                            | 83.90   | 78.36    |
|                | 0 – 8 h                                 | 91.27                                                            | 96.89   | 91.47    |
|                | 0 – 10 h                                | 97.20                                                            | 100     | 100      |
| Stanley        | 0 – 2 h                                 | 33.77                                                            | 39.87   | 36.30    |
|                | 0 – 4 h                                 | 60.63                                                            | 65.40   | 64.97    |
|                | 0 – 6 h                                 | 82.19                                                            | 84.12   | 84.50    |
|                | 0 – 8 h                                 | 93.65                                                            | 94.72   | 96.78    |
|                | 0 – 10 h                                | 100                                                              | 100     | –        |

In cultivars 'Nada' and 'Stanley', the greatest loss of water was in dipped fruit, 40% and 39.87%, respectively, compared to control and frozen fruit. Dipping fruits in boiling water removes the wax bloom from the fruit skin, because it

represents a hydrophobic part of the skin disturbing the undisturbed water evaporation during drying (Goyal et al., 2007), due to which the intensity of water evaporation at the drying start was more intense compared to control that also leads to a higher drying rate, which is in agreement with results of Mitrović et al. (2018b) and Doymaz and Pala (2002). Ismail et al. (2008) tested the influence of fatty acid bases and esters, as dipping means, during drying of grapes at different temperatures have come to conclusion that each dipping leads to reduction of drying time. While drying frozen fruits, one part of the air-drying heat is directed to transition of water crystals into water, and then to the free water evaporation due to which in cultivars 'Nada' and 'Stanley', quantity of evaporated water at the drying start (2h after) was lower compared to dipped fruit. The exception was 'Čačanska Rodna' in whose frozen fruits the greatest loss of free water was at the drying start in comparison to dipped fruits and control, which can be explained by varietal characteristic. This is the reason why frozen fruits of 'Čačanska Rodna' dried in the shortest time as water loss at the drying start was over 50%, and in other cultivars frozen fruits dried in shorter time compared to control.

## CONCLUSION

Based on the results of our examinations, it can be concluded that pretreatments applied (dipping and freezing) affect drying rate increase at the temperature of 90 °C, i.e. reduce the drying rate in tested cultivars of 'Čačanska Rodna', 'Nada' and 'Stanley'. Frozen fruits in all tested cultivars dried in shorter time compared to control. Fruits of 'Čačanska Rodna' dried in the shortest time compared to cultivars 'Nada' and 'Stanley' in all variants of the experiment (control, dipping and freezing), that can be explained by varietal characteristic. Freezing can be recommended as a pretreatment in plum drying technology with the aim to extend the plum drying season.

## ACKNOWLEDGEMENTS

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## **ZOOTECHNICS**



## THE PROTECTIVE EFFECT OF MORINGA OLEIFERA LEAVES POWDER ON THE CHEMICAL, MICROBIAL AND SENSORY EVALUATION OF CATFISH PRODUCT

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### Abstract:

*Fish is considered as one of the cheapest sources of animal protein all over the globe. It provides protein, vitamins, minerals and omega-3 fatty acids for human diets. It represents one of the national income sources in Egypt. The Egyptian Fisheries Authority declared in 2020 that, the production of fish reached 1 million 920 thousand tons. Since African catfish is one of the fish species consumed in Egypt, this study was done to use the fresh (Nile Karmout) catfish (*Clarias gariepinus*) to produce catfish burger, and study the effect of adding 1% of Moringa leaves powder to the formula on the chemical composition, microbiological stability, and sensory evaluation of the product. The data showed that the addition of 1 % moringa leaves powder to the catfish burger caused reduction in the water holding capacity, and increased the cooking loss while reduced the number of the examined bacterial count and improved the sensory evaluation compared to the control samples.*

**Key words:** catfish burger, moringa leaves powder

### Introduction

Fish is one of the best and cheapest sources of animal protein, minerals, and other essential nutrients for human diets across the globe. It is an important source of protein, vitamins, minerals, and omega-3 fatty acids in poor countries. In Egypt, fish is one of the most important sources of national income. Furthermore, fish is also considered a source of safe protein which is used to meet food needs and develop other industries. The Egyptian fisheries cover more than 13 million acres, which equates to about 150 percent of the land. African catfish is one of the most popular fish species for fish farming on the African continent. It has high growth rates, a high resistance to environmental stress, and a low level of handling stress during farming operations (Suleiman, 2009). Its meat tastes good, so there has been a lot of interest in its farming in recent years. Several species of catfish live in the Arab Republic of Egypt, including the following: 1- African catfish *Clarias*

gariepinus 2- Egyptian catfish *Clarias anguillaris*. The African catfish, *Clarias gariepinus*, is the most abundant species in Egypt. Catfish (*Clarias gariepinus*) are among the freshwater fishes which are well adapted to enclosed waters and resistant to diseases. They are produced in large quantities.

According to Aref et al., (2018) they determined that the moisture content of minced catfish fillet (*Clarias gariepinus*) was 76.48%, that the ash content was 1.22%, that the crude protein content was 19.88%, and that the crude acid extract was 1.63%. Meanwhile (Zhu et al., 2015) discovered that the chemical composition of minced catfish fillet (*Clarias gariepinus*)  $68.77 \pm 2.21\%$ ,  $16.01 \pm 0.35\%$ ,  $13.47 \pm 0.81\%$ , and  $1.10 \pm 0.05\%$  for moisture, crude protein, crude either extract and total ash contents respectively. Ozogul et al., (2011), found that in good quality fish, the maximum TBA value was 5 mg malonaldehyde (MA/kg), and fish with high TBA value (8 mg MA/kg) can be consumed. Development of off-flavors and odors in fish and seafood products, which indicates of quality loss could be resulted from Lipid oxidation.

Level of total volatile basic nitrogen (TVB-N) can be used as an effective and useful indicator to measure seafood spoilage According to (EU, 1995). The European Commission stated that, the amount of (TVB-N) can be used as indicator when sensory methods increment doubts about the freshness of seafood products. Water-holding capacity, is closely related to fish and meat juiciness, texture, and other quality attributes (Jiang et al., 2018). It can be significantly affected by some processing methods such as freezing (Jiang et al., 2018).

*Moringa oleifera* (*Moringa oleifera* Lam.) is considered to be an important source of natural antioxidant, and antimicrobials (Dillard et al., 2000; and Hazra et al., 2012). Its leaves provide an excellent source of natural antioxidants, which can extend the shelf life of fat- containing foods due to the availability of flavonoids, ascorbic acid carotenoids, and polyphenols (Al-Juhaimi et al., 2015). Food products such as fish and other sea foods are extremely sensitive to chemical and microbiological spoilage during processing and storage. Therefore, adequate preservation methods are needed in order to maintain the safety and quality of such products and to extend their shelf life (Hassoun and Çoban, 2017). Most sea food products deteriorate due to microorganisms. It is believed that bacterial growth is the main cause of fish and fish product spoilage. Therefore, bacterial count should be used to monitor the quality of food products (Aref et al., 2018).

Sensory evaluation is a type of testing or assessment that shows how consumers react to food products using their senses. This is a way of determining whether or not a product is accepted or rejected by the consumers.

Boran and Köse., (2007), found a correlation between lipid oxidation and sensory deterioration in prepared fish products stored in the freezer. Consequently, lipid

oxidation should be retarded to keep sensory and nutritional values of fish products during processing or storage.

The following were the goals of this investigation:

- 1- Produce minced catfish flesh from fresh (Nile Karmout) catfish (*Clarias gariepinus*).
- 2- 2-Adding 1% Moringa oleifera leaf powder to minced catfish flesh to make ready-to-eat catfish burgers and evaluating moisture, crude protein, Ash, pH, total acidity, color, water holding capacity (WHC), TBA values, TVB-N values, microbiological quality, and sensory assessment for the product.

### Materials and Methods

**Moringa leaves powder:** Moringa plant was gained from the university farm, El-Minia, Egypt. The leaves were thoroughly cleaned, air dried for 24 hours at room temperature, then mechanically ground into 60 mesh particles.

**Spices** (black pepper, cumin powder, thyme powder, onion powder, and garlic powder), salt, and wheat flour were acquired from Minia city's local market.

**Methods:**

**Preparation of cat fish product:** : sixty kg of live karmout catfish (*Clarias gariepinus*) utilised in this study came from a local market in El-Minia, Egypt, with an average length of 572 cm and a weight of  $1450 \pm 10$  g. Fish were iced and delivered directly to Minia University's Department of Food Science Laboratory. The fish were thoroughly washed, gutted, manually made into fillet and mechanically minced by a meat grinder using 4 mm (coarse). Minced fish was stored refrigerated at 4°C until use.

Catfish burger product was prepared according to the recipe described by Magdy (2010) and shown in (table 1).

Table (1) Formula of catfish burger product.

| Ingredient           | %   |
|----------------------|-----|
| Ground catfish Flesh | 90  |
| Wheat flour          | 3.5 |
| Salt                 | 2.5 |
| Cumin powder         | 1   |
| Garlic powder        | 1   |
| Onion powder         | 1   |
| Black pepper powder  | 1   |



After thoroughly mixing all the ingredients, the bulk was divided into two equal portions. The first one was left as control (with no additives), while dried Moringa leaves powder was added to the other one in the ratio of (1 %).

Each portion was divided into small balls 50 g each, and formed into a burger-like shape 10cm diameter and 0.5 cm thickness, then placed in polystyrene tray with LDPE sheets in between (Figure 1).

Analytical methods:

Chemical composition: Moisture, crude protein, crude fat, and ash, contents were determined as described in the (A.O.A.C, 1995).

Determination of pH: pH was measured by digital pH meter according to (Ramadhan et al., 2011). About 10 g of sample was mixed with 90 ml of distilled water using homogenizer and the pH was noted.

Determination of total acidity: The total acidity for fish burger was determined by titration at zero time according to the method described by (Keeton and Melton, 1978). The acidity was expressed as lactic acid.

Determination of Total volatile basic nitrogen (TVB-N): The total volatile bases were estimated as described by A.O.A.C (1995). 10g of sample was added to the heating flask containing 300 ml distilled water plus 2 g magnesium oxide. In the receiving flask 25 ml of boric acid (2%) and few drops of methyl red indicator were added. After 25 minutes of distillation, the content of the receiving flask was transferred to another flask and titrated with 0.05N (H<sub>2</sub>SO<sub>4</sub>).

The total volatile basic nitrogen (TVB-N) was determined as follows:

$$(\text{TVB-N}) = \frac{(V \times N \times 100 \times 14)}{W}$$

Where:

V=volume (ml) H<sub>2</sub>SO<sub>4</sub> used for sample.

N=normality of H<sub>2</sub>SO<sub>4</sub>.

W=weight of sample in grams.

Determination of Thiobarbituric acid (TBA): The thiobarbituric acid (TBA) was performed as described by Tarladgis et al. (1969).

Physical analysis:

Determination of water holding capacity (W.H.C): Water holding capacity (W.H.C) was measured according to Alvarez, et al. (1992).

While, expressible water is calculated as follow:

$$\text{Expressible water (\%)} = \frac{\text{PPW} - \text{APW}}{\text{PPW}} \times 100$$

Where:

PPW = pre-pressed weight and APW = after-pressed weight

Water holding capacity is calculated as follows:

$$\text{Water holding capacity (\%)} = \% \text{Moisture} - \% \text{Expressible water}$$

Determination of color (L, a, and b) and whiteness: Color values (L\*, a\*, and b\*) were measured for fish fillet mince, by a colorimeter (Color Tec PCM Color Meter Tec. NJ. USA). The value L\* is a measure of lightness, a\* represents the chromatic scale from +a redness to –a greenness and b\* represents the chromatic scale from +b yellowness to –b blueness. Three random measurement spots on each sample were made and the average data were recorded (Foh et al., 2011).

Cooking loss measurement: Cooking loss was measured according to the method of Niamnuy et al., (2008) and was calculated from the differences in the mass of minced before and after cooking as follow:

$$\% \text{Cooking loss} = \frac{(\text{Mass before cooking} - \text{Mass after cooking})}{\text{Mass before cooking}} \times 100$$

#### Microbiological analysis:

Total plate count (TPC): Total plate count (TPC) was determined according to the method of Harrigan and McCance, (1976). The results were described as (CFU/g).

Psychrophilic bacteria: Psychrophilic bacteria was made as (CFU/g) according to the methods described by Collins et al. (1989).

Coliform bacterial count: The method of (Difco Laboratories Manual, 1998) was used to determine the coilform bacterial count.

Sensory evaluation: Sensory evaluation for color, taste, odor, texture, and overall acceptability for the cooked untreated and treated catfish burger were carried out in order to determine the consumer acceptability for the product according to the methods described by Eyo, (1983). Ten judges were participated in this test. A numerical hedonic scale ranged between 1 and 10 (1 for very bad, and 10 for excellent) was used for sensory evaluation.

## Results and Discussion

Table 1 shows the impact of 1 % moringa leaf powder on the chemical composition, pH value, and total acidity (lactic acid) of catfish burgers.

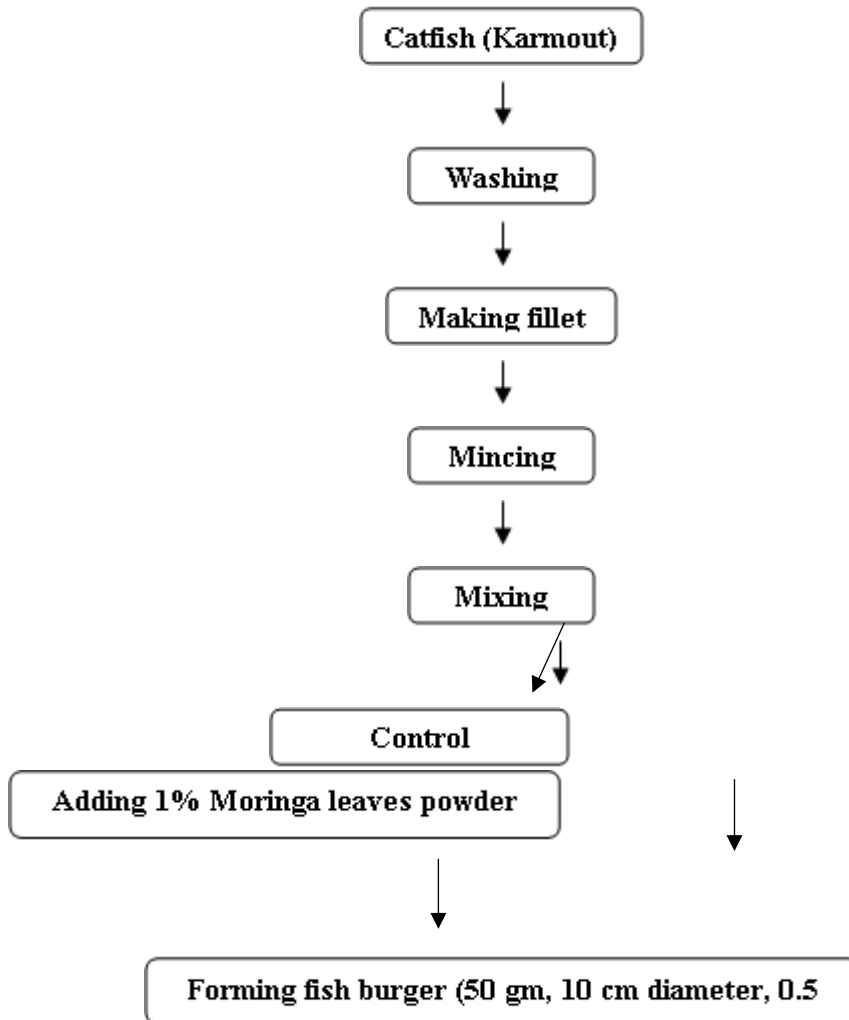


Fig. 1. Flow diagram of production of catfish burger.

The moisture content values reduced as a result of the moringa powder addition due to the lower moisture content of dried moringa leaves compared to catfish meat, but the crude protein, crude ether extract, and ash levels were unaffected. The sample containing 1 % moringa leaves powder had a higher overall acidity value (reported as lactic acid) than the control, resulting in a lower pH value for the moringa powder containing sample. The findings are consistent with those of (Mahmoudzadah et al., 2010) and (Zhu et al., 2015).

Table (2) Effect of adding 1 % moringa leaves powder on the chemical composition of catfish burger % (wet weight basis).

| Parameters                  | Control    | 1 % moringa powder |
|-----------------------------|------------|--------------------|
| Moisture content            | 69.31±0.15 | 67.77±0.57         |
| Crude protein (N x 6.26)    | 17.69±0.23 | 17.40±0.59         |
| Crude either extract        | 4.54±0.11  | 4.44±0.19          |
| Ash content                 | 4.13±0.10  | 4.09±0.07          |
| pH                          | 6.31±0.01  | 6.26±0.01          |
| Total acidity (lactic acid) | 0.66±0.01  | 0.72±0.05          |

Means of 3 replicates ±SD

Total volatile basic nitrogen compounds (TVB-N) or volatile amines are three substances that include dimethylamine (DMA), trimethylamine (TMA), and ammonia (NH<sub>3</sub>). The activity of spoilage bacteria intensified after the death of the fish, resulting in the conversion of trimethylamine oxide to trimethylamine (TMA), dimethylamine (DMA), and other chemicals as a result of this action.

Table 1 shows the influence of 1 % moringa leaves powder on total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA), and color values (L\*, a\*, b\*) of catfish burger (3). For the sample containing 1 % moringa leaves powder, the findings showed a reduction in both (TVB-N) and (TBA) values. Because moringa leaves have been recognized as an effective source of natural antioxidants, the addition of moringa powder reduced the (TVB-N) and (TBA) values while suppressing the development of total volatile basic nitrogen (TVB-N) (Al-Juhaimi et al., 2015). The addition of 1 % moringa leaves powder resulted in a decrease in L\* and b\* values as well as an increase in a\* value (Avile's, et al.).

Table (3) Effect of adding 1 % moringa leaves powder on the (TVB-N), (TBA), and color values (L\*, a\*,b\*) of catfish burger % (wet weight basis).

| Parameters                 | Control    | 1 % moringa powder |
|----------------------------|------------|--------------------|
| TVB-N (mg/100g)            | 9.09±0.56  | 8.79±0.39          |
| TBA (mg malonaldihiyde/kg) | 1.20±0.01  | 1.06±0.01          |
| L*                         | 42.33±0.46 | 37.67±0.66         |
| a*                         | 14.09±0.14 | 16.98±0.29         |
| b*                         | 21.89±0.13 | 18.90±0.37         |

Means of 3 replicates ±SD

Figure 2 shows the effect of adding 1 % moringa leaves powder to a catfish burger's water holding capacity (WHC), expressible water (EW), and cooking loss. The addition of 1 % moringa leaves powder resulted in a decrease in water holding capacity and an increase in both expressible water and cooking loss, according to the findings. The control sample's reduced cooking loss was due to the high WHC value, which indicates a high power to collect water in the protein matrix.

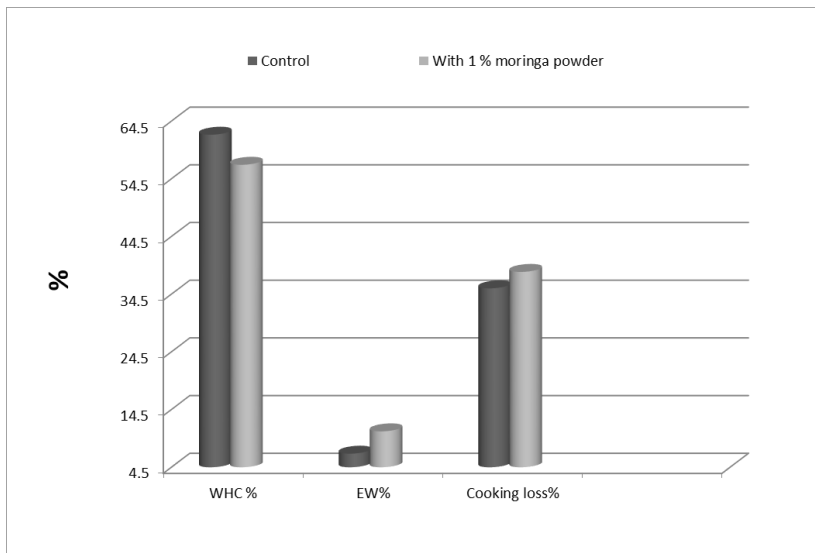


Figure (2) Effect of adding 1 % moringa leaves powder on water holding capacity (WHC), expressible water (EW), and cooking loss of catfish burger.

The microbiological safety and quality of fish and fish products are very important to processors, retailers, and of course consumers. Due to its nature, fish is generally susceptible to contaminate with pathogens and other microorganisms. The effect of adding 1 % moringa leaves powder on the total count, the psychrophilic bacterial count, and E.coli of catfish burger (log CFU/g) is shown in figure (3). The data showed that the addition of 1 % moringa leaves powder to the catfish burger reduced all the examined bacterial counts (total, psychrophilic, and E.coli) due to the antimicrobial effect of moringa leaves powder. That means the addition of moringa powder to the formula negatively affected the surviving of the microorganisms that contaminating the catfish burger. The results come in agreement with (Zhu et al., 2015).

Quality of catfish products is generally determined by sensory evaluation. Figure 4, illustrates the effect of 1% moringa leaves powder on the sensory evaluation

(color, taste, odor, texture, and general acceptability) of catfish burger. The results showed that adding 1% moringa leaves powder to the catfish formula increased the color, taste, odor, texture, and general acceptability values compared to the control sample.

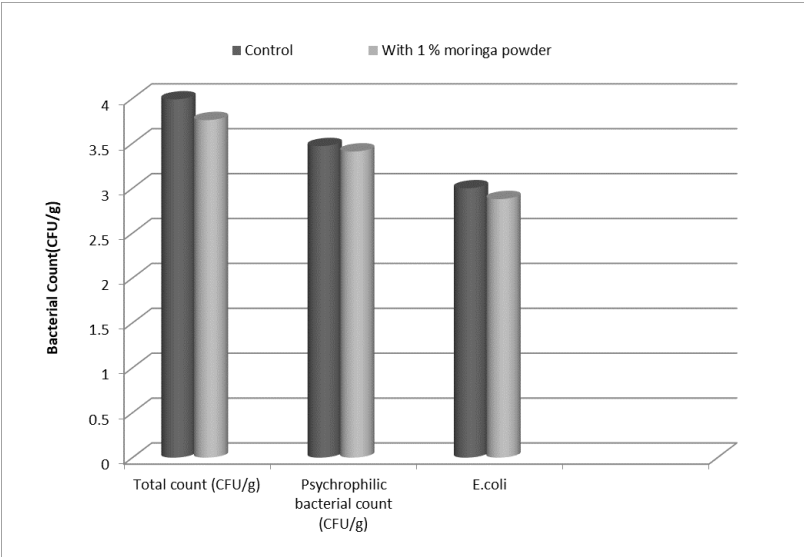


Figure (3) Effect of adding 1 % moringa leaves powder on the total count, the psychrophilic bacterial count, and E.coli of catfish burger (log CFU/g).

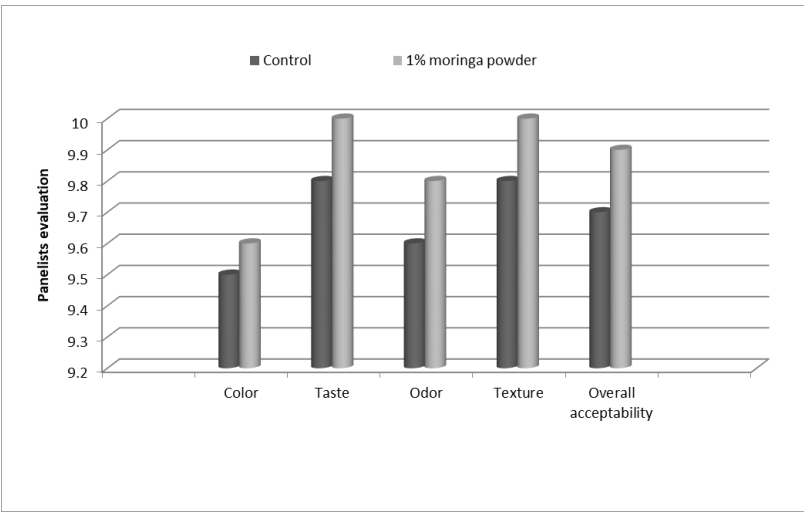


Figure (4) Effect of adding 1 % moringa leaves powder on the sensory evaluation of catfish burger.

## Conclusion

Fish provides proteins, vitamins, minerals and omega-3 fatty acids for human consumption. Since African catfish is one of the fish species consumed in Egypt, this study was done to use fresh (Nile Carmout) catfish (*Claries gariepinus*) for the production of catfish burgers and studied the effect of adding 1% Moringa leaf powder to the chemical formula. composition, microbiological stability and sensory evaluation of the product. The obtained research results showed that the addition of 1% moringa leaf powder to catfish burgers leads to a decrease in water retention capacity and increased cooking losses, while the number of tested bacteria is reduced and sensory evaluation is improved compared to control samples. This paves the way for increasing the range of fish used in human nutrition.

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## BEES NOSEMOSIS IN ROUMANIA - THERAPEUTIC EFFICACY OF PLANT DIETARY SUPPLEMENT

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**Abstract:** *Honeybee nosemosis, produced by two microsporidii species, Nosema ceranae and Nosema apis, is a serious disease which develops diarrhea, reducing honey production, increased mortality in winter and underdeveloped colony passed through illness. For treatment were tested over 200 compounds but only fumagillin and several organic compounds of mercury gave acceptable results, with some disadvantages: Nosema spores does not affect; residues in bee products. In this context, the aim of study was to evaluate the therapeutic efficacy of a plant dietary supplement which is a patent application and it is based on the use of conventional active principles from different herbs and digestive tract content acidification of bees with vinegar. The study was performed in Romania, at the families of bees from different hives located in different counties in the country. In order to establish the level of infection with Nosema spp., before and after treatment, were examined individually bee intestines through macroscopic and microscopic examinations. Infestation levels were correlated with symptoms more or less obvious, and the data obtained before and after treatment were statistically analyzed using SPSS-IBM software (cross-tab analyzes and Chi-Square test). The results showed o high efficacy of plant dietary supplement which destroys the parasites; no remanence hive products; can be applied to both nutritious and sprayed onto the surface of the frame; no side effects.*

**Key words:** *Nosemosis, bees, Plant dietary supplement*

Nosemosis is one of the most common and widespread diseases of adult honeybees. The causative agents, *Nosema apis* and *Nosema ceranae*, belong to microsporidia some obligate intracellular eukaryotic parasites and they develop a serious disease which determine diarrhea, reducing honey production, increased mortality in winter and underdeveloped colony passed through illness [1, 2, 3, 4, 5, 6].

Whether it is *Nosema apis* or *Nosema ceranae* involved in the ethiology of disease, therapeutic intervention is imperative. The use of synthetic or biosynthetic products, topically applied or as a supplement to food, is known in the treatment of bees' nosemosis. The best results are obtained by combining the two ways of administration, thus reducing environmental contamination [7, 8, 9].

According to EU requirements, it is mandatory to give up allopathic therapies that leave residues in these products and find natural remedies that are effective but which, to the same extent, not to hurt the hive.

In this context, the aim of the study was to test the therapeutic efficacy of Plant Dietary Supplement used for prevention and control of nosemosis in bees from Timis, Bihor, Brasov, Ilfov, Piatra Neamt and Hunedoara Counties.

## Material and methods

The study was performed in Romania, at the families of bees from different hives located in 6 counties in the country (13 localities). Bee colonies were divided into 2 groups in each county: group I treated with Plant dietary supplement and group 2 - control group that received only sugar syrup 1: 1. From each family were collected samples bee before and after treatment and were kept in alcohol 60 ° (8070 honeybee samples). In order to establish the level of infection with *Nosema spp.*, before and after treatment, were examined individually bee intestines through macroscopic and microscopic examinations. Bee samples were examined in the Parasitology Laboratory – Faculty of Veterinary Medicine Timisoara by qualitative method. The Plant dietary supplement is a Patent application no 000113/B0 administered in therapeutically nutritious at 200 ml / colony in the first treatment day and 100 ml / colony in the second and third therapeutic activity. For spraying inside the hive, apple vinegar, fresh grape wine and garlic tincture are added to the dietary supplement. Infestation levels were correlated with symptoms more or less obvious, and the data obtained before and after treatment were statistically analyzed using the SPSS-IBM software (cross-tab analyzes and Chi-Square test).

## Results and discussions

Bee samples collected were positive as a whole, variations in the level of infestation by *Nosema* from + (poor infestation) to +++ (massive infestation). We emphasized spores of *Nosema spp.* by microscopic examination (fig.1).



Fig. 1. *Nosema* spp. – spores

The degree of honeybee infestation before treatment according to the location of samples collection is presented in Tabel 1 and figure 2.

Table 1. Degree of infestation before treatment by microscopic examination

| Nrcrt | Location   | Level of infestation before treatment |       |       |       | Total  |
|-------|------------|---------------------------------------|-------|-------|-------|--------|
|       |            | 0                                     | 1     | 2     | 3     |        |
| 1     | Alios      | 7                                     | 17    | 22    | 25    | 71     |
|       |            | 9.9%                                  | 23.9% | 31.0% | 35.2% | 100.0% |
| 2     | Barateaz   | 3                                     | 7     | 0     | 0     | 10     |
|       |            | 30.0%                                 | 70.0% | 0.0%  | 0.0%  | 100.0% |
| 3     | Denta      | 21                                    | 42    | 25    | 9     | 97     |
|       |            | 21.6%                                 | 43.3% | 25.8% | 9.3%  | 100.0% |
| 4     | Gad        | 3                                     | 8     | 0     | 0     | 11     |
|       |            | 27.3%                                 | 72.7% | 0.0%  | 0.0%  | 100.0% |
| 5     | Herandasti | 60                                    | 46    | 193   | 137   | 436    |
|       |            | 13.8%                                 | 10.6% | 44.3% | 31.4% | 100.0% |
| 6     | Hisias     | 9                                     | 11    | 10    | 1     | 31     |
|       |            | 29.0%                                 | 35.5% | 32.3% | 3.2%  | 100.0% |
| 7     | Ianova     | 6                                     | 0     | 0     | 0     | 6      |
|       |            | 100.0%                                | 0.0%  | 0.0%  | 0.0%  | 100.0% |
| 8     | Mierlău    | 4                                     | 0     | 0     | 0     | 4      |
|       |            | 100.0%                                | 0.0%  | 0.0%  | 0.0%  | 100.0% |

|    |           |       |        |       |       |        |
|----|-----------|-------|--------|-------|-------|--------|
| 9  | Balotesti | 0     | 8      | 0     | 0     | 8      |
|    |           | 0.0%  | 100.0% | 0.0%  | 0.0%  | 100.0% |
| 10 | Gârcina   | 3     | 7      | 5     | 0     | 15     |
|    |           | 20.0% | 46.7%  | 33.3% | 0.0%  | 100.0% |
| 11 | Opatia    | 19    | 20     | 22    | 4     | 65     |
|    |           | 29.2% | 30.8%  | 33.8% | 6.2%  | 100.0% |
| 12 | P. Brasov | 27    | 13     | 0     | 0     | 40     |
|    |           | 67.5% | 32.5%  | 0.0%  | 0.0%  | 100.0% |
| 13 | Resita    | 0     | 0      | 10    | 3     | 13     |
|    |           | 0.0%  | 0.0%   | 76.9% | 23.1% | 100.0% |
|    | Total     | 162   | 179    | 287   | 179   | 807    |
|    |           | 20.1% | 22.2%  | 35.6% | 22.2% | 100.0% |

#### Legenda

1 positive sample – 10 examined bees

0 – control group

1 - + poor infestation

2 – ++ medium infestation

3 - +++ massive infestation

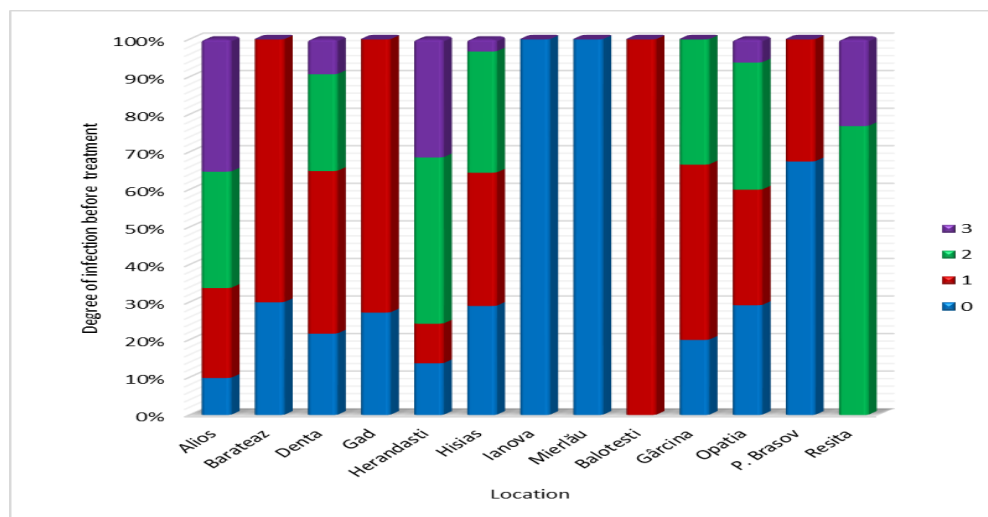


Fig. 2. Descriptive statistics of infestation degree before treatment

Following administration of the plant dietary supplement there is a remarkable increase in negative samples and free of *Nosema* colony. The degree of honeybee infestation after treatment according to the location of samples collection is shown in the table 2 and figure 3.

Table 2. Degree of infestation after treatment by microscopic examination

| No | Localitatea | Level of infestation after treatment |       | Total  |
|----|-------------|--------------------------------------|-------|--------|
|    |             | 0                                    | 1     |        |
| 1  | Alios       | 63                                   | 8     | 71     |
|    |             | 88.7%                                | 11.3% | 100.0% |
| 2  | Barateaz    | 10                                   | 0     | 10     |
|    |             | 100.0%                               | 0.0%  | 100.0% |
| 3  | Denta       | 88                                   | 9     | 97     |
|    |             | 90.7%                                | 9.3%  | 100.0% |
| 4  | Gad         | 11                                   | 0     | 11     |
|    |             | 100.0%                               | 0.0%  | 100.0% |
| 5  | Herandasti  | 409                                  | 27    | 436    |
|    |             | 93.8%                                | 6.2%  | 100.0% |
| 6  | Hisias      | 30                                   | 1     | 31     |
|    |             | 96.8%                                | 3.2%  | 100.0% |
| 7  | Ianova      | 6                                    | 0     | 6      |
|    |             | 100.0%                               | 0.0%  | 100.0% |
| 8  | Mierlau     | 4                                    | 0     | 4      |
|    |             | 100.0%                               | 0.0%  | 100.0% |
| 9  | Balotesti   | 8                                    | 0     | 8      |
|    |             | 100.0%                               | 0.0%  | 100.0% |
| 10 | Gârcina     | 13                                   | 2     | 15     |
|    |             | 86.7%                                | 13.3% | 100.0% |
| 11 | Opatia      | 65                                   | 0     | 65     |
|    |             | 100.0%                               | 0.0%  | 100.0% |

|    |           |        |       |        |
|----|-----------|--------|-------|--------|
| 12 | P. Brasov | 40     | 0     | 40     |
|    |           | 100.0% | 0.0%  | 100.0% |
| 13 | Resita    | 11     | 2     | 13     |
|    |           | 84.6%  | 15.4% | 100.0% |
|    | Total     | 758    | 49    | 807    |
|    |           | 93.9%  | 6.1%  | 100.0% |

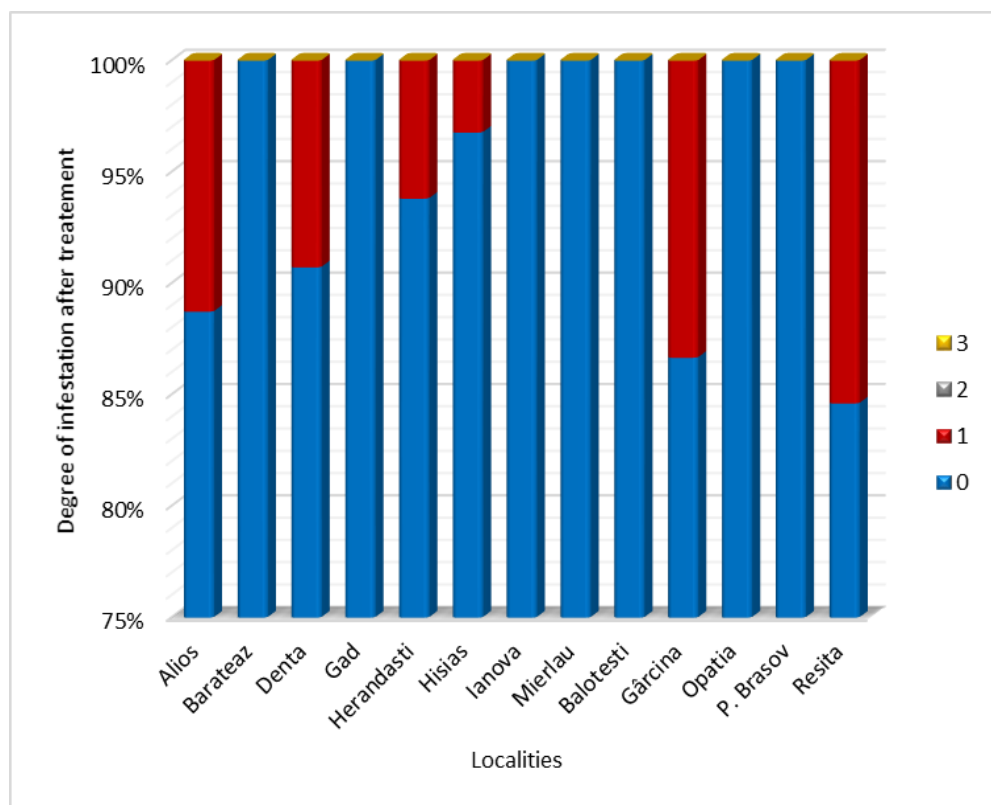


Fig. 3. Degree of infestation after Plant dietary supplement administration

In this study, the proposed invention is in line with European requirements, proving effective in reducing *Nosema spp.* infestation and without side effects. It is based on the use of active principles in different medicinal herbs and on the acidification of the content of the bee's digestive tract with apple vinegar. The data collected before and after the treatment administration were statistically analyzed and significant differences were obtained ( $\chi^2 = 926.227, p = 0.000$ ), which demonstrates the efficacy of the treatment used. The

degree of efficacy of the treatment is 100% if the initial degree of infestation was "1" (+ poor infestation), as observed in the localities: Barateaz, Gad, Balotesti and Poiana Brasov. The results of descriptive statistics show that the efficacy of Plant dietary supplement is 92%.

Some of the feasible alternative treatments that are used to control this disease are plant extracts. There are studies, in Roumania and in other countries which emphasised the importance of alternative therapy used in prevention and control of honeybee nosemosis [10, 11, 12, 13, 14].

Ten sulphated polysaccharides from algae were evaluated for their antimicrosporidian activity. The most efficient polysaccharides were then tested for their ability to inhibit the growth of *Nosema ceranae* in experimentally-infected adult honeybees. A decrease in parasite abundance but not in mortality rate was also observed with an iota carrageenan. The results of this study suggest that algal sulphated polysaccharides could be used to prevent and/or control bee nosemosis [15].

In the other study, the authors have evaluated the effects of essential oils of Chilean plant species (*Cryptocarya alba*), which is used against *N. ceranae*, and to identify and quantify the majority active compounds in the EO as well as their potential use for the control of nosemosis. The results showed that *C. alba* oil may be a candidate for the treatment or prevention of nosemosis [16].

## Conclusions

- The Plant dietary supplement which contains only natural ingredients with antiseptic and antibiotic properties is recommended for prevention and control of nosemosis in bees.
- The advantages of this invention are: 92% efficacy, no remanence hive products; can be applied to both nutritious and sprayed onto the surface of the frame; no side effects.

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## GRANULATED MINERALS IN THE RATIONS OF LACTATING COWS

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### Abstract

*Research has been conducted on the cows of Ayrshire breeds. In the course of the research were studied the chemical composition of feed and their residues, excretions (feces, urine) of animals according to the classical methods of zootechnical analysis. Milk production was determined individually from each cow on the basis of control milking according to the schedule. A milk sample was taken once a month to determine the quality indicators of milk. These samples were used to evaluate the Share of fat and protein, Dry matter (DM), Dry skimmed milk residue (DSMR).*

*Cows of the 2nd experimental group, which received a granulated minerals with the diet (option 2 recipe 2) were superior in average daily milk yield. The difference in their favor compared to the control was 5,12 %. The cows that received the studied additive (option 2 recipe 1) also outperformed the cows from the control group by 3,38 % in this indicator.*

*Tested granulated minerals were introduced into the rations of cows, there were changes in the content of the fraction of fat and protein in milk. In the milk of cows in the control group these figures were 4,22 % and 3,2 %, respectively. In the 2nd experimental group, the fat content was the highest and amounted up to 4,2 %. A similar situation was with the protein content in milk, this figure was at the level of 3,24 %.*

*The introduction of the studied feed additives into the diets also contributed to an increase in the content of dry matter (DM) and DSMR in milk. The highest rates were observed in the 2nd experimental group (12,87 % and 8,62 %, respectively), where the cows were fed a granulated minerals (option 2 recipe 2).*

**Key words:** cows, ration, granulated minerals, milk productivity

## INTRODUCTION

The condition for the effectiveness of dairy cattle breeding is the search and introduction of feed products based on the rational use of local resources. For lactating cows, a balanced diet for minerals is of great importance. The role of the macro- and microelements in the metabolic processes of lactating cows is known - with a deficiency of individual elements a decrease in productivity is observed and there is a threat of diseases. It has been proven that minerals play an important role - they are part of body tissues and complex organic compounds. Also they are involved in metabolism. In the body of an animal there are 65 mineral elements that are not synthesized in tissues, therefore, they must be supplied with feed and water.

In the rumen of ruminants, under the action of proteolytic enzymes of microorganisms, vegetable feed proteins are broken down to peptides, amino acids, and then to ammonia. Rumen microorganisms can use not only protein, but also non-protein nitrogenous substances. Therefore, part of the protein in the diet of ruminants can be replaced with synthetic urea (carbamide). Carbamide contains 45 % nitrogen, it is advisable to add it to the feed both to save protein and as a nitrogen source for microorganisms. Carbamide in the rumen of adult ruminants is broken down by the bacterial enzyme urease to ammonia and carbamic acid. From ammonia and digestion products of feed carbohydrates, microorganisms synthesize a more complete protein of their body, which includes many independent amino acids.

Commercial urea contains about 42 % nitrogen. Since protein contains 16 % nitrogen, one weight part of carbamide can replace 2,6 weight parts of protein. One kilogram of carbamide in combination with feed carbohydrates can replace 6-7 kg of protein-rich cake.

Inclusion in the main diet of cows of extruded amidoconcentrate additives based on carbamide and polysaccharide, as well as fillers from soybeans and sunflower meal in the amount of 500 g/head provided (Udovitskaya A.V., 2015) an increase in milk productivity by 8,9 % ( $p < 0,01$ ) (up to 16,9 kg of average daily milk yield).

When cows were fed a complex mineral supplement with vitamins A, D, E in animals of the experimental group, the average daily milk yield (Samokhina A.A., Gamko L.N., 2018) increased by 1,52 kg, or 10,67 % ( $P < 0,01$ ). The inclusion of a complex mineral supplement with carbamide and vitamins A, D, E in the composition of the feed mixture for lactating cows made it possible to increase the average daily milk yield in the experimental group by 1,08 kg, or 7,67 % ( $P < 0,05$ ).

The use of urea in animal nutrition allows for an increase in the protein content in the diet of animals and, consequently, in products of animal origin. Also, the use of carbamide is more cost-effective in comparison with the use of roughage from rapeseed, sunflower, soybeans.

## **MATERIAL AND METHOD**

The study of the effectiveness of the use of a granulated minerals was carried out at two enterprises in the Volgograd region on cows of the Holstein and Ayrshire breeds.

The granulated materials includes urea, monoammonium phosphate, chalk, brucite, sodium chloride, which makes it possible to use this product in cattle diets.

At each of the enterprises for the experiment were formed 3 groups of lactating cows - 1 control and 2 experimental. Animals were selected according to the principle of pair-analogues, taking into account breed, age, live weight, productivity and number of lactations. Each group had 10 heads. The duration of the experiment was 133 days, including 7 days of the preliminary period, 6 of the transition period and 120 of the basic period.

In the course of the scientific and economic experiment were studied the chemical composition of feed and their residues, excretions (feces, urine) of animals according to the classical methods of zootechnical analysis.

Milk production was determined individually from each cow on the basis of control milking according to the schedule. During the control milking, a milk sample was taken once a month to determine the quality indicators of milk. These samples were used to evaluate the Share of fat and protein, Dry matter (DM), Dry skimmed milk residue (DSMR), the density was determined using a milk analyzer. Protein content in milk samples was estimated by colorimetric method.

Accounting of milk and the selection of average samples for analysis were conducted at each milking. Milk samples were about 1-2 % of milk yield. The minimum daily sample size is 100 ml.

The physiological state of the animals was determined by clinical and hematological parameters. To study clinical parameters the pulse (heart rate), body temperature, and respiratory rate were determined. Rumination was calculated for 2 minutes by movement of rumen.

The control of the physiological state of cows was carried out by examining blood taken from 3 experimental animals of each group from the jugular vein.

Studies on the effectiveness of the use of granulated minerals based on mineral raw materials in feeding dairy cows were carried out in the "Agrofirma" Vostok" Nikolaevsky district of the Volgograd region. The scheme of the experiment carried out on lactating cows of the Ayrshire breed is presented in Table 1.

Table 1– Scheme of the experiment

| Group          | Number of heads | Feeding conditions                                   |
|----------------|-----------------|------------------------------------------------------|
| Control        | 10              | Regular ration (RR)                                  |
| 1 Experimental | 10              | RR + granulated minerals complex (option 2 recipe 1) |
| 2 Experimental | 10              | RR + granulated minerals complex (option 2 recipe 2) |

The composition of the regular ration for dairy cows included alfalfa hay - 3.2 kg, cereal-bean haylage - 20 kg, corn silage - 18 kg, compound feed - 4 kg, as well as amido-mineral supplement in the amount of 200 g. A mineral supplement in the amount of 200 g was in the 1st experimental group and 250 g in the 2nd experimental group. The recipes of the studied additives are presented in Table 2.

Analysis of the content of nutrients, macro- and microelements allowed us to conclude that cows of all groups received them in the required amount, according to the feeding standards.

Table 2 – Recipe for granulated minerals for lactating cows

| Composition            | Mineral Balance Additive, % |          |
|------------------------|-----------------------------|----------|
|                        | recipe 1                    | recipe 2 |
| Carbamide              | 22,7                        | 29,4     |
| Monoammonium phosphate | 22,7                        | 29,4     |
| Chalk                  | 45,5                        | 29,4     |
| Brucite                | 9,1                         | 11,8     |
| Sodium chloride        | 0                           | 0        |

Necessary to take into account milk productivity and assess the effect of a particular additive in the feeding of dairy cows. In the course of scientific and economic experience it was revealed that the use of the studied granular minerals

had a positive effect on the average daily milk yield of cows and milk quality indicators, which is reflected in Table 3.

According to obtained data the cows of the 2nd experimental group, which received a granulated minerals with the diet (option 2 recipe 2) were superior in average daily milk yield. The difference in their favor compared to the control was 5,12 %. The cows that received the studied additive (recipe 1) also outperformed the cows from the control group by 3,38 % in this indicator.

At the same time, it should be noted that when the tested granulated minerals were introduced into the diets of cows, there were slight changes in the content of the mass fraction of fat and protein in milk. In the milk of cows in the control group these figures were 4,22 % and 3,2 %, respectively. In the 2nd experimental group, the fat content was the highest and amounted up to 4,2 %. A similar situation was with the protein content in milk, this figure was at the level of 3,24 %.

Table 3 - Milk productivity of cows and milk quality, ( $X \pm m$ )

| Indicator                          | Group              |                                    |                    |
|------------------------------------|--------------------|------------------------------------|--------------------|
|                                    | Control            | 1<br>Experimental                  | 2<br>Experimental  |
| Average daily milk yield, kg       | 17,430 $\pm$ 0,510 | <b>18,020<math>\pm</math>0,130</b> | 18,320 $\pm$ 0,610 |
| Share of fat, %                    | 4,220 $\pm$ 0,020  | 4,230 $\pm$ 0,030                  | 4,250 $\pm$ 0,040  |
| Share of protein, %                | 3,200 $\pm$ 0,070  | 3,220 $\pm$ 0,080                  | 3,240 $\pm$ 0,040  |
| Dry matter (DM), %                 | 12,680 $\pm$ 0,160 | 12,780 $\pm$ 0,140                 | 12,870 $\pm$ 0,180 |
| Dry skimmed milk residue (DSMR), % | 8,460 $\pm$ 0,070  | 8,550 $\pm$ 0,090                  | 8,620 $\pm$ 0,070  |
| Lactose, %                         | 4,580 $\pm$ 0,050  | 4,590 $\pm$ 0,040                  | 4,640 $\pm$ 0,070  |
| Cinder, %                          | 0,690 $\pm$ 0,006  | 0,740 $\pm$ 0,007                  | 0,740 $\pm$ 0,006  |
| Calcium, %                         | 0,129 $\pm$ 0,002  | 0,131 $\pm$ 0,001                  | 0,132 $\pm$ 0,002  |
| Phosphorus, %                      | 0,097 $\pm$ 0,001  | 0,099 $\pm$ 0,001                  | 0,100 $\pm$ 0,002  |
| Density, g/cm <sup>3</sup>         | 1,029 $\pm$ 0,007  | 1,030 $\pm$ 0,009                  | 1,031 $\pm$ 0,007  |
| Acidity, T°                        | 17,890 $\pm$ 0,08  | 17,910 $\pm$ 0,090                 | 17,91 $\pm$ 0,110  |

The introduction of the studied feed additives into the diets also contributed to an increase in the content of dry matter (DM) and DSMR in milk. The highest rates were observed in the 2nd experimental group (12,87 % and 8,62 %, respectively), where the cows were fed a granulated minerals (option 2 recipe 2).

A similar results was observed for the content of calcium and phosphorus in milk. Due to the introduction of the studied additives, these indicators were higher compared to the control.

There were no significant changes in the density and acidity of milk. Thus, the use of granulated minerals based on mineral raw materials in the diets of dairy cows had a positive effect on their milk productivity. During the scientific experiment were studied the digestibility of nutrients in the diets of experimental cows. These studies included physiological experience, determination of the balance of nitrogen and minerals. They were carried out on 9 experimental cows-analogues.

Table 4 - Coefficients of digestibility of diet nutrients by cows, %

| Indicator                       | Group      |                |                |
|---------------------------------|------------|----------------|----------------|
|                                 | Control    | 1 Experimental | 2 Experimental |
| Dry matter                      | 67,26±0,78 | 68,68±0,83     | 68,83±0,87     |
| Organic matter                  | 69,01±0,98 | 70,29±1,05     | 70,44±0,99     |
| Crude protein                   | 65,06±0,77 | 66,08±0,92     | 66,23±0,98     |
| Crude fiber                     | 59,41±0,72 | 60,85±0,75     | 61,00±0,81     |
| Crude fat                       | 66,44±0,61 | 67,40±0,72     | 67,55±0,68     |
| Nitrogen-free extractives (NFE) | 78,64±0,86 | 79,67±0,92     | 79,82±0,93     |

Analysis of the obtained results showed that throughout the scientific study, the consumption of feed and nutrients in total in cows of all groups was the same. This suggests that the animals of all groups ate the given diet in full.

An experiment on the digestibility of nutrients, taking into account data on feces, made it possible to determine the amount of digested nutrients (Table 4).

The use of granulated minerals in the diets of cows contributed to better digestion of nutrients compared to the control, which was reflected in the digestibility coefficients.

The coefficient of digestibility of dry matter in the control group was 67,26 %, and in the experimental group – 68,68 % and 68,83 %, which is higher compared to the control by 1.42 % and 1,57 %, respectively. Organic matter was digested better by cows receiving the studied mineral complex by 1,28 % and 1,43 %, respectively. The superiority of the experimental groups in the digestion



of crude protein was 1,02 % and 1,17 %, crude fiber – 1,44 % and 1,59 %, crude fat – 0,96 % and 1,11 %, NFE – 1,03 % and 1,18 % respectively.

In order to more fully explore the effectiveness of the use of granular minerals by dairy cows was studied the balance of nitrogen in their body. It should be noted that the diets of the experimental animals differed in the amount of nitrogen supplied with the feed; the addition of the studied minerals to the diet increased this indicator depending on the recipe and dose of input.

Based on the data obtained in the balance experiment and on the basis of the chemical composition of feed, their residues, feces, urine, the nitrogen balance was studied, which serves as an indicator of the use of protein in the animal body (Table 5.)

Table 5 - Balance and use of nitrogen in cows, g/head

| Indicator         | Group       |                |                |
|-------------------|-------------|----------------|----------------|
|                   | Control     | 1 Experimental | 2 Experimental |
| Taken with food   | 378,05±1,25 | 382,39±1,33    | 387,93±1,36*   |
| Leave:            |             |                |                |
| with feces        | 132,09±1,84 | 129,71±1,96    | 131,01±1,89    |
| with urine        | 148,60±2,19 | 147,66±2,32    | 147,98±2,26    |
| with milk         | 89,24±1,57  | 92,84±1,72     | 94,97±1,64     |
| Total leave       | 369,93±2,47 | 370,21±2,64    | 373,96±2,68    |
| Digested          | 245,96±1,91 | 252,68±2,11    | 256,93±2,04*   |
| Balance           | 8,12±0,72   | 12,18±0,89*    | 13,98±0,97*    |
| Used for milk, %: |             |                |                |
| from accepted     | 23,61±0,31  | 24,28±0,42     | 24,48±0,37     |
| from digested     | 36,28±0,47  | 36,74±0,65     | 36,96±0,59     |

The table 5 shows that cows of different groups consumed different amounts of nitrogen. So, analogues of the 1st experimental group consumed nitrogen by 1,15 %, 2nd experimental group - by 2,61 % more than their peers from the control. Since the milk yields of cows in the first and second experimental groups were higher than in the control group, their nitrogen excretion with milk was higher. So, in the 1st and 2nd experimental groups, this indicator was higher by 4,03 % and 6,42 %, respectively, compared with the control group.

It should be noted that during the physiological experiment, all experimental cows had a positive nitrogen balance: in the control group it was 8,12 g (the lowest indicator), in the 1st experimental group – 12,18 g (which is

4,06 g more than the control) , and in the 2nd experimental group – 13,98 g (which is 5,86 % more than the control).

The use of granulated minerals in the diets of cows contributed to an increase in the protein nutritional value of the feed. For the synthesis of milk proteins, experimental cows used more nitrogen per milk. Thus, the peers of the control yielded to the analogues of the experimental groups by 0,67 % and 0,87 % - from the accepted, and by 0,46 % and 0,68 % - from the digested.

Consequently, the use of the studied granular minerals in feeding lactating cows had a positive effect on the exchange of nitrogen in their bodies and contributed to an increase in the coefficient of its use. Higher rates of digestion, deposition in the body, usage, including for milk, were established when cows were fed diets with the studied additive option 2 recipe 2.

Mineral substances play an extremely important role in the vital activity of the organism of animals, their productive and reproductive health.

Of all the mineral compounds of the animal's body, up to 75 % are macronutrients such as calcium and phosphorus. That is why data on the use of calcium and phosphorus are the main indicators characterizing the metabolism in the body and the availability of minerals.

To identify the effect of feeding the studied granular mineral complexes on calcium metabolism in the body, its balance was studied in lactating cows (Table 6).

Table 6 - Balance and use of calcium in cows, g/head

| Indicator                    | Group       |                |                |
|------------------------------|-------------|----------------|----------------|
|                              | Control     | 1 Experimental | 2 Experimental |
| Taken with food              | 122,19±0,95 | 123,87±1,15    | 124,68±1,07    |
| Leave: with feces            | 83,32±1,52  | 82,14±1,64     | 81,70±1,35     |
| with urine                   | 5,81±0,09   | 5,53±0,11      | 5,39±0,08*     |
| with milk                    | 22,48±0,63  | 23,43±0,69     | 24,00±0,58     |
| Balance                      | 10,58±0,75  | 12,59±0,83     | 13,41±0,92     |
| Used for milk from intake, % | 18,40±0,55  | 19,06±0,67     | 19,40±0,57     |
| Total used from accepted, %  | 33,46±0,57  | 34,44±0,64     | 34,95±0,69     |

Calcium intake in all groups was different - it was the lowest in animals of the control group (122,19 g), while the experimental analogues received it in the diet by 1,68 g and 2,49 g more. The cows of the control group excreted 22,48 g of calcium with milk, which turned out to be less than in the 1st experimental group by 4,22 % and the 2nd experimental group - by 6,76 %.

As well as the nitrogen balance, the calcium balance in all experimental groups was positive, however, the difference between the experimental groups over the control was higher in the 1st experimental group by 2,01 g and in the 2nd experimental group by 2,83 g.

Due to the different amounts taken with feed and excreted calcium, as well as deposited in the body, its use from that taken was 33,46 % in the control group, 34,44 % and 35,95 % in the experimental groups. At the same time, this macronutrient was used for milk from the accepted 18,40 % (control), 19,06 % and 19,40 % in the experimental groups.

Thus, all cows were provided with calcium, only the cows of the experimental groups used it better, in contrast to the analogues of the control group.

Along with nitrogen and calcium in the balance experiment, the exchange of phosphorus in the body of dairy cows of the control and experimental groups was considered. It should be noted that a similar pattern was observed for the use of phosphorus by the cows of the experimental groups (Table 7).

Table 7 - Balance and use of phosphorus in cows, g/head

| Indicator                    | Group      |                |                |
|------------------------------|------------|----------------|----------------|
|                              | Control    | 1 Experimental | 2 Experimental |
| Taken with food              | 68,04±0,51 | 69,44±0,57     | 70,22±0,59     |
| Leave: with feces            | 46,24±0,54 | 46,11±0,63     | 46,23±0,66     |
| with urine                   | 3,32±0,14  | 3,43±0,11      | 3,35±0,15      |
| with milk                    | 16,91±0,29 | 17,84±0,32     | 18,32±0,33*    |
| Balance                      | 1,57±0,12  | 2,06±0,15      | 2,32±0,13*     |
| Used for milk from intake, % | 24,85±0,69 | 25,69±0,72     | 26,09±0,74     |
| Total used from accepted, %  | 27,16±0,69 | 28,66±0,78     | 29,39±0,72     |

The level of phosphorus consumption was in the range of 68,04-70,22 g. At the same time, the animals of the experimental groups consumed more phosphorus, the difference with the analogues of the control group was 1,4 g (2,05 % higher) and 2,18 g (3,20 % above) respectively.

With milk, the animals of the control group excreted 16,91 g of phosphorus, which is less than the same indicator of the 1st experimental group by 5,5 %, and the 2nd experimental group - by 8,34 %. Phosphorus balance in all groups was also positive. In the control group, it was 1,57 g, which turned out to be less than in the 1st experimental group by 0,49 g and in the 2nd experimental group by 0,75 g. At the same time, more phosphorus was used for milk synthesis in the experimental groups compared to the control: from the taken by 0,84 % and 1,24 %, and from the digested - by 1,50 % and 2,23 % compared with the control.

It can be noted that the introduction of the studied granulated minerals into the diet contributed to an increase in the use of calcium and phosphorus in the body of experimental animals.

## CONCLUSION

Based on the results of the research, it can be concluded that there were changes in the content of the fraction of fat and protein in milk. In the milk of cows in the control group these figures were 4,22 % and 3,2 %, respectively. In the 2nd experimental group, the fat content was the highest and amounted up to 4,2 %. A similar situation was with the protein content in milk, this figure was at the level of 3,24 %.

The introduction of the studied feed additives into the diets contributed to an increase in the content of dry matter (DM) and DSMR in milk. The highest rates were observed in the 2nd experimental group (12,87 % and 8,62 %, respectively), where the cows were fed a granulated minerals (option 2 recipe 2). Cows of the 2nd experimental group, which received a granulated minerals with the diet (option 2 recipe 2) were superior in average daily milk yield. The difference in their favor compared to the control was 5,12 %. The cows that received the studied additive (option 2 recipe 1) also outperformed the cows from the control group by 3,38 % in this indicator.

Granulated minerals in the diets of cows contributed to better digestion of nutrients compared to the control, which was reflected in the digestibility coefficients. The coefficient of digestibility of dry matter in the control group was 67,26 %, and in the experimental group – 68,68 % and 68,83 %, which is higher

compared to the control by 1.42 % and 1.57 %, respectively. Organic matter was digested better by cows receiving the studied mineral complex by 1.28 % and 1.43 %, respectively. The superiority of the experimental groups in the digestion of crude protein was 1.02 % and 1.17 %, crude fiber – 1.44 % and 1.59 %, crude fat – 0.96 % and 1.11 %, NFE – 1.03 % and 1.18 % respectively.

During the physiological experiment, all experimental cows had a positive nitrogen balance: in the control group it was 8.12 g (the lowest indicator), in the 1st experimental group – 12.18 g (which is 4.06 g more than the control) , and in the 2nd experimental group – 13.98 g (which is 5.86 % more than the control).

Calcium intake in all groups was different - it was the lowest in animals of the control group (122.19 g), while the experimental analogues received it in the diet by 1.68 g and 2.49 g more. The cows of the control group excreted 22.48 g of calcium with milk, which turned out to be less than in the 1st experimental group by 4.22 % and the 2nd experimental group - by 6.76 %.

The level of phosphorus consumption was in the range of 68.04-70.22 g. At the same time, the animals of the experimental groups consumed more phosphorus, the difference with the analogues of the control group was 1.4 g (2.05 % higher) and 2.18 g (3.20 % above) respectively.

Introduction of the studied granulated minerals into the diet contributed to an increase in the use of calcium and phosphorus in the body of experimental animals.

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## EXAMINATION OF GENETIC AND PHENOTYPIC TRENDS OF SOME BREEDING AND REPRODUCTIVE TRAITS OF THE SOUTH KAZAKH SHEEP POPULATION

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**Abstract:** *The data of scientific research are reflected and proved in the paper, which can serve as a certain contribution to zootechnical science for the purpose of using in practice the breeding-tribal work in conditions of different forms of ownership, as well as at improvement of breeding-tribal work and production technology of high quality, competitive and ecologically clean sheep production in conditions of maximum year-round seasonal use of foothill and foothill-steppe pastures in the south of Kazakhstan.*

**Keywords:** *selection, genotypic variability, wool cutting, heredity, correlation, recurrence, erythrocytes, inbreed leukocytes.*

### INTRODUCTION

Sheep breeding is a strategic and traditional branch of livestock breeding of the Republic of Kazakhstan and plays a huge role in meeting the needs of the national economy in specific raw materials and food products.

The achieved results in sheep breeding of Zhambyl region cannot be recognized as high due to insignificant specific weight of fine and equated wool. Besides, quality indicators and wool cutting vary greatly by years. Growth of modification variability of features does not allow to estimate precisely genotype of animals by their phenotype and, as a result, the efficiency of selection work decreases.

The main reasons can be cited as insufficient specificity of selected features on individual fine wool varieties, incomplete understanding of the gradation of wool length in individual parts of the animal body, undeveloped perfect genetic methods of breeding, weak study of the peculiarities of variability, inheritance, and the relationship of selected features [1].

As the genetic basis of selection is variability, the study of regularities of variability of productive features of animals in populations of thin-wool sheep breed South-Kazakh merino is of fundamental importance for theoretical and practical purposes of selection.

The progress of each breed and the increase of its genetic value largely depend on the presence in it of animals of different intra-breed types with their 8 distinctive productive qualities and biological features. The higher or lower level of development of separate leading economic useful features is undoubtedly connected with biological features of animals. They consist in increased viability and efficiency of animals of separate types [2]. At breeding animals "in purity" first of all requires studying of economic and biological features of the main structural units of the breed, leading from which is the presence of intra-breed types and revealing of the most desirable of them and development of scientifically based principles and methods of transformation of undesirable ones. In this aspect, the study of variability of selected features in new natural climatic and forage conditions for animals of different inbred types of the given breed of sheep in the conditions of "Batay-Shu" LLP of Zhambyl region is of scientific and practical interest that determines the urgency of this work.

The purpose and objectives of the research - to establish the regularities of intra-population correlated variability of the main economic characteristics for the development of genetic bases for the improvement of reproductive qualities and productivity increase of South-Kazakh merinos bred in the conditions of "Batay-Shu" LLP of Zhambyl region.

## **MATERIAL AND METHODS**

The research and production experience was conducted in "Batay-Shu" LLP, Shu District, Zhambyl region.

The object of research were the South Caucasian sheep merino (group I) and sheep mix (♂ Australian merino x ♀ South Kazakhstan merino) (group II).

Age-related changes in body weight of the resulting offspring were studied by weighing them at birth, at 4, 7 and 18 months of age. At the same time, external body mass measurements were taken from these animals at the same time.

Absolute, average daily and relative increases in their live weight were determined based on the weighting data. Exterior physique features were studied



based on the results of the main body measurements (height in the crest, height in the sacrum, oblique length of the body, chest depth, width in the breasts, chest girth and pelvic girth), as well as on the indices of physique (length, stretch, hip, pelvic, knee, massiveness, chest and bone).

When boning and shearing test sheep, the following items are individually taken into account: live weight, cut of unwashed wool and staple length, as well as samples of wool for laboratory tests.

When studying the reproductive capacity of mothers, fertility was taken into account, as well as the safety of young animals during chops. Fertility and fecundity of rams were determined by the number of viable lambs received from each hundred of mothers according to mating and calving data. At the same time, the survival rate of lambs from birth to chop was taken into account.

For biochemical studies of animal blood (types of transferrin and hemoglobin) the method of horizontal electrophoresis in starch gel was used, followed by coloring of gels by conventional methods. Genetic analysis of the population was carried out using mathematical indicators, where the frequency of alleles and genotypes, the estimation of gene balance was determined in accordance with the Hardy-Weinberg law.

Economical efficiency was calculated on the basis of animal productivity indicators taking into account the cost of growing, the cost per cent of growth (tenge), profits from the sale of meat and young animals in live weight.

## RESULTS AND DISCUSSION

Theoretical concepts of correlative variability are the basis for many fundamental generalizations of modern biology and zootechnology. Since correlative variability is of general biological nature, the study of this phenomenon has always been promising in cognitive and applied terms. Therefore, interest in the problem of correlative variability does not weaken in all periods of scientific development.

The correlative variability of blood and productivity indices of South Caucasian sheep merino has been studied on 1.5-year-old lambs of "Batay-Shu" LLP.

Productivity indices of sheep were determined for 6 features and also blood was taken to study hematological indices for 14 features. The results of determination of average development level and indicators of variability of these signs are given in Table 1.

Table 1. The correlative variability of blood and productivity indices

| Symbol                                       | n  | $\bar{X} \pm m_x$ | $\sigma$ | Cv   |
|----------------------------------------------|----|-------------------|----------|------|
| Dressing of unwashed wool, kg                | 50 | 6,81 $\pm$ 0,053  | 0,81     | 12,1 |
| Staple length, cm                            | 50 | 9,36 $\pm$ 0,059  | 0,90     | 9,6  |
| Live weight, kg                              | 50 | 54,0 $\pm$ 2,784  | 4,26     | 7,9  |
| Pure fiber output, %                         | 50 | 39,5 $\pm$ 0,390  | 5,07     | 12,9 |
| Slicing of pure fiber, kg                    | 50 | 2,68 $\pm$ 0,034  | 0,44     | 16,4 |
| Coefficient of woolliness, g                 | 50 | 57,0 $\pm$ 0,823  | 10,7     | 18,8 |
| Erythrocytes, million in 1 mm <sup>3</sup> . | 50 | 8,99 $\pm$ 0,048  | 0,66     | 7,4  |
| Leukocytes, thousand in 1 mm <sup>3</sup> .  | 50 | 6,90 $\pm$ 0,138  | 1,74     | 25,9 |
| Hemoglobin, g%                               | 50 | 8,85 $\pm$ 0,174  | 2,48     | 27,9 |
| Catalase, mg H <sub>2</sub> O                | 50 | 2,16 $\pm$ 0,039  | 0,55     | 25,9 |
| Peroxidase, with                             | 50 | 37,5 $\pm$ 0,379  | 5,36     | 14,3 |
| Acid capacity, mg%                           | 50 | 35,6 $\pm$ 11,39  | 161      | 45,2 |
| Acid phosphatase, BE                         | 50 | 1,01 $\pm$ 0,030  | 0,40     | 39,8 |
| Alkaline phosphatase, CU                     | 50 | 9,29 $\pm$ 0,368  | 4,98     | 53,7 |
| AST, 1 mm <sup>3</sup> .unit.                | 50 | 50,9 $\pm$ 0,412  | 5,44     | 10,7 |
| Alt, 1 mm <sup>3</sup> .                     | 50 | 28,4 $\pm$ 0,171  | 2,23     | 7,8  |
| Aldolase, uh...                              | 50 | 2,73 $\pm$ 0,125  | 1,43     | 52,4 |
| Albumins, g                                  | 50 | 4,84 $\pm$ 0,106  | 1,33     | 27,6 |
| Globulins, g                                 | 50 | 2,04 $\pm$ 0,089  | 1,11     | 54,5 |
| Total protein, g%                            | 50 | 6,88 $\pm$ 0,046  | 0,58     | 8,4  |

As can be seen, the productivity of test lambs was quite high, and blood parameters are within physiological norms. Nevertheless, comparatively high values of coefficients of variability of acid blood capacity, globulin content, aldolase activity, alkaline and acid phosphatase should be noted. A similar pattern with respect to variation of interior and biochemical characteristics has been observed before in studies of sheep and other animal species [3].

Proceeding from general genetic concepts about intochromosomal interaction of genes and their inheritance, the theoretical premise of the genetic mechanism of supposed links between polymorphic blood systems and economically useful features of animals, some authors consider pleiotropic effect, when the gene of this or that blood group has direct or indirect influence on the other feature, linked to the study of genes, located in one chromosome, as blood groups can be in the same chromosome with genes, which control the blood group.

Most scientists believe that productive and breeding qualities, as well as adaptive properties of animals are determined by the level of biochemical processes in the body [3]. Researches of many scientists proved the possibility of using in selection the polymorphism of proteins, including serum proteins [4].

In our studies we studied genotypic features of serum proteins composition, the level of activity of aminotransferral enzymes, as well as their inheritance and relationship to the productivity of rams, South-Kazakh merino (group I) and in the mixed rams (♂ Australian merino x ♀ South Kazakhstan merino) (group II).

Significant inter-breed differences in the content of total protein, its different fractions and in the value of albumin-globulin coefficient have been revealed (Table 2).

As it can be seen from the data of Table 2, the largest content of total protein and its albuminous fraction is found in the animals of Group II, the superiority of which over animals of Group I was 1.9, 3.2 and 23.3; 15.9%.

Depending on the ratio of protein fractions, the albumin-globulin coefficient (A/G) in the sheep of group I was 0.66, and in group II - 0.96. The highest indexes of globulin fraction had rams-producers of group I - 47,7% against 40,1% in rams of group II.

Table 2. Protein fractions and immunoglobulin in the blood serum of two rams genotypes

| Indicator              | Group |       |
|------------------------|-------|-------|
|                        | I     | II    |
| Total protein, g%      | 7,28  | 7,42  |
| Albumins, % prealbumin | 2,43  | 2,24  |
| Albumin                | 22,70 | 27,64 |
| Postalbumin            | 6,25  | 8,80  |

|                         |                 |                 |
|-------------------------|-----------------|-----------------|
| Globulin, %             | 16,0            | 12,9            |
| $\beta$                 | 12,6            | 12,6            |
| $\gamma$                | 19,1            | 14,6            |
| Haptoglobin, %          | 7,8             | 9,7             |
| Transferrin, %          | 5,9             | 4,9             |
| Ceruloplasmine, %       | 8,4             | 7,8             |
| Immunoglobulins, mg/ml. | 43,66 $\pm$ 2,3 | 32,35 $\pm$ 1,2 |

It is known that gamma globulins participate in creation and maintenance of active and passive immunity in animals. As for the content of globulins, including gamma globulin fraction, group II sheep exceeded group I sheep by 15.2% and 9.0%.

We also isolated transferrin, haptoglobin, and ferru-plasmine fractions. The greater amount of haptoglobin was found in sheep of group II (9.7%), smaller amount - in group I (7.8%). As for the content of ceruloplasmine, the animals of group I were 7.7 and 21.7% superior to the animals of group II. The largest specific weight of transference fraction was found in sheep of group II, which exceeded sheep of group I and II by 5.1 and 26.5%.

As is known, the number of immunoglobulins in blood serum is an indicator of the protective properties of the animal body. Our research has established that the largest number of immunoglobulins is contained in the blood serum of rams of group I, the smallest - in group II.

It should be noted that the level of this indicator kneaded rams almost no different from the rams of group I, which indicates an adequate response of their bodies to the conditions of the environment. The latter is confirmed by the results of studies by some scientists 6 on the survival rate of young sheep and the conservation of adult sheep of different genotypes.

In modern selection strategy the development of theoretical bases of gene pool conservation and improvement of local breeds of domestic animals is a fundamental problem of biological science.

Breeding-tribal work with animals on the basis of the main economically useful features directly or indirectly leads to changes in the gene pool of animals and the structure of the herd [5].

Preservation of an optimal level of genetic variability and heterozygosity in animal populations is connected with adaptive abilities of animals to changing environmental conditions.

Without genetic diversity in the population, animals lose their evolutionary adaptability and stability, which leads to significant economic damage [6,7].

In the practice of zootechnics genetic diversity in animal populations is usually determined by the genealogical structure of the breed or its structural units. According to scientists [8,9], for the approach to differential analysis of genetic variability it is necessary to have a method, which would allow simultaneously to judge about the variability of specific structural genes and provide information about the variability of discrete genes that are part of an integral integrated phenotype. Such methods include the analysis of genetic markers. In the last decades the method of estimation of genetic structure of animal populations was often supplemented by analysis of peculiarities of investigated populations by polymorphic proteins of animal blood.

On materials of results of studying of genetic polymorphism of transferrin's and hemoglobin in the serum of blood of sheep of the South Caucasian merino, it is established that in breeding flocks at selection of lambs for cultivation it is necessary to give preference to animals with types of transferrin's AA, SS, AV, AL, CE, BC, and at selection in group of repair lambs - with types AA, BC, AL, CE as having the best indicators of breeding and productive qualities. According to scientists, this method allows the selection of genetically determined highly productive animals for cultivation at an early stage of their development [10,11].

Immunogenic methods, which reveal in animals genetically determined, codominant inherited and unchangeable types of polymorphic proteins and enzymes in postnatal ontogenesis, make it possible to use them in solving problems of monitoring of breeding processes in populations. Polymorphic proteins of biological fluids do not change in ontogenesis, are easily determined at early stages of animal development and, as a rule, have a codominant type of inheritance. Due to this, they are ideal genetic markers and are widely used in the study of the genetic structure of populations and the development of methods to control breeding and genetic processes. Besides, the comparative study of intra-breed populations using immunogenic methods is important for understanding the mechanisms ensuring relative constancy of the rock and its development [12].

Control of genetic variability is of great interest, because line breeding, blood refreshing, and inbreeding can change the homogeneity and heterozygosity of a breed. In this connection, a genetic analysis of the structure of the South Caucasian merino sheep herd was carried out. It was found out that 5 allelic systems of transferrin A, B, C, D, E, which in combination can give 15 phenotypes, were found in test sheep of both inbreed types.

We detected 14 phenotypes of AA, BB, SS, DD, AV, AS, AD, AE, BC, VD, SD, CE, EE, and no Tf EE type was detected.

Table 3: The phenotypes frequency distribution of sheep by type of Tf

| Tf         | I - group |      | II - group |      | Total for the household |      |
|------------|-----------|------|------------|------|-------------------------|------|
|            |           |      |            |      | Actual                  |      |
|            | n         | %    | n          | %    | n                       | %    |
| AA         | 7         | 6,3  | 7,3        | 5,5  | 14,3                    | 5,9  |
| BB         | 6         | 3,2  | 5          | 3,4  | 11                      | 3,1  |
| SS         | 9         | 7,8  | 11         | 8,6  | 20                      | 8,0  |
| AV         | 6         | 5,5  | 5          | 4,2  | 11                      | 4,3  |
| AC         | 43        | 35,5 | 49         | 36,7 | 92                      | 36,4 |
| AD         | 5         | 4,2  | 6          | 4,3  | 11                      | 4,3  |
| SUN        | 39        | 32,0 | 46         | 34,7 | 85                      | 33,6 |
| VD         | 3         | 2,1  | 2          | 1,7  | 5                       | 2,0  |
| SD         | 4         | 3,3  | 1,2        | 0,1  | 4                       | 1,6  |
| $\Sigma$   | 121       | 100  | 132        | 100  | 253                     | 100  |
| I - group  | 22        | 17,4 | 24         | 18,2 | 45                      | 17,8 |
| II - group | 100       | 82,6 | 108        | 81,2 | 208                     | 82,2 |
| $\chi^2$   |           |      |            |      |                         | 58,1 |

The frequency of distribution of phenotypes varies markedly depending on the genotype of the sheep. The alleles Tf C (0.54), (0.49); Tf A (0.21), (0.23), and the lowest frequency was Tf E (0.04), (0.06). The average level is Tf D (0.12), (0.16) and Tf B (0.09) and (0.06), respectively. In terms of sex and age groups, types of transferrin significantly differ in frequency of distribution. But the general trend remains in the direction of the highest concentration in sheep of four types Tf AB (5.5%), BC (32.0%), AC (35.5%), AD (4.2%), whose share in the population is equal to 77.2%. The smallest distribution was observed for types AE, EE, BE (2.3%).

Tf BC and AS types had the highest prevalence in all age and sex groups. (67,5%). In ewes of the second group the tendency remains in the direction of the highest concentration of the four types Tf AA BB, SS and AS, whose share in the population is 66%. The smallest distribution was observed for the types AD, VD, SD (19.3 percent). Tf SS and AC types had the highest prevalence in all age and

sex groups. From the above data it follows that in terms of Tf types' distribution frequencies, the population of the 1st group is close to the population of the 2nd group, which indicates the following about their genetic similarities.

The 6.2% superiority of heterozygous types over homozygous types in the studied population of Group I sheep, and the 13% superiority in Group II characterizes the level of polymorphonostittransferrin locus of both breeds. Absence in adult sheep of group I consisting of elite animals and weak concentration in other age groups of types Tf AA, DD, EE, EE, EE, CE and in adult sheep of the second type with types Tf DD, CE, EE (6,7 %) indicates less selective significance of animals with the listed types.

Synthesis of hemoglobin types in South Caucasian merino sheep of both groups is controlled by two codominant alleles Hv B and Hv A with the corresponding frequency 0.77, (0.72); 0.23, (0.28) to which the three types of hemoglobin AA, BB and AB correspond. The HBB and AB types differ the most in distribution both for the entire population and for individual age and sex groups and vary from 52.3 to 63.0 and from 23.3 to 37.3, respectively (Table 3).

The value of  $\chi^2$  at the transferrin locus in ewes is 58.1, which indicates a reliable difference between the empirical and theoretical frequencies of genotypes. This means that the population uses a rather intensive selection that disturbed the genetic balance in both populations at the transferrin locus. The value of  $\chi^2$  at the hemoglobin locus is 0.06, below the table.

The genetic equilibrium in the hemoglobin locus is maintained, probably due to the low polymorphism. Low polymorphism of the hemoglobin locus slightly reduces the selective significance of this indicator.

According to our data, in the transferrin locus of South Caucasian merino sheep superiority of heterozygotes is 3.6%, and in hemoglobin the ratio of these genotypes is almost the same and, accordingly, the degree of homozygosity is higher ( $Ca = 3.01$ ) than in the transferrin locus (0.72).

The increase in the degree of homozygosity ( $Ca$ ) is observed in both loci and is accompanied by a decrease in the number of active alleles (and indeed in loci Tf their 5, in hemoglobin loci 2). Accordingly, the increase in its value leads to a decrease in genetic and phenotypic diversity and exacerbates the homogeneity of the population, which is undesirable in the breeding process.

The low polymorphism level of Na (0.24) indicates that the number of active alleles of the population for the Hv locus is less than possible for the Tf locus (1.7). The indicator of the state of the population is the coefficient V, which means

the degree of realization of the possible variability, the value of which is lower than the desirable value in both populations.

Typically, tribal animals are selected at an early age based on phenotypic indicators of origin. Early forecasting is particularly necessary in an intensification industry.

In our studies, sheep of the South Caucasian merino of group I differ in live weight indices depending on the type of transferrin.

Its indices vary from 60.4 kg (TfCC) to 35.0 kg (Tf BB) for the group of adult mothers, the difference is 25.4 kg ( $P>0.99$ ), for the group of lambs the maximum weight can be traced in animals with types Tf BB 55.3 kg and minimum with type Tf BB (36.4 kg), the difference is 18.9 kg ( $P>0.99$ ).

The difference between the maximum (47.8 kg with TfC) and minimum (31.0 kg with TfB) indicators was 16.8 kg ( $P>0.99$ ) for the group of yards. It should be noted that for all age and sex groups there is a tendency of higher live weight in animals with types of transferrin CC, AC and BC, whose specific weight in the groups is 65.0; 64.7; 61.0% respectively.

Live-weight indices of intra-breed type II, depending on the type of transferrin, range from 64.8 kg (TfS) to 59.8 kg (Tf AC) for the group of adult sheepdogs, maximum weight for the group of annual gages is observed for animals with types (TfVD) of 61.4 kg and minimum weight for animals with type (TfAV) of 56.3 kg.

The trend of the best live weight is observed in animals with types of transferrin CC, AC, VD, whose specific gravity in the groups is 59.0 and 60.0 percent, respectively. The analysis of wool productivity of sheep of group I depending on types of transferrin shows that animals with types Tf SS, AS, BC, VD were the best performers.

The difference between the best performance of the animals with Tf CC (3.5 kg); Tf AC, BC, VD and the worst performance of Tf AV in the group is 0.8; 0.3; 0.5; 0.7 kg ( $P<0.95$ )<sup>7</sup> In group II sheep, the best performance was observed in animals with Tf BC, AD, VD types, whose specific gravity was 52.0 %. The difference between the best performance of the animals with TfVS (2.3 kg); Tf AD, SD, and the worst performance of the group with TfAD, DD, VD is 0.5; 0.8 kg ( $P<0.95$ ) [13].

Reliability of the received indicators can be connected with a small quantity of investigated animals that has entailed weak variability of a sign and low level of used polymorphic systems.

It is necessary to note, that advantage, both on live weight, and on wool cutting had animals of both intra-breed types with transferrin SS, AC, BC and VD. This



testifies to the possibility of using polymorphic systems as markers for the best productivity of South Kazakh merino sheep. The study of protein content in blood serum has shown that sheep-producers, sheep-breeders, annual vivid, as well as newborn sheep-breeders and vultures of group I are inferior by this indicator to their counterparts of group II - by 0.56%; 1.45%; 4.44%; 10.1% and 9.98% respectively.

Thus, the study of polymorphic systems of sheep's blood serum proteins of South Kazakhstan merino of both groups by age and sex revealed the presence of certain allele combinations and the ratio of transferrin and hemoglobin genotypes. It was shown that these animals have their specific spectrum of alleles and combinations of genotypes.

On the basis of the obtained results the possibility of using genetic markers of blood in early evaluation of productive qualities of animals was established.

The interrelation of biochemical indices with the main selected features of rams depending on their breed affiliation has been studied. The data show that in general, a closer relationship is observed between live weight and biochemical indicators of experimental animals. In the considered groups of animals between the content of total protein and live weight a positive relation of average value ( $r_s=0.35-0.51$ ) was revealed. Coefficients of correlation of blood biochemical indices with the productivity of sheep-producers, a similar connection of this index with wool cutting was found only in the mixed sheep of group II ( $r_s = 0.38$ ) in the absence of it in animals of group I and II. High positive correlation between live weight and AST activity was observed in group II animals ( $r_s=0.62$ ), while group I and II animals had almost the same average correlation coefficients ( $r_s=0.54-0.51$ ).

We determined the degree of heredity of activity of aminotransferases enzymes by the dispersion method. The inheritance of polymorphic proteins and enzymes is, as a rule, controlled by the out some-dominant alleles. In this case, the phenotype is the same as the genotype. Polymorphic structures do not change during individual life and are preserved in animals in the set in which they are received from parents with genetic information. The studied feature (AST) is found to be highly heritable ( $h^2=0.62-0.68$ ), and its degree of inheritance was much higher than that of AST. Heredity coefficients of aminotransferases enzyme activity.

Calculation of economic efficiency of the study was based on the determination of the difference between the total actual revenue from the sale of sheep meat and wool less the cost per animal. Predatory live weight of sheep in "Batay-Shu" LLP turned out to be higher and carcass weight was 31,5 kg, the

average revenue from sales of wool in the experimental group was 268 tenge, while for the farm - 220 tenge.

Table 4: Calculation of economic efficiency of the study

| Indicator                                | Batai Shu LLP   |                 |
|------------------------------------------|-----------------|-----------------|
|                                          | management team | management team |
| Predwarping live weight, kg              | 61,2            | 56,3            |
| Carcass weight, kg                       | 31,5            | 27,7            |
| Produced wool, kg                        | 5,9             | 5,2             |
| Realization price of 1 kg of lamb, tenge | 900             | 900             |
| Realization price 1 kg of wool, tenge    | 268             | 220             |
| Total costs, tenge                       | 21650           | 21650           |
| The products were sold in total, tenge   | 29931           | 26074           |
| Cost of young growth, kg                 | 21650           | 21650           |
| Profit, tenge                            | 8281            | 4424            |
| Profitability, %                         | 38,25           | 20,43           |

When comparing the productivity of the breeding groups of sheep with the average herd, in 1-group, the average additional income per sheep was 3857 tenge, and in 2-group - 2282 tenge. These differences are based on the fact that the selling price of products in 1-group amounted to an average of KZT 28002.5, and in 2-group - KZT 25123.0. High profits from the sale of lamb and wool were received in the experimental group in 1-group - 29931 tenge and, accordingly, in 2-group - 26264 tenge, at a level of profitability of 38.25 and 26.85% respectively. The level of profitability on farms is 20,43 and 15,83% respectively.

**Conclusions** The study of polymorphic systems of sheep's blood serum proteins of different intra-breed types by sex and age groups revealed the presence of certain allele combinations and the ratio of transferrin and hemoglobin genotypes. It was found that these animals have their specific spectrum of alleles and combinations of genotypes. On the basis of the obtained results the possibility of using genetic markers of blood in early evaluation of productive qualities of animals was established.

When breeding South-Kazakhstan merino in the zone of their distribution, it is recommended to use Australian meat merino to improve and raise the quality

of productivity, because kneaded animals give 8-10% more cutting of wool in washed fibers and have 10-15% more live weight than purebred animals.

## CONCLUSION

These studies have shown that study of polymorphic systems of blood serum proteins of South Kazakh merino sheep revealed the presence of certain combinations of alleles and that these animals have their own specific spectrum of alleles and combinations of genotypes.

In addition, according to the obtained results, the possibility of using genetic blood markers in the early assessment of the production qualities of animals was determined.

The determined economic efficiency of the study was based on determining the difference between the total real income.

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## OCCURENCE OF GIARDIA SP. IN RUMINANTS IN SERBIA

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### **Abstract**

*Giardia spp. are flagellates that are found in the intestinal tract of humans and other mammals, birds and amphibians. Infections with giardia have been reported widely in livestock and companion animals in different parts of the world. Evidence of infection in humans and animals of Giardia duodenalis, especially of assemblage A and B has firmly established giardiasis as a zoonotic disease. At ruminants giardiasis usually result with diarrhea, especially in young animals, which in turn adversely affect production resulting in economic losses. So far, no research has been done related to giardiasis in small ruminant and cattle in Serbia, and here we present the results of the first studies of the presence of giardiasis in ruminants in our country.*

**Keywords:** *Giardia duodenalis.*, cattle, goat, sheep, Serbia

### **Introduction**

*G. duodenalis* (syn. *Giardia lamblia*, *Giardia intestinalis*), a flagellate parasite, is one of the most prevalent and widespread intestinal parasite in humans and several vertebrate animal (mammals, birds, amphibian) worldwide. Parasites takes on two morphologically distinct forms during its life cycle. The replicative form is a motile pear-shaped cell that survives only in host small intestines called a trophozoite. Trophozoites swim through the intestinal mucus until they attaches to the epithelium by a ventral adhesive disc or sucker (Cernikova et al.,2018). To the host intestinal epithelium then divide by binary fission, forming either more

trophozoites or the non-replicative cyst stage. Cysts pass through large intestine of the host and are shed in the feces. Giardiasis represents a major public health concern in both developing and developed countries and infected animals (pets or domesticated species) present a main source of human infection because transmission of the *G. duodenalis* occurs directly (fecal/oral) by cyst contaminated food or water (Thompson et al.2008; Robertson,2009).

Parasites causing a diarrheal condition known as giardiasis. According morphology and genetic evidence six species have been recognized in the genus *Giardia*. These include *G. duodenalis* in humans and other mammals. Phylogenetic analysis and enzyme electrophoresis examination *G. duodenalis* revealed the existence of eight assemblages A–H within the species. Assemblage A and B is usually occurred at human but in ruminants higher occurrence genotype E, with genotypes A and B being less frequent (Giangaspero et al.2005; Castro-Hermida et al.2006; Gomez-Munoz et al.2009; Lim et al.,2013)

In small ruminants (sheep and goats), data tend to indicate an occurrence of around 20%–25%, ranging from <10% to >40%, with similar data for cattle. On farms where *Giardia* infection has been diagnosed in ruminant stock, a cumulative occurrence of close to 100% may be expected. In general, very young animals (neonates) are less likely to be infected, although animals <6 months of age tend to be more susceptible to infection with signs of disease. Frequently, however, even infections with very high cyst excretion are not associated with clinical signs. Nevertheless, diarrhea, weight loss, ill thrift, and even death have also been associated with *Giardia* infection in some animals.

So far, no research has been done related to goat giardiasis in Serbia, and here we present the results of the first studies of the presence of giardiasis in goats in our country performed in period from 2018 to 2021.

## Materials and methods

In the period 2018-2021 we examined 1274 fecal samples from 24 goats herds, 937 fecal samples from 31 sheeps herds and 197 fecal samples from 31 cattle herds and in central and south part of Serbia.

The diagnosis of giardiasis is commonly established by microscopic identification of cysts or less commonly trophozoites in faecal wet smear stained with iodine. During examination we did not include molecular identification of the giardia genotype. Examinations we performed with Carl Zeiss AxioLab A1 microscope with the AxioCam 105 Color microscope camera and Zen Lite software.

## Results and Discussion

During our examination infection with giardia we established in all ruminant species.

### CATTLE

Infection with giardia we established in seven herd and to 37 animals. In younger calves, especially below 6 months of age, the excretion of watery faeces with a mucoid appearance may be the only indication of infection with the parasite. Chronic cases of giardiasis in calves may impact negatively on performance which may be reflected in reduced weight gain, impaired feed efficiency and decreased carcass weight. Calves have been reported to be infected with *G. duodenalis* as early as 4 days of age, and the highest intensity of cyst excretion between the ages of 1 and 3 months. Transmission occurs among infected calves as well as chronically infected adults.

*Giardia* has been found in both beef and dairy cattle throughout the world with varying prevalence (Xiao and Herd, 1994, O'Handley et al.1999, Ralston et al., 2003). The infection pattern of *Giardia* appears similar between beef and dairy cattle with cysts appearing in the faeces at approximately 4 weeks of age (Trout et al.2005, Mendonca et al.2007). Both dairy and beef calves may harbour more than one genotype of *G. duodenalis*, which can be of zoonotic significance (O'Handley et al.1999, Trout et al.2005, Mendonca et al.2007).

*Giardia* has been implicated as an aetiological agent alone and in combination with other enteric pathogens in calf diarrhoea. Infection may also result in numerous diarrhoea episodes which in turn adversely affects production and result in economic losses for farmers. In calves, and to a lesser extent in other production animals, giardiasis can result in diarrhea that does not respond to antimicrobial or coccidiostatic treatment. The excretion of pasty to fluid feces with a mucoid appearance may indicate giardiasis, especially when the diarrhea occurs in young animals (1–6 months old). Chronic cases of giardiasis in calves may impact negatively on performance which may be reflected in reduced weight gain, impaired feed efficiency and decreased carcass weight (Huetink et al.,2001).

### SHEEP

During our examination infection with giardia we established only in five herd and to 64 animals. Infection we found in pre-weaned lambs having a



much higher compared to those that were over 3 months. In infected lambs we found clinical signs like foul-smelling diarrhoea which is lightly colored, greasy and mixed with mucous and reduced weight gain.

The prevalence of *G. duodenalis* infection in sheep varies considerably and was higher in lambs than in adults. All the findings from these studies suggest that the infection rates of *Giardia* tend to decline as the age of the animals increases (Ryan et al., 2005, Castro-Hermida et al., 2006, 2007). In most cases, infections are asymptomatic but infected animals are carriers shedding large numbers of cysts into the environment (Castro-Hermida et al., 2006). Even if most infections are asymptomatic, infections in lambs may result in a malabsorption syndrome, decreased feed efficiency and subsequently a decreased weight gain and sometimes death.

Host age and immune status of the host affect the severity of the disease but other factors such as the number of specimens examined, the age structure of the herds, management procedures and the health status of the animals may account for the discrepancies or variations in the infection rates in the different populations.

## GOAT

During our examination infection with giardia we established only in two goats herd and to 42 animals. A prevalence we established in pre-weaned animals ( $\leq 3$  months) having a much higher compared to those that were over 3 months. That confirmed results of numerous examination that infections are normally significantly higher in pre-weaned kids compared to that in older one (Xiao, 1994; Geurden et al., 2008; Taminelli and Eckert, 2008, Ma et al., 2014).

Examination of goat giardiasis established that most infections with giardia are asymptomatic (Castro-Hermida et al., 2007). Clinical signs that may be observed, mostly in young animals that are foul-smelling diarrhoea which is lightly colored, greasy and mixed with mucous and reduced weight gain (Olson et al., 1995; Aloisio et al., 2006, Pavlović and Ivanović, 2022). This symptoms we found during our research too.

In small ruminants, giardiasis more surveys from sheep than goat populations and therefore fewer publications on giardia in goats and that confirmed during our examination

As these were the first complete studies of giardiasis in ruminants in Serbia, they did not include molecular identification of the giardia genotype. First occurrence of giardiasis in goats was established by Pavlović during 2018 and it

was later confirmed in sheep and cattle (Pavlovic et al.,2020, 2021a,b, 2022). A numerous genotyping studies of *G. duodenalis* in ruminants report a higher occurrence of genotype E, with genotypes A and B being less frequent (Ruiz et al.,2008). Studies performed in Belgium have reported zoonotic genotype A infections in goats and sheep (Geurden et al.2008) and in study in Malaysia and India reported genotypes A and B in cattle and goats (Lym et al.,2013, Utaaker et al.,2017). These findings suggest that ruminants could be a potential source of zoonotic infection with giardia.

In the all cases in therapy were used febendazole an effective molecule, at least in terms of complete clinical recovery. They confirming observations of other authors, who successfully treated infected with *G.duodenalis* using similar therapy protocols (Xiao et al., 1996).

## Conclusions

The present study confirmed the presence of *Giardia duodenalis* in ruminats herds in Serbia. At infected animals giardiasis usually result with diarrhea, especially in young animals, which in turn adversely affect production resulting in economic loss. At the same time, diseased animal pose an epidemiological danger because they excrete infectious forms of gardia in their feces. In order to get acquainted with the genotype of established parasites, further research will be focused on the molecular identification of established parasites in goats.

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## **SERUM ENZYME ACTIVITIES IN THE BLOOD AND MILK IN THE DIFFERENT STAGE OF LACTATION IN HOLSTEIN DAIRY COWS**

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### **Abstract**

*The objective of this study was to determine correlation between serum blood and milk enzyme activities of aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), alkaline-phosphatase (ALP) in the 36 dairy Holstein cows divided into three groups according to production period. Group 1 consisted cows in the start of lactation (n = 12); Group 2 -consisted of early lactation cows (n=12) and Group 3 included mid lactation cows (n=15). Statistically significant higher (P<0.01) activity of AST in blood serum was established in early lactation groups of cows as compared to mid lactation group of cows. ALT activity showed a lower (P<0.01) serum activities in early lactation groups of cows than in the mid lactation cows. Higher values ALP in blood and milk are determined in early lactation groups of cows as compared to mid lactation cows, but without statistical significance (P>0.05). Research results showed possibility of mild degree of hepatic lesions, probably due to fat infiltration in early lactation cows. No significant difference (P>0.05) was observed in milk serum value for AST, ALT and ALP between the three groups of*

cows. No significant correlations among AST, ALT and ALP activities in blood and milk serum were determined ( $P>0.05$ ) and shows that activity of these enzyme in the milk are not used as markers for early diagnosis of subclinical disease.

**Keys words-** dairy cows, blood, milk, enzymes activities, early lactation periods, mid lactation

## Introduction

The metabolic profile, a series of specific blood analytical tests, is routinely used to reveal metabolic problems in dairy cattle (Oetzel, 2004; Stengarde et al., 2008; Gross et al., 2011). Evaluation of the blood and milk biochemical parameters to assess the animal health and milk yield has always been interested by authors and the various discrepancies have been observed in both blood and milk yield results (Nozad et al., 2011; Jozwik et al., 2012).

Milk parameters originate from blood and food component and clarifying the appropriate relationships among these parameters individually in food blood and milk are useful in understanding the health and production status in animals (Jozwik et al., 2012; Liu et al., 2012, 2013; Ghadaa, 2014). Major health disorders in high-yielding cows occur around parturition and during lactation. Metabolic conditions of negative energy balance (fasting, parturition and lactation) lead to an increased uncontrolled rate of mobilization of body fat and its increased accumulation in liver cells, resulting in disturbance of the physiological and morphology integrity of the liver (Vazquez-Anon et al., 1994; Overton and Waldron, 2004; Bobe et al., 2004). Fatty liver and diffuse infiltration of hepatocytes involve cell membrane damage and hepatocyte destruction accompanied by the release of cytoplasmic enzymes (AST, GGT, LDH), the activity there of in the blood being considerably elevated (Oetzel, 2004; Stojevic et al., 2005; Lubojacka et al., 2005).

Blood plasma and serum ALT, AST, ALP and GGT activities were reported to be useful indicator of liver function for postpartum dairy cows (Bobe et al., 2004; Stojevic et al., 2005). While little information is available concerning about the activity changes of ALT, AST, GGT and ALP in milk. The activities of these enzymes were monitored in milk and blood serum of cows and results of correlation analysis and regressive models showed a close relation between them (Liu et al., 2012, 2013; Ghadaa, 2014). More practical attention has been given to detection of enzyme activity in milk and many enzymes have been proposed and listed as reliable markers for early diagnosis of subclinical disease (Babaei et al., 2007; Katsoulos et al., 2010).

The objective of this study was to determine correlation between serum blood and milk enzyme activities in the different stage of lactation in the dairy cows.

### Materials and methods

Animals, diets and protocol design: A total of 36 dairy cows were randomly selected from the same Holstein herd containing 445 cows (Farm: Šarulja, Knić, Central Serbia). The cows were high-yielding with a preceding lactation of about 8500 l. Three groups of clinically healthy cows were chosen from the herd. Group 1 consisted cows in the start of lactation ( $n = 12$ ) in period of  $5 \pm 3$  days after calving; Group 2 -consisted of early lactation cows ( $n=12$ ) in the first month of lactation ( $22 \pm 15$  days), and Group 3 included mid lactation cows ( $n=15$ ) between 90 to 150 days of lactation ( $133 \pm 75$  days). The experimental cows were free in open-stall barns. Diet and housing facilities were adapted to research purposes, with diet suited to the energy requirement of early and mid lactation cows. Diet in early lactation consisted of 4 kg grass hay, 10 kg corn silage (30% Dry Matter, DM), 20 kg sweet corn silage, 12 kg beet nodule silage, 4 kg concentrate (18% crude protein, CP) and 1 kg molasses. Diet in mid lactation consisted of 4.5 kg lucerne hay, 19 kg corn silage (30% Dry Matter, DM), 16 kg beet nodule silage, 9 kg concentrate (18% crude protein, CP) and 1.2 kg soybean expeller.

The chemical composition of total mixed rations offered to early lactation and mid lactation dairy cows are given in Table 1.

Table 1: Chemical composition of total mixed rations offered to early lactation and mid lactation dairy cows.

|                                            | Early lactation | Mid lactation |
|--------------------------------------------|-----------------|---------------|
| Dry Matter (DM) (kg)                       | 15.60           | 19.58         |
| Net Energy of Lactation (NEL) (MJ)         | 95.52           | 128.65        |
| Crude Protein (CP) (% of DM)               | 11.31           | 16.88         |
| Rumen undegradable protein (RUP) (% of CP) | 33.91           | 26.33         |
| Fat (% of DM)                              | 3.47            | 4.68          |
| Fiber (% of DM)                            | 22.17           | 18.85         |



Blood and milk samples were taken simultaneously from each lactating cow during morning milking. Blood samples were taken by jugular veni puncture into a sterile tube from each animal, and the blood serum was separated by centrifugation at room temperature ( $1,800\times g$ , 15 min). Milk samples were centrifuged at  $12,000\times g$  for 30 min at  $4^{\circ}\text{C}$  and the supernatant was transferred into the new sterile tubes. Blood serum and milk were stored at  $-20^{\circ}\text{C}$  until being used for biochemical measurements.

The blood and milk serum activities of aspartate-transaminase (AST) alanine-aminotransferases (ALT) and alkaline-phosphatase (ALP) were measured in the biochemical laboratory „OXUS“ (Kragujevac, Serbia) by spectrophotometric techniques using a BT 1000, (Biotechnica Italia) and the corresponding commercial kits (DIALAB, YUNICOM).

The statistical analysis of the obtained data was carried out by ANOVA-procedure (Statgraphic Centurion, Statpoint Technologies Inc. Warrenton, Va, Virginia, USA). The analysis of variance were used to evaluate the probability of the significance of the statistical differences between mean serum enzyme activities in each group and the Pearson test was performed for evidencing significant correlations. Differences were considered as significant when P values were below 0.05 or 0.01.

## Results and Discussion

Modern milk production often puts the production capabilities of cows at risk, which can result in metabolic disorders. In order to predict such disorders and eventual subclinical diseases it is necessary to determine physiological ranges of biochemical parameters in a clinically healthy herd (Oezel, 2004; Overton and Waldron, 2004). The present study compared the serum enzyme activities in blood and milk serum in dairy cows during early and mid lactation period.

The results of the serum blood and milk activities of AST, ALT, ALP in cows in the early and mid lactation period and correlations among blood and milk serum enzyme activities are given in Tables 2 and 3.

Table 2. Blood and milk serum enzyme activities in the start of lactation (Group 1), early (Group 2) and mid lactation (Group 3) of dairy cows ( $n=12$  in each group). Results are expressed as mean  $\pm$  standard deviation (SD).

|                     | Group 1            | Group 2            | Group 3            |
|---------------------|--------------------|--------------------|--------------------|
| AST (blood)<br>IU/l | $90.81\pm 21.98^A$ | $84.18\pm 16.19^A$ | $59.72\pm 10.95^B$ |

|                 |                            |                            |                           |
|-----------------|----------------------------|----------------------------|---------------------------|
| ALT(blood) IU/l | 28.00±8.46 <sup>A</sup>    | 28.54 ±3.96 <sup>A</sup>   | 36.45±9.62 <sup>B</sup>   |
| ALP(blood) IU/l | 162.36±193.25 <sup>a</sup> | 117.64±22.28 <sup>a</sup>  | 96.81 ±31.94 <sup>a</sup> |
| AST (milk) IU/l | 33.82±23.76 <sup>a</sup>   | 33.27±9.65 <sup>a</sup>    | 25.36±11.87 <sup>a</sup>  |
| ALT(milk) IU/l  | 20.05±12.47 <sup>a</sup>   | 20.27±14.66 <sup>a</sup>   | 29.55±19.83 <sup>a</sup>  |
| ALP(milk) IU/l  | 199.23±186.23 <sup>a</sup> | 241.11±109.31 <sup>a</sup> | 121.64±32.56 <sup>a</sup> |

Legends: Mean values within a row with no common superscript differ significantly, values marked by small letter differ significantly ( $P < 0.05$ ); values marked by capital letter differ high- significantly ( $P < 0.01$ ).

Table 3. Correlation coefficients among the biochemical parameters in the blood and milk calculated for all cows in the present study.

|            | AST (blood)    | ALT (blood)    | ALP (blood)    |
|------------|----------------|----------------|----------------|
| AST (milk) | $r=0.14^{ns}$  | $r=0.16^{ns}$  | $r=-0.09^{ns}$ |
| ALT(milk)  | $r=-0.23^{ns}$ | $r=-0.03^{ns}$ | $r=0.17^{ns}$  |
| ALP(milk)  | $r=-0.06^{ns}$ | $r=0.11^{ns}$  | $r=0.15^{ns}$  |

Legends: ns-non-significant ( $P>0.05$ ).

Lactation has a great impact on biochemical parameters in the blood of cows, reflecting on metabolic demands. The activity of AST in blood is very important. AST act as a catalyst in connecting the metabolism of amino-acids and carbohydrates. Accordingly, changes in their activity in the blood can be a consequence of their increased activity in cells (primarily liver), but also a reflection of cell structure damage. AST is considered as the most sensitive indicator in the diagnosis of fatty liver in cows (Pechova *et al.*, 1997; Bobe *et al.*, 2004; Lubojacka *et al.*, 2005; Stojevic *et al.*, 2005). In this study, statistically significant higher ( $P<0.01$ ) activity of AST in blood serum was established in early lactation groups of cows as compared to mid lactation group of cows. No significant difference ( $P>0.05$ ) was observed in milk serum value for AST between the three groups of cows.

ALT activity in cows differs during certain production periods. The lowest ALT activity was measured during early lactation, while activity increased in the second and third periods of lactation. In the dry period enzyme activity decreased, but it was still statistically much higher than in the first period of lactation. The authors consider that the role of ALT in predicting liver damage in ketosis is not significant (Tainturier *et al.*, 1984; Stojevic *et al.*, 2005). Our results confirm this because in the period of mid lactation (third period) we measured the highest ( $P < 0.01$ ) concentration of ALT. No significant difference ( $P > 0.05$ ) was observed in milk serum value for ALT between the three groups of cows.

ALP are used as biochemical marker in diagnosis of osteoporosis, hepatobiliar disease and fatty liver in the dairy cows. The activity of ALP in blood serum are increased in periods from puerperium to mid lactation in the dairy cows, especially in cows with liver lipidosis. (Bobe *et al.*, 2004; Liu *et al.*, 2012). In this study, higher values ALP in blood and milk are determined in early lactation groups of cows as compared to mid lactation cows, but without statistical significance ( $P > 0.05$ ) as consequence high individual variabilites. On the basis changes in blood and milk AST, ALT and ALP activities in the different stage of lactation, our result suggested that early lactation cows had mild degree of hepatic lesions, probably due to fat infiltration.

Significant correlations among AST, ALT and ALP activities in blood and milk serum are not determined ( $P > 0.05$ ) in this study, and shows that activities of these enzymes are not used as markers for early diagnosis of subclinical disease. These results are in opposite with results (Liu *et al.*, 2012, 2013; Ghadaa, 2014), who found a strong correlation between them. Further investigations will confirm or not these statements.

### Conclusions

On the basis changes in blood and milk AST, ALT and ALP activities in the different stage of lactation, our result suggested that early lactation cows had mild degree of hepatic lesions, probably due to fat infiltration. No significant correlations among AST, ALT and ALP activities in blood and milk serum are determined ( $P > 0.05$ ) and shows that activity of these enzymes in the milk are not used as markers for early diagnosis of subclinical disease.

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## SIGNIFICANCE OF HEAT SHOCK PROTEIN HSP70 IN EARLY LACTATION COWS

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### **Abstrakt:**

*Heat shock protein (Hsp70) is a relatively new biomarker that has been poorly studied in veterinary medicine, especially in bujatrlica. Elevated Hsp70 values in the cell are known to help better cell survival and stability. However, recent research from the past few years indicates that if these proteins are found extracellularly, they show proinflammatory effects and are related to the development of insulin resistance and diabetes. The peripartum period in dairy cows is a model for studying metabolic stress. Metabolic stress in early lactating cows is characterized by lipolysis, ketogenesis, insulin resistance and inflammation as a result of negative energy balance and increased use of lipids for energy purposes. As inflammation, insulin resistance and metabolic adaptations caused by parturition and early lactation are the most important elements for successfully overcoming the period of early lactation, Hsp70 can be an important indicator that can be used to assess the metabolic adaptation of cows.*

**Key words:** HSP 70, peripartal period, metabolic stress.

## **Physiological significance of heat shock protein Hsp70 in early lactation**

Heat shock proteins (Hsp) are also called chaperones, are proteins that are important for the proper formation of the polypeptide chain and play a role in its translocation within the cell. These molecular companions play a key role in maintaining protein homeostasis in the cell (proteostasis) (Zhao et al., 2020). They prevent misfolding and aggregation of proteins, which is achieved by their action on the folding of intermediates (Hartl et al., 2011; Kim et al., 2013; Vabulas et al., 2010). Hsp70 in cells helps to restore the native conformation of proteins that have been denatured under the action of various stressors, by preventing their aggregation, which results in protecting cells from apoptosis and have an anti-inflammatory effect. The discovery of these proteins is related to experiments during which animals are exposed to heat stress, when the universal heat shock response (HSR) is developed, so the name heat shock proteins is defined (Mahat et al., 2016; Fujimoto and Nakai 2010). Because of this, they are called heat shock proteins, when an increase in their concentration was observed, and then increased gene expression for the production of these proteins was determined, although over time they have been shown to play indispensable and important roles in various physiological aspects.

These proteins are classified on the basis of molecular mass, and the most important is the heat shock protein 70 (Hsp70) with a molecular mass of about 70 kDa, which is marked as a "master player in protein homeostasis" (Fernández-Fernández and Valpuesta 2018).

Based on molecular mass, heat shock proteins are classified as follows: 10 kDa (Hsp10), 20-30 kDa (Hsp27, HspB1), 40 kDa (Hsp40), 60 kDa (Hsp60), 70 kDa (Hsp70, Hsp71, Hsp72, Grp78, Hsc70), 90 kDa (Hsp90, Grp94) and 100 kDa (Hsp104, Hsp110). Hsp70 is important in cattle, and multiple genes have been found to determine the production of this chaperone, while iRNK for Hsp70 has been found in cells of various tissues as well as in blood plasma in cattle (Agnew and Colditz 2008; Asea 2007). Heat shock proteins can be said to be phylogenetically conserved and ubiquitous molecules, which confirms their importance in the functioning of the organism. In addition to the molecular weight classification, guidelines for heat shock protein nomenclature in humans based on systematic gene symbols assigned by the Genome Nomenclature Committee are used (Kampinga et al., 2009).

Stimulation of Hsp synthesis and expression of genes that stimulate them can be played by various intrinsic and extrinsic factors such as physiological

factors including growth and hormone factors, pathophysiological factors including infection, inflammation, ischemia, oxidative damage and toxin action, factors from environments such as heat stress or heavy metals (Prohaszka and Fust 2004). Hsp manifests its physiological role in the pathways associated with adenosine triphosphate, indicating that the activation of heat shock proteins in the body is an active process (Csermely 1999). Its physiological roles in protein metabolism are diverse and include, transport / sorting of proteins into accurate subcellular barriers, cell cycle control and signaling and protection of cells from stress and apoptosis (Borges and Ramos 2005). The most studied heat shock proteins are the stress-stimulated heat shock proteins, which include HSP70 / HSP72 (HSPA1A), followed by the constitutive forms HSP70 / HSP73 / HSP78 (HSPA8), a form from the endoplasmic reticulum (Grp78 / BiP). and the form most commonly found in mitochondria HSP75 / mtHSP70 / mortalin / TRAP-1 (HSPA9) (Tavaria et al., 1996). Due to the fact that stress induces the production of Hsp70 and that it exhibits antiapoptotic, cytoprotective and immune effects, and that it regulates these processes, this chaperone is also the most studied of all heat shock protein families. There are various experimental protocols in which increased expression of Hsp70 has been demonstrated, such as models of sepsis, stroke, acute respiratory distress syndrome, renal failure, and ischemia of the heart, and in certain circumstances its increased expression has been shown to increase survival (Jo et al., 2006; Weiss et al., 2002; Chen et al., 2003; Giffard and Yenari 2004). In addition, the role of Hsp70 in the prenatal, embryonic period has been proven, where the expression of this chaperone has been shown to play an indispensable role in maintaining normal fetal development by regulating cell cycle and apoptosis and protecting against stressors that can harm the embryo (Luft and Dix 1999).

Hsp70 is detected in cells, but also in blood serum, and depending on the localization, it has the ability to show completely different physiological effects (Rodrigues-Krause et al., 2012). Hsp70 enters the circulation through two mechanisms. The first mechanism involves the passive entry of Hsp70 into the bloodstream originating from necrotic or stress-laden cells, while the second mechanism involves the active secretion of Hsp70 into the bloodstream by various cells (Asea 2007). There is no clear knowledge about which part of Hsp enters the bloodstream through the active and which part through the passive route. The contradictory role and expression of Hsp in various pathophysiological processes and conditions further complicates this interpretation. Namely, its concentration increases in various diseases, and on the other hand, increasing the concentration means better survival, while in certain circumstances reduced Hsp



production leads to various metabolic disorders in humans (Agnew and Colditz 2008; Asea 2007; Kampinga et al., 2009; Pittet et al., 2002). Hsp70 stimulates the immune response, but also inhibits it so that too strong immune stimulation would not lead to damage to the host tissue. Its role in pathology but also in cell protection, and the difference in concentrations and expression in different diseases is still confusing when we try to form a unique law of action and even the origin of Hsp70 in the systemic circulation.

Hsp70 has a dual role in the body depending on whether it is intracellular or extracellular, so iHsp70 has a protective role and eHsp70 a proinflammatory role. The study of eHsp70 has become relevant due to the availability of diagnostic kits for determining its concentration, and the latest results show that it is a very useful predictor of mortality in patients with septic shock (Bautista - Carbajal 2021). Hsp70 enters the extracellular space in several ways: by exiting necrotic cells, under the action of various stressors and inflammation in intact cells, it can be produced in the liver as an acute phase protein, and exosome transport and direct contact with cell lipid membrane has been described (Merchant and Korbelik 2011; Gupta et al., 2013). The proinflammatory effect of eHsp70 is achieved by induction of innate immune cells, induction of secretion of inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-6), inducible expression of nitric oxide synthase (iNOS) and nuclear translocation of nuclear factor  $\kappa$ B (NF- $\kappa$ B) (Asea 2003). According to the chaperone equilibrium theory, the higher the value of eHsp70 compared to iHsp70, the more pronounced its proinflammatory effects (Krause et al., 2015).

Intracellular Hsp70 (iHsp70) shows its protective and anti-inflammatory effect. Induced iHsp70 protects the cell from apoptosis by reducing or blocking caspase activation, binding to apoptosis-inducing factor (AIF) and inhibiting AIF-induced chromatin condensation or preventing mitochondrial damage and nuclear fragmentation (Buzzard et al., 1988; Ravagnan et al., 2001). It blocks morphological changes in cells caused by apoptosis induced by tumor necrosis factor, and has been found to help repair cells in inflammatory damage (Muralidharan et al., 2014; Jaattela et al., 1998). The anti-inflammatory effect of iHsp70 is reflected in the fact that it inhibits the response to lipopolysaccharides and blocks the production of inflammatory mediators such as TNF- $\alpha$ , and other mechanisms have been described (Borges et al., 2012). Hsp70 production gene expression has been well studied in ruminants or their cell cultures exposed to high ambient temperatures, and multiple increases in iHsp70 in cells provide better adaptation to heat stress (Kim et al., 2020; Dangi et al., 2016; Sheikh et al., 2017).

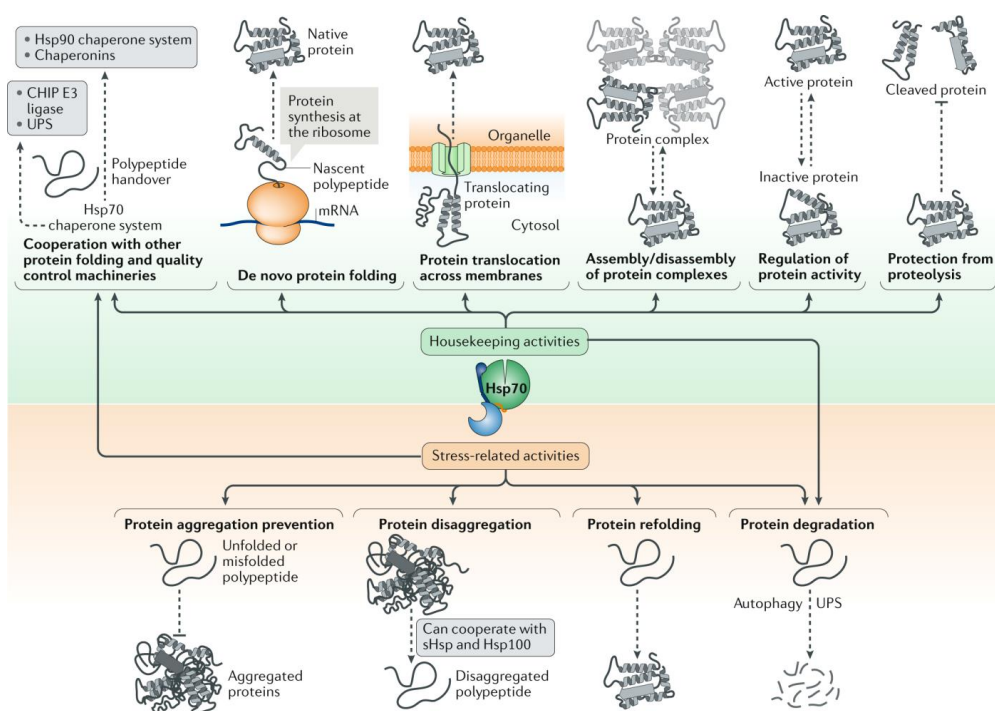


Figure 1. Physiological roles of Hsp70 (Rosenzweig et al., 2019).

Intracellular HSP (iHsp70) has a strong anti-inflammatory effect, while extracellular HSP (eHsp70) has the opposite role, inducing activation of several proinflammatory pathways. Extracellular eHsp70 enters the bloodstream from living cells that are exposed to stress, most likely through vesicular secretion, exosomes or lysosomes and through intact lipid membranes that are independent of protein transport through the endoplasmic reticulum-Golgi apparatus, while passively it comes from damaged and necrotic cells (Molvarec et al., 2010; de Maio 2011). Multiple inflammatory pathways can be triggered as a consequence of exposure to extracellular eHsp70, most likely through its binding to Toll receptors on cell membranes (Ehses et al., 2010; Borges et al., 2012). On the other hand, intracellular iHsp70 blocks the activation of nuclear factor  $\kappa$ B (NF- $\kappa$ B), thus achieving its anti-inflammatory effect (Jones et al., 2011). NF- $\kappa$ B is a general transcription factor necessary for triggering inflammatory responses to various signals, and has been detected in B-lymphocytes (Barnes and Karin 1997; Chan et al., 2004; Chen et al., 2005). In the inflammatory response of the liver, as an important organ for the production of acute phase proteins and cytokines, the association of iHSP70 with NF- $\kappa$ B / I $\kappa$ B complex in the hepatocyte cytosol is of

great importance, which prevents the transcription of TNF- $\alpha$  and inducible nitric oxide synthase genes, thus achieving the anti-inflammatory effect of chaperone (Gabai et al., 1997). Cell survival is enabled after inhibition of c-Jun N-terminal kinase- (JNK-) signal transduction by intracellular Hsp70 after its increased production during the stress response (Beere et al., 2000). Cell protection basically inhibits apoptosis, which is achieved in several ways. iHSP70 prevents caspase activation (Tsuchiya et al., 2003; Zheng, et al., 2008), increases Bcl-2 expression and inhibits cytochrome C release (Creagh and Martin, 2003), in cerebral infarction increased expression of this heat shock protein decreases infarction and apoptosis (Geiger and Gupte 2011), Hsp70 reduces oxidative stress (Gutierrez et al., 2008), and cyclopentenone prostaglandins (cp-PGs), which under certain circumstances can cause Hsp70 expression and thus become potent anti-inflammatory autocoids (de Bittencourt and Curi 2001; Rossi et al., 2000). Intracellular Hsp70 also shows interference with proinflammatory cytokines, which is achieved at the gene level where the region of the gene that promotes TNF- $\alpha$  contains an HSF1 binding site that suppresses TNF- $\alpha$  transcription, which means that activation of HSF1 will lead to reduced expression of TNF- $\alpha$ , which may be a method of anti-inflammatory action (Xiao et al., 1999; Knowlton 2006). However, this process is two-way, so TNF- $\alpha$  can stop the activation of HSF1 (Dai et al., 2000; Li et al., 2010; Ghosh et al., 1998). Due to such relationships at the genetic level, the induction of Hsp72 (HSPA1A) reduces the expression of genes for cytokines such as TNF- $\alpha$ , IL-1, IL-12, IL-10 and IL-18 (Asea et al., 2000). Unlike intracellular Hsp70, eHSP70 has been shown to be of great importance in inflammatory responses, achieved by transducing MyD88 / IRAK / NF- $\kappa$ B signals after binding to Toll-like receptor 2 (TLR2) and TLR4, via CD14-dependent responses (Asea et al., 2002), which promotes innate immune activation (Kim and Sears 2010). eHSP70 has a paracrine role in the bloodstream (de Maio 2011). eHSP70 induces signals that give a typical proinflammatory response with increased production of NO and proinflammatory cytokines TNF- $\alpha$  and IL-1- $\beta$  (Campisi et al., 2003). eHSP70 is positively correlated with clinically significant indicators of inflammation such as CRP, fibrinogen, and monocyte count (Njemini et al., 2004).

The peripartum period in dairy cows is a model for studying metabolic stress. Metabolic stress in cows in early lactation occurs as a consequence of calving and the beginning of lactation, when there are changes in metabolism that support the upcoming lactation. Metabolic stress is characterized by a negative energy balance, increased milk production, directing glucose to the mammary gland, while lipolysis and ketogenesis meet the energy needs of other tissues, which affect the overall metabolic adaptation of cows (Pascottini et al., 2020; Belic et al., 2018). Increased influx of fatty acids in the liver, along with reduced

lipoprotein transport in the body leads to the development of fatty liver in cows and the development of ketosis (Mc Fadden and Review 2020). The only lipogenic hormone in the body of cows is insulin, whose values are reduced, and its action is inhibited under the influence of pronounced lipolysis and ketogenesis, resulting in insulin resistance that further stimulates lipolysis (Cincović et al., 2017). Increased serum TNF- $\alpha$  activity was found in cows with moderate to severe fatty liver syndrome (Ohtsuka et al., 2001). Several experimental studies have shown a direct influence of NEFA on inflammatory processes by influencing the activation of Toli-like receptors (TLRs), especially TLR4. Activation of TLR4 can lead to an inflammatory response with the secretion of proinflammatory cytokines (Contreras and Sordillo 2011; Sordillo et al., 2009). The period of lactation and milk production in cows affect the values of eHsp70. Thus, it was found that after delivery in early lactation and with increasing milk production, the concentration of eHsp70 in blood serum and saliva in cows increases (Catalani et al., 2010; Lamy et al., 2017; Kristensen, et al., 2004). The dynamic changes in the values of eHsp70 and TNF- $\alpha$  are almost identical in cows in the first weeks after calving when there is metabolic stress, so that the values of both parameters increase (Catalani et al., 2010). In addition, Hsp70 affects many metabolic flows related to energy metabolism, liver lipid metabolism, and insulin resistance, and exerts its activity via TLR4 (Archer et al., 2018; Shi et al., 2019).

## Conclusions

Hsp70 has a dual role in the body depending on whether it is intra- or extracellular.

Intracellular Hsp70 (iHsp70) shows its protective and anti-inflammatory effect.

The proinflammatory effect of eHsp70 is achieved by induction of innate immune cells, induces the secretion of inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-6), inducible expression of nitric oxide synthase (iNOS) and nuclear translocation of nuclear factor  $\kappa$ B (NF- $\kappa$ B).

The period of lactation and milk production in cows affect the values of eHsp70. After parturition in early lactation and with an increase in milk production, the concentration of eHsp70 in the blood serum and saliva of cows also increases.

According to the chaperone equilibrium theory, the higher the value of eHsp70 compared to iHsp70, the more pronounced its proinflammatory effects.

In addition, Hsp70 affects many metabolic flows related to energy metabolism, liver lipid metabolism, and insulin resistance.

As inflammation, insulin resistance and metabolic adaptations caused by parturition and early lactation are the most important elements for successfully overcoming the period of early lactation, Hsp70 can be an important indicator that can be used to assess the metabolic adaptation of cows.

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## **PROTECTION OF PLANTS**



## INTEGRATED STRATEGIES FOR MANAGING FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL CONTAMINATION IN WHEAT

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Review paper

### Abstract

*Fusarium head blight (FHB) is economically the most important wheat disease, causing yield and quality losses. Fungal species from the genus Fusarium are the main causative agents of FHB, with Fusarium graminearum as the predominant species. F. graminearum synthesizes more different secondary metabolites (mycotoxins). In wheat, the most studied mycotoxins are trichothecenes and zearalenone produced by F. graminearum. Trichothecene deoxynivalenol (DON) and its acetylated forms 3-acetyl-deoxynivalenol (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON) are the most detected in wheat grains. Strategies for controlling FHB and DON in wheat include different preharvest and postharvest measures, emphasizing integrated approaches. The main aim of this review was to present some preharvest and postharvest strategies for integrated FHB management in wheat production.*

**Keywords:** *integrated pest management, Fusarium head blight, deoxynivalenol, wheat*

### Introduction

Among main cereal crops (rice, wheat, maize), wheat is the second most important cereal crop worldwide, after rice. In 2020, Serbia harvested an area of wheat was 600,000 ha, producing 2,873,503 tons of wheat grains (Statistical Yearbook of the Republic of Serbia, 2021). The average yield was 4.9 tons per hectare. It is one of the major staple foods for humans. The wheat grain consists

of 80-85% starch and proteins, 9-20% dietary fibres, and minor contents of lipids, vitamins, minerals, and bioactive compounds (Prasadi and Joye, 2020).

*Fusarium* head blight (FHB) is one of the most devastating diseases of small grains (wheat, barley, rye) caused by various fungal species of the *Fusarium* genus, of which *F. graminearum* Schwabe (sexual stage – *Giberrella zeae* (Schwein.) Petch) is the most common in temperate climatic regions Europe, America and Asia (Wegulo et al., 2011; Krnjaja et al., 2011a,b; Župunski et al., 2019). *Fusarium* species that cause FHB infect wheat crops during the anthesis stage under favorable weather conditions, such as high temperatures, humidity, and abundant rain. Maize, wheat, and barley residues are usually the primary inoculum for the initial FHB infection (Shah et al., 2018). Cereal grains infected by *Fusarium* species often contain high concentrations of mycotoxins causing harm to humans and animals. Mycotoxins are secondary metabolites produced by fungal species. Food contaminated with mycotoxin can cause diseases in humans and animals (mycotoxicoses) (Zain, 2011).

*F. graminearum*, as the predominant FHB pathogen in the asexual cycle, produces macroconidia which are transported from soil or infected leaves to flowering spikes by rain and wind and proliferated to spikelets. FHB symptoms manifest as necrosis and bleaching of the spike with shriveled kernels. Orange sporodochia, which comprise macroconidia, are formed on infected spikelets. In the sexual cycle, *F. graminearum* produces abundant perithecia in the crop residues. Ascospores (sexual spores) are released from perithecia, which mature during warm and humid weather in spring and spread by wind, rain, or insects, causing initial FHB infection (Shah et al., 2018; Francesconi et al., 2019).

*F. graminearum* synthesizes more different mycotoxins, mainly trichothecenes and zearalenone. Trichothecenes are of sesquiterpenoid mycotoxins. They are divided into four groups, types A, B, C, and D. Mycotoxins from types A and B are the most important in cereal crops. B-type trichothecenes include the mycotoxins deoxynivalenol (DON), its derivatives (3-acetyl-deoxynivalenol (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON)), and nivalenol (NIV). DON represents the most frequently found mycotoxin in wheat grains worldwide (Piacentini et al., 2019). FHB symptoms can be improved by mycotoxin DON. DON can be a virulent factor in FHB pathogenesis (Tian et al., 2016; Krnjaja et al., 2021).

It is noticed that DON can contaminate straw and chaff, with higher levels in chaff than in the grains (Cowger and Arellano, 2013). It is very important in pig and turkey husbandry on a litter of straw and chaff. Wheat straw provides 12% of weaner pig's feed intake in straw-based systems. In dairy production, wheat straw and chaff are used as roughage feed. Additionally, wheat grains are

integrally part of ensiled forage for dairy and beef cattle. Mycotoxins also survive in ensiled grains and present a risk to animal health (Cowger and Arellano, 2013). Consumption of contaminated feed, DON causes intoxication of animals, inhibiting protein synthesis (Pestka, 2010). DON is a neurotoxin and immunotoxin, which can cause lower feed intake (anorexia), emesis, and feed refusal because it is called vomitoxin (Haidukowski et al., 2005).

The occurrence and development of FHB and DON contamination depend on many factors such as climatic, agroecological, and environmental conditions, especially during flowering stages, and also agricultural practices (crop rotation, tillage, cultivar susceptibility, fungicide applications, etc.) Fungal growth and DON synthesis in wheat grains can appear during preharvest and postharvest. Preharvest contamination of grains is influenced by environmental conditions (moisture, temperature, humidity, droughts, insects) and agronomic practices. In addition, some preventive measures such as drying and different types of decontamination are applied during storage and processing (Blandino et al., 2012; Los et al., 2018). Based on mentioned, the main aim of this research was to review and consider some of the sustainable control strategies in integrated management of FHB and DON in wheat grains, focusing on preharvest and postharvest preventive measures for mitigation of these contaminants in wheat grains.

## **Preharvest strategies for the control of FHB and DON**

Agronomic measures for preharvest wheat protection from FHB and DON contamination include tillage, crop rotation, early sowing, optimal fertilization, choosing resistant cultivars, and chemical and biological control. Integrated approaches are recommended as a more effective way to reduce FHB and DON occurrences. Likewise, the effectiveness of these measures individually or in combination depends on climatic and environmental conditions during the growing period, especially during the flowering wheat stages.

### *Tillage, crop rotation, sowing time, nitrogen fertilization, resistant cultivars*

*F. graminearum* survives and overwinters as a saprophyte on cereal residues that are the major source of inoculum for the next season. Tillage to bury crop residues and crop rotation with non-hosts are used as effective agronomic measures to reduce inoculum potential of pathogen and DON accumulation in wheat-growing (Dill-Macky and Jones, 2000). Sowing time is an important preventive measure for controlling FHB severity and DON in wheat crops. The



most risk of FHB infection is in 10-20 days from anthesis of wheat. Compared to late sowing time, early sowing time can contribute to avoiding favorable weather conditions for FHB infection during wheat flowering. However, this measure is recommended depending on the climatic conditions in which the crop is grown, especially during the anthesis and grain filling wheat stages (Shah et al., 2018). The effect of nitrogen fertilization on the development of FHB and DON synthesis is inconsistent. There are reports of a significant effect of increased N rates on FHB development and the level of DON (Lemmens et al., 2004), as well as reports that weather conditions affect FHB severity and DON synthesis more than N rates (Krnjaja et al., 2015). Additionally, Subedi et al. (2007) have reported small and inconsistent differences among nitrogen treatments and concluded that the effects of nitrogen on FHB incidence and severity were not of practical significance in spring wheat.

High yields of wheat are provided by sowing quality and healthy seeds. The main aim of the seed industry should also be to improve host resistance. There are several types of FHB resistance: type I - resistance to initial infection, type II - resistance to spread of *Fusarium* spp. within the spike, type III - resistance to seed infection, type IV - tolerance to FHB and DON, and type V - resistance to DON accumulation. Types I and II FHB resistance are the most described (Mesterházy, 1995; Boutigny et al., 2008). Cultivation of resistant cultivars reduces the occurrence of *Fusarium* species and the concentration of their mycotoxins. One of the ways to achieve this aim is to find sources of resistance to FHB and to determine quantitative trait loci (QTL) (genes) in a segregating population. This is a lengthy procedure because FHB resistance is determined by several genes which are influenced by the environment. An additional challenge in the breeding of wheat cultivars is the need to combine FHB resistance with exceptional agronomic and qualitative characteristics (Miedaner et al., 2017). However, knowledge of wheat genetic resistance to FHB has increased in recent years based on numerous studies of genetic resources in wheat relatives, cultivars, and breeding lines. There are numerous discovered and reported germplasms (FHB-resistant loci) available for breeding, for example, wheat genotypes from East Asia (Buerstmayr et al., 2020; Góral et al., 2021). Plant height significantly affects FHB. Buerstmayr et al. (2020) have established a significant dependence between FHB resistance and plant height, with taller plants as more resistant to FHB than shorter plants. Generally, there is a high risk of FHB infection for shorter plants because the distance from the inoculum source in plant residues on the soil to the spike is shorter than for taller plants. Furthermore, parameters such as the presence of awns and the percentage of flower opening at the flowering stage also contribute to the incidence and severity of FHB (Shah et al., 2018).

## Chemical and biological control

The application of fungicides is another important preharvest measure for controlling FHB and DON. The most commonly used fungicides are demethylation inhibitor (DMI) fungicides (metconazole, propiconazole, prothioconazole, tebuconazole, epoxiconazole), showing significant effectiveness in suppressing FHB and DON (Krnjaja et al., 2014; Wegulo et al., 2015). Additionally, to be effective, fungicides must be sprayed during flowering time. During anthesis, warm and wet climatic conditions intensify FHB symptoms, as the conidia infect spikelets during a short period at anthesis. Therefore, the timing of fungicide application is a key factor for successful FHB control. However, growers often have trouble doing fungicide treatment on time, because of the unfavorable weather conditions for crop spraying (Shah et al., 2018).

Biological agents (bio-fungicides) are also used in the FHB control strategy. Their application is especially useful in organic wheat production. Bio-fungicides can be many beneficial fungi, bacteria, and yeasts, which infect and control the development of plant pathogens. Bacteria from the genera *Bacillus*, *Pseudomonas*, and *Streptomyces* have already been used in the field with successful results in the reduction of FHB severity (Legrand et al., 2017). In addition, *in vitro* tested strains of *Lactobacillus plantarum* and *Bacillus amyloliquefaciens* have also shown antimicrobial activity against tested strains of *F. graminearum* and *F. culmorum* isolated from wheat (Baffoni et al., 2015). The strain of *Bacillus subtilis* isolated from wheat anthers has shown an antagonistic effect against *F. graminearum* (Palazzini et al., 2016). Furthermore, the yeast species *Aureobasidium pullulans* reduced FHB severity by 20% on winter wheat in greenhouse conditions (Sarrocco and Vannacci, 2018). Then, fungal species from the genus *Trichoderma* are used in biological control against *Fusarium* spp., with *T. harzianum* as the most researched species (Filizola et al., 2019). However, *T. games* can also significantly reduce FHB severity and DON production. Mycoparasitic species *Clonostachys rosea* is used as a beneficial fungus against FHB, reducing perithecial production. Co-culture of *Cryptococcus flavescens* and *C. aureus* reduced FHB severity in wheat greenhouse trials (Sarrocco and Vannacci, 2018). Metabolites produced by plants (phenolic compounds and essential oils) are effective inhibitors of *F. graminearum* infections. Likewise, essential oils (EOs) or plant extracts are used as bio-fungicides (Grahovac et al., 2009). Due to the negative impact of pesticides on the environment and human health, EOs are used as biocontrol products. The steam distillation method is used for their obtaining from different plant organs. The chemical composition of EOs varies, it depends on the plant organ from which the EO is extracted (Raveau et

al., 2020). By testing the efficiency of different EOs to reduce DON accumulation, such as extracts from cinnamon, clove, lemongrass, oregano, and palmarosa, the EO extract of clove was the most effective to reduce the accumulation of DON in wheat grain infected by *F. graminearum*. However, the mycotoxigenic activity of EOs affected by environmental factors (temperature and water activity) (Shah et al., 2018).

## Postharvest strategies for the control of FHB and DON

Protection of wheat grains from fungal contaminants is also necessary during the postharvest period. Due to fungal postharvest contamination, the grains lose nutritive and qualitative values and become unusable for human and animal consumption. Therefore, physical, biological, and chemical methods are recommended as post-harvest management strategies for wheat grains protection.

Physical methods are based on preventive approaches. The moisture content of wheat grains should be 13.5% or lower during harvest and storage (Wegulo et al., 2015). Storage temperature and humidity must be adequate to prevent fungal growth and mycotoxin synthesis. Aeration of storage can also reduce the risk of fungal development (Mielniczuk and Skwaryło-Bednarz, 2020). Since insects can be fungal vectors, it is important to protect storage wheat grains from insects by using synthetic (methyl bromide and phosphine) and natural insecticides (plant essential oils), traps, and sticky traps (Kumar and Kalita, 2017; Kljajić et al., 2021). Ozonation prevents fungal development during storage and reduces DON in wheat grains. DON degradation is positively correlated with ozone concentration and the time of grain exposure to ozone (Li et al., 2015). In contaminated cereals, mycotoxins can be reduced by using adsorbents such as aluminosilicate minerals (zeolite, clay minerals) (Krnjaja et al., 2009).

Biological methods include the application of microorganisms for the neutralization of mycotoxins in wheat grain after harvest. Lactic acid bacteria (LAB), from the genera *Lactobacillus* and *Pediococcus*, reduced DON levels in malting wheat grains by adsorption mechanisms or microbiological binding. Modified glucomannan obtained from *Saccharomyces cerevisiae* yeast cell walls successfully reduces certain individual and combined harmful effects of mycotoxins. Another biological method based on the reduction of mycotoxins is the application of microorganisms that degrade mycotoxins into lower toxic compounds than the initial substance. Trichothecene mycotoxins are degraded by *Curtobacterium* spp. (Krnjaja et al., 2009; Mielniczuk and Skwaryło-Bednarz, 2020).

Chemical methods are based on detoxification by different chemical compounds. Organic acids such as citric and lactic acid are used for reducing DON and its derivatives in feed (Humer et al., 2016). Chlorine, hydrogen sulfates and ammonium hydroxide are also used for inactivating one or more mycotoxins and microorganisms. However, the use of any chemical compounds for conversing or disrupting mycotoxins may have negative effects on the sensory and nutritional properties of wheat and its products (Los et al., 2018; Mielniczuk and Skwaryło-Bednarz, 2020).

## Conclusion

Individual preharvest and postharvest measures can be usefully for reducing FHB incidence, but integrated control strategies for FHB are more efficient, especially under favorable environmental conditions for *Fusarium* infection. The selection of resistant cultivars to FHB is the most effective preventive measure in wheat protection. However, the complexity of creating resistant cultivars leads to applying multiple strategies in FHB management, including the combination of measures such as fungicides and other agronomic practices. After harvest, grains should be stored in adequate storage with increased aeration capacity. Implementation of postharvest strategies is used to protect wheat grains from fungal contaminants and DON accumulation, of which all physical and biological methods are especially recommended.

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## **Интегралне мере у заштити пшенице од фузариоза класа и деоксиниваленола**

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Ревизијални рад

### **Апстракт**

*Фузариоза класа (ФХБ) је економски најважнија болест пшенице која проузрокује губитке у приносу и квалитету. Врсте гљива из рода Fusarium су главни проузроковачи ФХБ, са Fusarium graminearum као примарном врстом. F. graminearum синтетиче више различитих секундарних метаболита (микотоксина). У пшеници, највише проучавани микотоксини су трихотецени и зеараленон продуктовани од F. graminearum. Трихотецен деоксиниваленол (ДОН) и његови ацетиловани облици 3-ацетил-деоксиниваленол (3-АДОН) и 15-ацетил-деоксиниваленол (15-АДОН) су најчешће детектовани микотоксини у зрну пшенице. Стратегије за контролу ФХБ и ДОН у пшеници укључују коришћење различитих мера заштите пре и после жетве, наглашавајући интегралне приступе. Главни циљ овог прегледног рада био је да представи неке стратегије заштите пре и после жетве, као део интегралног концепта управљања фузариозама у производњи пшенице.*

*Кључне речи: интегралне мере заштите, фузариоза класа, деоксиниваленол, пшеница*



## Integralne mere u zaštiti pšenice od fuzarioza klasa i deoksinivalenola

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### Apstrakt

*Fuzarioza klasa (FHB) je ekonomski najvažnija bolest pšenice koja prouzrokuje gubitke u prinosu i kvalitetu. Vrste gljiva iz roda Fusarium su glavni prouzrokovatori FHB, sa Fusarium graminearum kao primarnom vrstom. F. graminearum sintetizira više različitih sekundarnih metabolita (mikotoksina). U pšenici, najviše proučavani mikotoksini su trihoteceni i zearalenon produkovani od F. graminearum. Trihotecen deoksinivalenol (DON) i njegovi acetilovani oblici 3-acetil-deoksinivalenol (3-ADON) i 15-acetil-deoksinivalenol (15-ADON) su najčešće detektovani mikotoksini u zrnu pšenice. Strategije za kontrolu FHB i DON u pšenici uključuju korišćenje različitih mera zaštite pre i posle žetve, naglašavajući integralne pristupe. Glavni cilj ovog preglednog rada bio je da predstavi neke strategije zaštite pre i posle žetve, kao deo integralnog koncepta upravljanja fuzariozom u proizvodnji pšenice.*

*Ključne reči: integralne mere zaštite, fuzarioza klasa, deoksinivalenol, pšenica*

## YIELD AND YIELD COMPONENTS GRAINS OF PERSPECTIVE GENOTYPES OF WINTER WHEAT

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### Abstract

*In the experimental field of the Center for Small Grains in Kragujevac in two growing seasons, plant height, 1,000 kernel weight, test weight, water content in grain, and grain yield of 10 wheat genotypes were analyzed (G-1/6, G-2/98, G-3/7, G-4/7, G-5/24, G-6/2, G-7 / 5-9, G-8 / 5-4, G-9/36 and Victory). The experiment was laid out in a randomized block design with three replications on a Vertisol soil. In both growing seasons, precipitation and average annual temperature were above the multi-year. The highest yield during two years has the genotype G-7/5-9, then G-6/2, and the lowest G-4/7. The 1,000-kernel weight for all genotypes was greater than 40 g. Geotype G-7/5-9 had an average 1,000-kernel weight greater than 50 g this year, is an important parameter for assessing variety breeding. All genotypes has good resistance to lodging and a satisfactory height of the stem, which ranged from 76 cm to 96 cm in the first vegetation season, and from 72 cm to 90 cm in the second.*

**Keywords:** wheat, grain yield, 1,000-kernel weight, plant height, lodging

### INTRODUCTION

Worldwide, wheat is produced on areas that vary from 209 to 232 million hectares, with a grain yield of 560 to 750 million tons (Knežević et al. 2022). In the Republic of Serbia, compared to the ten-year average (2011-2020), the area under wheat increased by 9.4%, and the average wheat yields range from 4.5-8 t ha<sup>-1</sup> (SYRS 2020). Annual wheat consumption per capita varies depending on the region from 20 kg in Central America and sub-Saharan Africa, 70 kg in India, to over 150 kg in the Middle East (Shewry and Hey, 2015) and about 130 kg in the

Republic of Serbia (Knežević et al., 2022). To increase the yield, producers can use the variety, as a biological agent and cultivation technology as a technological solution that allows different degrees of expression of the genetic potential of the variety (Denčić et al., 2009; Knežević et al., 2020). A large number of properties play a decisive role in the formation of yields. The contribution of each of them may be different in different genotypes and in different environmental conditions, as a result of the interaction between traits within each genotype and the interaction between genotype and environmental conditions (Denčić et al., 2006; Denčić and Kobiljski, 2007; Đekić et al., 2013).

Plant height is one of the basic factors influencing lodging resistance (Berry et al., 2004). Stem morphology, as well as chemical characteristics of basal internodes are the basic determinants of stem strength or elasticity (Berry et al., 2003; Tripathi et al., 2005; Kong et al., 2013) while the strength of root-soil connection is related to the development and depth and mechanical characteristics of the soil itself (Foulkes et al., 2011). Stem bending in wheat (*Triticum aestivum* L.) is a significant agronomic problem, which greatly affects the economics of production (Singh et al., 2019) and can cause a reduction in yield of up to 40%, if it occurs immediately after classification (Kelbert et al., 2004).

Values of test weight depend on a large number of factors primarily on the tested material and method of execution. Wheat grain quality is a complex concept and includes several properties: physical characteristics of grain (test weight, 1,000-kernel weight, specific weight, grain size and shape, glassiness and flour content, grain color, grain purity), grain health and freshness, grain chemical composition and others. To assess the quality based on the physical properties of grain, it is easiest to determine the properties of test weight, 1,000 kernel weight and specific grain mass, and lower mass indicates more shells in grain mass and lower endosperm compactness (Kovačević and Rastija, 2014).

The aim of this study was to analyze plant height, lodging resistance, 1,000-kernel weight, test weight, water content in grain and grain yield of 10 wheat genotypes in two growing seasons.

## MATERIALS AND METHODS

The research was conducted at Center for Small Grains in Kragujevac in the growing seasons 2018/19 (GS1) and 2019/20 (GS2). Ten wheat varieties G-1/6, G-2/98, G-3/7, G-4/7, G-5/24, G-6/2, G-7/5-9, G-8/5-4, G-9/36 and Victory were evaluated for their plant height, lodging resistance, 1,000-kernel weight, test weight, water content in grain, and grain yield. The experiment was set up according to the plan of a completely random block system in four repetitions, with an area of the basic plot of 5 m<sup>2</sup>, number of rows 10, spacing between rows

12.5 cm and distance of plants in a row of 3 cm. Sowing in both years was done with a micro-seed drill, in the optimal time in the second half of October. Maize was used as the preceding crop in each experimental year. During the growing season the standard measures of plant protection were used. At tillering, 50 kg ha<sup>-1</sup> nitrogen was applied Calcium ammonium nitrate (CAN) with a nitrogen content of 27% was used for top dressing. Results were subjected to a two-way ANOVA (year, cultivar) for the experimental period using the statistical package SAS/STAT, 2000. User's Guide, Version 9.1.3. The significance of difference between the means was assessed by LSD test at the 95% level.

### ***Meteorological conditions***

The average annual air temperature in both growing seasons (10.97 and 11.49°C) was higher than the 30-year average (10.5°C). According to the average air temperature (11.7°C), 2020 was the seventh warmest from 1951 until today.

Table 1. Average monthly temperature and amount of precipitation during the two growing seasons and multi-year average

| Growing season / month | Average monthly temperature (°C) |       |           | Precipitation sum (mm) |       |           |
|------------------------|----------------------------------|-------|-----------|------------------------|-------|-----------|
|                        | GS1                              | GS2   | 1981-2010 | GS1                    | GS2   | 1981-2010 |
| X                      | 13.9                             | 13.6  | 11.9      | 9.4                    | 196   | 48.9      |
| XI                     | 7.6                              | 11.7  | 6.4       | 41.8                   | 68.1  | 49.5      |
| XII                    | 2.6                              | 4.9   | 2.1       | 51.8                   | 57.6  | 45.8      |
| I                      | 0.1                              | 1.3   | 0.9       | 85.3                   | 23.3  | 37.9      |
| II                     | 4.2                              | 6.2   | 2.3       | 2.2                    | 47.6  | 37.0      |
| III                    | 9.1                              | 7.8   | 6.6       | 10                     | 55.7  | 42.3      |
| IV                     | 13.2                             | 11.8  | 11.7      | 35.2                   | 17.8  | 53.9      |
| V                      | 14.5                             | 15.7  | 16.7      | 125.3                  | 7.9   | 58.7      |
| VI                     | 22.4                             | 19.9  | 20.0      | 143                    | 192.9 | 76.4      |
| VII                    | 22.3                             | 22.0  | 21.9      | 83.2                   | 61.6  | 57.7      |
| <i>Average/Sum</i>     | 10.97                            | 11.49 | 10.5      | 627.2                  | 728.5 | 508.1     |

Average temperatures during the vegetation period (March-July) were mostly higher or at the level of the multi-year average. The amount of precipitation in the vegetation period 2018/19. was 627.2 mm, while 2020 was on average rainy, and

in some central parts of Serbia very rainy and extremely rainy. The amount of precipitation for the city of Kragujevac in the vegetation period 2019/20 was 728.5 mm. The highest amount of precipitation in 2019 and 2020 was in June and amounted to 143 mm in 2019, and 192.9 mm in 2020. The average annual temperature and precipitation in both vegetation periods were above the multi-year average (Table1).

## RESULTS AND DISCUSSION

Plant height is one of the most important initial selection criteria and is the main factor influencing the resistance to lodging. Although the height of wheat plants is controlled mainly by genetic factors, it varies depending on climatic conditions and applied production technology measures. Wheat genotypes differed in plant height, so that the highest value for this trait in both growing seasons was the G-5/24 line, and the lowest was L-1/6 (Table 2). In the first growing season, a slightly higher plant height was recorded, ranging from 76 cm to 96 cm, and in the second from 72 cm to 90 cm. Plant height in cereals has been associated with lodging resistance at all developmental stages (Navabi et al., 2006; Berry et al., 2015).

Genotypes of longer stems are more sensitive to lodging, in contrast to lower genotypes that do not lie down in conditions that cause lodging. Analyzing genetic variability for lodging resistance in a divergent wheat population (140 genotypes) in groups of genotypes formed based on stem height (low, medium and high genotypes) Navabi et al. (2006) concluded that grain yield is negatively correlated with lodging, ie that lodging sensitivity is positively correlated with plant height.

Table 2. Plant height, test weight and 1,000-kernel weight of wheat during the two growing seasons

| Genotype | Plant height (cm) |       | Test weight |       | 1,000-kernel weight (g) |       |
|----------|-------------------|-------|-------------|-------|-------------------------|-------|
|          | GS1               | GS2   | GS1         | GS2   | GS1                     | GS2   |
| G-1/6    | 76.00             | 72.38 | 81.10       | 79.50 | 44.00                   | 42.36 |
| G-2/98   | 84.20             | 83.50 | 82.10       | 82.90 | 44.50                   | 47.74 |
| G -3/7   | 90.15             | 88.25 | 82.10       | 83.30 | 44.35                   | 44.52 |
| G -4/7   | 74.10             | 75.13 | 80.30       | 82.30 | 45.70                   | 46.86 |
| G -5/24  | 96.00             | 90.00 | 81,70       | 82,50 | 45.25                   | 50.52 |

|          |       |       |       |       |       |       |
|----------|-------|-------|-------|-------|-------|-------|
| G -6/2   | 90.17 | 88.00 | 85,50 | 82,30 | 47.30 | 44.56 |
| G -7/5-9 | 86.30 | 87.88 | 83,30 | 81,10 | 50.38 | 50.82 |
| G -8/5-4 | 84.35 | 88.25 | 84,90 | 83,30 | 47.48 | 47.26 |
| G -9-2/3 | 80.23 | 78.63 | 85,30 | 83,50 | 42.53 | 45.80 |
| Victory  | 84.74 | 83.50 | 81.30 | 82.70 | 45.70 | 46.86 |
| Average  | 84.62 | 83.55 | 82.76 | 82,14 | 45.72 | 46.73 |
| LSD 0.05 | 3.34  | 3.15  | 2.45  | 2.38  | 3.65  | 3.51  |

Test weight is accepted as a measure of wheat quality due to its simple and fast performance. Test weight can also serve as an orientation value for assessing grindiness. Values of test weight depend on a large number of factors primarily on the tested material and method of execution. The increase in test weight is influenced by: rapid filling during filling, smooth grain surface, round grains, glassiness of grains, mixed grains of different sizes, broken and small grains, etc. Test weight varies from 60 to 84 kg hL<sup>-1</sup> for wheat, and good wheat must have a hectolitre weight above 76 kg hL<sup>-1</sup>. In the two-year period, the value of test weight ranged from 80.3 to 84.4 kg hL<sup>-1</sup>. The genotypes G-9-2/3 (84.4 kg hL<sup>-1</sup>), G-7/5-4 (84.1 kg hL<sup>-1</sup>) has the highest average value per test weight, while the G-1/6 (80.3 kg hL<sup>-1</sup>) had the lowest two-year average value (Table 2).

1,000-kernel weight is not only directly related to grain yield and milling quality of grain, but also has an impact on germination energy and growth indirectly affecting the yield (Botwright et al., 2002).

Table3. Grain yield and water content at harvest time of wheat during the two growing seasons

| Genotyp  | Grain yield (t ha <sup>-1</sup> ) |       |         | Water content (%) |       |
|----------|-----------------------------------|-------|---------|-------------------|-------|
|          | GS1                               | GS2   | Average | GS1               | GS2   |
| G-1/6    | 6.360                             | 5.808 | 6.084   | 12.95             | 10.40 |
| G-2/98   | 6.530                             | 6.200 | 6.365   | 13.88             | 13.15 |
| G -3/7   | 6.000                             | 6.462 | 6.231   | 9.85              | 10.85 |
| G -4/7   | 5.715                             | 5.978 | 5.846   | 12.23             | 11.23 |
| G -5/24  | 6.580                             | 5.833 | 6.206   | 12.05             | 12.80 |
| G -6/2   | 6.710                             | 6.120 | 6.415   | 12.20             | 10.93 |
| G -7/5-9 | 6.320                             | 6.650 | 6.485   | 13.40             | 12.30 |

|          |       |       |       |       |       |
|----------|-------|-------|-------|-------|-------|
| G -8/5-4 | 5.870 | 6.540 | 6.205 | 12.96 | 10.48 |
| G -9-2/3 | 5.705 | 6.420 | 6.062 | 13.03 | 13.65 |
| Pobeda   | 6.025 | 6.320 | 6.172 | 12.95 | 9.87  |
| Average  | 6.182 | 6.233 |       | 12.55 | 12    |

The 1,000-kernel weight is usually expressed as the 1,000-kernel weight dry and undamaged grains and depends on the variety, agroecological conditions and agricultural technology. In our country, it ranges from 33 to 45 g, and on average about 38 g and 50 g is an indicator of high grain yield (Kovačević and Rastija, 2014). Sufficient precipitation and optimal temperatures in both growing season during the seed sowing period increased the 1,000-kernel weight, so that all genotypes had a 1,000-kernel weight greater than 40 g (Table 2). Geotype G-7/5-9 in both years has an average 1,000-kernel weight greater than 50 g, an important parameter for the evaluation of variety breeding.

Wheat yields in the field are usually low and vary with weather, soil, and crop management practices. In the first year, the highest grain yield ( $6.710 \text{ t ha}^{-1}$ ) was achieved by line G-246/2, followed by L-5/24 ( $6.580 \text{ t ha}^{-1}$ ), while L-9-2/3 has the lowest yield ( $5.705 \text{ t ha}^{-1}$ ) (Table 3). In the second year, the highest grain yield was achieved by line L-7/5-9 ( $6.650 \text{ t ha}^{-1}$ ), followed by L-7/5-9 ( $6.540 \text{ t ha}^{-1}$ ), and the lowest yield by line L-1/6 ( $5.808 \text{ t ha}^{-1}$ ).

The highest average yield during two years has the genotype G-7/5-9 ( $6.485 \text{ t ha}^{-1}$ ), followed by G-6/2 ( $6.415 \text{ t ha}^{-1}$ ), and the lowest yield of the line G-4/7 ( $5.846 \text{ t ha}^{-1}$ ). The lowest water content at harvest time (9.85%) in the first year has the G-3/7 and the highest G-2/98 (13.8%). In the second growing season, the genotype L-9-2/3 has the highest water content (13.65), and the G-1/6 had the lowest water content (10.40%).

## CONCLUSION

The average annual temperature and the amount of precipitation in both growing seasons were above the multi-year average and had a positive effect on the yield and components of wheat yield. The highest grain yield and test mass were genotype G-7/5-9 ( $6.485 \text{ t ha}^{-1}$ ), followed by G-6/2 ( $6.415 \text{ t ha}^{-1}$ ), and the lowest yield of lines G-4/7 ( $5.846 \text{ t ha}^{-1}$ ). Sufficient precipitation and optimal temperature in both growing seasons during the seed sowing period influenced the increase in the 1,000-kernel weight, so that all genotypes have been an 1,000-kernel weight greater than 40 g. Genotype G-7/5-9 in both years have been an 1,000-kernel weight greater than 50 g, is an important parameter for the

evaluation of variety breeding. Wheat genotypes differed in plant height. In the first growing season, a slightly higher plant height was recorded, ranging from 76 cm to 96 cm, and in the second from 72 cm to 90 cm. In both growing seasons, the genotype G-5/24 had the highest value of plant height, and the lowest G-1/6. All genotypes they were good lodging resistance.

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## APPLICATION OF NEW STRATEGIES FOR ANALYSIS OF PESTICIDE RESIDUES IN FRUIT

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### ABSTRACT

*Due to many years of application worldwide, pesticides have become significant pollutants, and at the same time due to their persistence, solubility and mobility can be found in all spheres of the environment. Since pesticides are used in the cultivation of conventional fruit to control disease, it is important to analysis and monitor the presence of pesticides in fruit. The presence of pesticides in fruit can be a consequence of direct use, but also indirect pollution from already contaminated soil or water. Therefore, pesticide residues in fruit can also be indicators of polluted environment and soil. For the analysis of the presence of pesticides in fruit, it is important to include as much testing of different pesticides as possible. On the other hand, it is essential to consider an appropriate method of preparation for specific pesticides and to apply a sensitive detection technique. The most widely used detection technique for the determination of pesticides in fruit is mass spectrometry combined with gas and/or liquid chromatography. In this paper sample preparation approaches for determination pesticide residues in fruit, as well as techniques used to quantification of pesticides, will be discussed. The latest research of the occurrences pesticides residues in fruit will be discussed, developed methods for pesticide preparation will be presented, as well as advantages in determination pesticides in fruit.*

**Key words:** pesticides residue, conventional fruit, preparation

### 1. INTRODUCTION

Nowadays, the fruit growing requires use of a wide range of insecticides, fungicides and herbicides (Dias et al, 2016; Sadlo et al, 2018). Thus, all this pesticide residues may occur in the fruit products and final processed fruit products. The use of pesticides has increased significantly during the last years. Although the use of pesticides brings many benefits of agriculture, but some of

pesticides residues can affect human health (Tette et al., 2016). Short-term exposure to pesticides does not have a significant harmful effect, while long-term exposure can lead to the development of various allergies, malignant diseases, cardiovascular diseases and damage to vital organs (Brantseter et al, 2017).

More than a thousand different pesticides and their formulations can be used in the different stages of cultivation and during postharvest storages against a range of pests and fungi (Zanella et al., 2012). Consequently, due to the different input sources, pesticides residue can be transferred to fruits and vegetables, which can be determined in them and their products. Contamination of fruit may include both direct application of pesticides and indirectly through environmental contamination. However, the use of pesticides often leads to the presence of pesticide residues below permitted limits (maximum residue levels, MRLs).

Control and monitoring of pesticides in food, and especially in fruit, is necessary to ensure consumer safety. The European Food Safety Authority (EFSA) provides an annual report on the number of pesticide samples tested in Europe each year. The report covers the type of food tested, the frequency of pesticides, the type and number of pesticides detected. Concerns about food safety have led to the strict regulation of maximum residue limit (MRL) of pesticide residues in food. Risk assessment of pesticides is typically based on the pesticide residues data from food monitoring and food consumption data. Another challenge is that in most cases residue data for maximum residue levels are only available for the raw agricultural products, and effects of processing including washing or peeling are difficult to address due to limited availability of processing factors (Vazquez et al, 2016; Tankiewicz and Biziuk, 2018).

In the last few years many studies were published aiming the development of sensitive and accurate methods for pesticides determination in all food matrices. Analysis of pesticide residues in fruit is a challenge for analytical chemists. Pesticide residues can be analyzed using gas chromatography with electron capture detection (GC/ECD) and gas or liquid chromatography coupled to mass spectrometric detection, and also using tandem mass spectrometry (Hildmann et al 2015; Lawal et al, 2018).

The aim of this paper is to present an overview about recent innovation in sample preparation methods that were developed for the determination of pesticides in fruit by gas and liquid chromatographic techniques. The new extraction and modified procedures were considered in this paper which include application for analysis of pesticides in real fruit samples. The aim of the paper is primarily to summarize the progress in research regarding pesticide residues in fruits.

## 2. METHODS FOR THE ANALYSIS OF PESTICIDES IN FRUIT

### 2.1. Sample preparation

The analysis of pesticides in fruit requires sample preparation, separation and detection. The preparation techniques for analyzing pesticides from fruit are: liquid-liquid extraction (LLE), liquid-liquid microextraction (LLME), solid-phase extraction (SPE), solid-phase microextraction (SPME) and gel permeable chromatography (GPC). Solid-phase extraction is currently the most popular and used extraction technique (Biziuk and Stocka, 2015). The first step in pesticide analysis is the choice of method, validation of the chosen method and optimization of the technique on the instrument on which the determination is performed. Validation guidelines are used for method validation, but the guide prescribed by European regulations, the so-called SANTE guidance, is most often used. This guide precisely defines the conditions for linearity, correlation coefficient, recovery, repeatability, accuracy, relative standard deviations and calculation of measurement uncertainty. This guidance allows the laboratories to have free choice of methods, which is beneficial for the continuous evaluation of the analytical methods. Laboratories performing analyses of pesticides residues also tend to work under a quality system and standard ISO/EC 17025. This involves the use of certified reference materials and participation in proficiency tests (Dehouck *et al.*, 2015).

For most of pesticides compounds, regulatory guidelines set maximum residue levels (MRLs) in all different classis of fruit. The MRL values list for a wide variety of commodities and pesticides and their metabolites is updated from time to time. Maximum residue levels were first introduced by European Regulation in 2005 (Regulation (EC) No. 396/2005), and control of testing of real samples began in 2008. All existing pesticides regulations are requesting analytical methods to be provided for validation.

QuEChERS method introduced by Ananstassiades *et al.* (2003) and based on the liquid-liquid partitioning with acetonitrile followed by a clean-up step with d-SPE, is the most important method for pesticide analysis. QuEChERS is an abbreviation of the following words Quick, Easy, Cheap, Effective, Rugged and Safe, that describe the method. It has been the most popular technique for sample preparation and the most commonly used method. The primary challenge for the modern QuEChERS multi-residue analysis is the possibility of modifying the method and adapting the method of preparation depending on the reliability of testing specific or required pesticides in the tested matrix. Some modification of

the original QuEChERS method have been carried out depending on the matrix analyzed and the characteristics of the analyzed pesticides.

The organic phase is purified by dispersive SPE using primary secondary amine (PSA) and graphite black carbon (GCB if necessary). The role of magnesium sulphate is to absorb the trace amount of water in the first step of extraction (Ananstassiades *et al*, 2003). Primary secondary amin (PSA) retains fatty acids from the acetonitrile extract with a weak anion exchange mechanism (Tankiewicz and Biziuk, 2018). The PSA-treated extract can be acidified with formic acid to increase the stability of base-sensitive pesticides. By applying QuEChERS extraction kits for the determination of pesticides in fruits and vegetables, the effects of the matrix are reduced and it is possible to determine a large number of analytes. The method can be modified depending on the type of sample and the target analytes. To improve the release of polar to the base environment sensitive pesticides, the method was modified using buffered salts, which achieve a pH value of 5. When testing citrus fruit samples, the wax protection can be removed by freezing the samples for at least one hour.

The QuEChERS method has been shown to be practical for pesticide analysis on a number of different pesticides groups and is increasingly being employed on all fruit matrices. From the point of view of analysis, sample preparation remains a challenge related to the evaluation of multiresidue methods for pesticide analysis. Wanwimolruk *et al*. (2019) reported washing and peeling experiments to guava fruit samples using 15 g of homogenized guava fruit. In the extraction experiment 15 mL of acetonitrile (with 1% acetic acid) saturated with 6 g of magnesium sulphate and 2.5 g of sodium chloride was used. In the second phase, 50 mg of PSA and 150 mg of magnesium sulfate were used for cleanup, and GC-MS/MS was used for detection. Sixty-seven pesticides and two metabolites were validated.

Most recently, a new sorbent based on charcoal-celite has been employed for clean-up step for analysis etoxazole from strawberry juice (Malhat and Anagnostopoulos, 2020). Multiresidue methods have become more popular and essential to control a growing number of active substances. Although provided for use in fruit and vegetables, the QuEChERS technique was later modified for the analysis of the fatty food of animal origin (Jeong, 2012).

A few methods are reported in the literature for multiresidue analysis in bananas. Carneiro *et al* (2013) developed a method to investigate the occurrence of 128 pesticides in bananas by a modified QuEChERS procedure and UHPLC-MS-MS analysis. Analysis of the samples confirmed the presence of the following pesticides: boscalid, carbendazim and imidacloprid were quantified, respectively, at the concentration  $(31.0 \pm 4.12 \text{ } \mu\text{g/kg})$ ,  $(24.0 \pm 3.65 \text{ } \mu\text{g/kg})$ , and  $(13.0 \pm 5.17$

µg/kg). The results presented in this report demonstrate that the validated method is feasible to be applied in pesticides routine analysis carried out in the matrices with high water content. Another study was conducted by Borges *et al* (2009) for analysis of 10 organophosphates pesticides (ethoprophos, dimethoate, malathion, malaoxon, chlorpyrifos, chlorpyrifos-methyl, diazinon, fenitrothion, fenamiphos and phosmet) and buprofezin in banana samples taken from the local markets of the Spain Islands. The analysis method was based on QuEChERS schemes and using gas chromatography with nitrogen-phosphorus detection (GC-NPD).

Due to the use of organic solvents such as acetonitrile, the obtained extracts typically contain a relatively large number of coextracted pigments. Different approaches based on novel sorbents used in dispersive solid-phase extraction have been evaluated in terms of pesticides recovery and matrix effects impact. The matrix effect is especially important when liquid chromatography with mass detection is used for pesticide detection.

## 2.2. Separation and detection

Continuous improvement of mass spectrometric detections has allowed in getting better pesticides residue analysis. Tandem mass spectrometry (MS/MS), operating in the multiple reaction mode, is routinely employed for the sensitive determination of pesticide residues in food and feed commodities. It is recommended for analysis pesticides in the entire world because its information rich content and explicit conformation (Nasiri *et al*, 2016).

Thus liqued chromatography-tandem mass spectrometry (LC-MS/MS) methods are based on triple quadrupole (QqQ) analyzers are frequently used in environmental and food analysis because of the high sensitivity achieved using Selected Reaction Monitoring (SRM) acquisition mode. As a compromise between sensitivity, acceptable chromatographic peak shape and conformation purposes, two SRM transitions are currently monitored. The last few years, mass spectrometry has been coupled with certain advances in chromatographic innovation such as Ultra-Fast Liquid Chromatography (UHPLC). These analyses have made possible the development of multiresidue determination covering many trace contaminants. Important information is also that UHPLC can reduce the analysis time and increase sensitivity (Carneiro *et al*, 2013).

The use of high resolution MS (HRMS) instruments has been introduced for quantitative pesticide residues analysis in grapes. One of the main attributes of the HRMS analyzers is their accurate mass measurements, increasing the

reliability of the analyte detection by providing extra selectivity by elemental composition of parent and fragment ion spectra (Grimalt and Dehouck, 2016).

For multiresidues determinations using gas chromatography, detection can be by selective and sensitive detectors such as flame photometric (FPD), pulsed flame photometric (PFPD), nitrogen-phosphorus (NPD), electron capture detectors (ECD) or mass spectrometer detectors (GC-MSD). Nowadays, the use of mass spectrometry is almost replaceable and unavoidable. Mass spectrometry is a very sensitive and selective technique for the determination of organic compounds and identification at the broad-spectrum level. Scanning in the MS system can be in full mode and selected individual ionic monitoring (SIM), adequate selection of appropriate target ions for each analyte, which gives great selectivity of the method. The condition for the determination of pesticides using gas chromatography is that it is volatile and thermally stable.

LC-MS/MS is suitable for the determination of heat-labile pesticides (carbamates) and polar pesticides (neonicotinoids and organophosphorus pesticides) that are challenging if not impossible to analyze with GC-MS/MS (Chamkasem and Harmon, 2015). Some of pesticides (for example, methamidophos, omethoate and thiabendazole) tend to show peak tailing via interaction with or adsorption onto active sites of the injector or stationary phase of columns during the GC separation. Many pesticides can be detected using both test techniques, as both techniques have the ability to achieve low levels of quantification, and this method of determination provides a complete insight into as many pesticides tested as possible.

Over time, the amount of pesticides present may change due to the physicochemical properties of the pesticide and environmental factors. In this way, the mentioned factors can influence the experimental data and safety assessment, as well as the registration and establishment of the maximum residue levels of pesticides. The course of the analysis can also be affected by degradation reactions, which include chemical reactions, primarily hydrolysis, oxidation-reduction, ionization and photodegradation, as well as microbiological degradation (Wanwimolruk et al, 2019). The stability of pesticides can be affected by the type of matrix, i.e. its composition, the presence of organic matter, pH value and water content (Malhat and Anagnostopoulos, 2020).

Recent study by Hou *et al* (2016) identified and confirmed the presence of 101 pesticides in green tea leaves using gas chromatography/tandem mass spectrometry. Tea leaves are a very complex matrix, but using PSA/GCB (500 mg) and NH<sub>2</sub> absorbent for cleaning provides satisfactory recovery and precision for all determined pesticides.

In these last years, a number of improvements in the analytical methodologies for the analysis of pesticides residues in fruit have been achieved. Some of the main ones are: a) the reduction sample size, use of toxic organic solvents and the quantity of solvents and other reagents in order to minimize the extraction process; b) the automation of the sample preparation which results loss reduction and which improved the reproducibility and repeatability of the analytical methods; c) satisfactory separation of pesticides and reliable quantification of pesticides.

### **3. CONCLUSION**

The determination of pesticides in all fruit is necessary for ensuring that human exposure to contaminants and is an important tool for the determination of environmental contamination by pesticide residues. Analysis of pesticides in fruit is a difficult and demanding laboratory task. Extraction and preparation of samples with satisfactory recovery is an important purpose in pesticide analysis. Optimizing the method for reliable sensitivity and selectivity is the main task for reliable quantification. The use of sophisticated instruments and techniques suitable for determining traces of analytes has led to modernization. The application of new techniques for pesticide analysis has paved the way for the determination of several hundred pesticides simultaneously. Another noticeable trend is the evaluated multiresidue method using two determination techniques simultaneously. The development of fast, safe and efficient methods of preparation has made it possible to determine different classes of compounds. There is hope that more attention will be paid to organic production and reduce the use of pesticides, thus safe a healthy environment. Thanks to new materials, such as modern modified QuEChERS preparation and sophisticated instrumental techniques, pesticide analysis becomes reliable and significant. The importance of testing and monitoring leads us to think about future generations who remain behind us, and whose uncontrolled use of pesticides harms and endangers the environment and human health.

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# **RURAL DEVELOPMENT, AGRO-ECONOMY AND COOPERATIVES**



## STRATEGIC ORGANIZATIONAL AND TECHNOLOGICAL PRODUCTION OF PORK IN HALF OF RED MANGULICA

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Original scientific paper

### Abstract

*The subject of this scientific paper is the analysis in pig breeding, on a private farm in the Nisava district in the village of Mekiš, Municipality of Doljevac. The economics of pig production in farm production were monitored. The study covers the Pig Farm at the Cvetković Bobana Farm in the village of Mekiš 18410 Doljevac, in the Nisava District of Serbia. In the period of 2021, the volume and production of fatteners on the said farm are monitored and the obtained economic results are analyzed. In the observed period, it was determined that the total number of fatteners on the farm is 80. The average weight of live pigs on the farm is 100 kg, which costs 95 euros, and pig carcasses cost 1.18 euros / kg. The average weight of pig carcasses is 80.5 kg.*

**Key words:** meat production, pig fattening, fat, price, economy, profitability of income, incentive.

### Introduction

Mangulica cultivation in pig breeding indoors in Serbia has been used for more than half a century. This way of growing mango trees has shown that favorable economic effects have been achieved.

On some rural farms where intensive cultivation of mangulica is organized, namely: blue or white mangulica; Lasasta Mangulica; Red mangulica and Moravian black mangulica.

For the needs of organic meat production, a variant of mango cultivation was used. The goal of this method of growing Red Mangulica is to get as much meat

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per throat. It is desirable that the farms are located nearby outside the settlement. Pavlovića put bb,76300

## Material and methods

Agricultural production of cereals for the red mangulica pig farm.

The economy of production of nutrients for feeding mangulica on the farm from the group of cereals: corn and triticale was monitored.

The farm has conditions and areas for field production.

Maize covered 3 ha, and economic indicators of triticale production on 3 ha.

The annual production of cereals on the pig farm is over 55 tons per year.

Natural and financial indicators for maize are given (Table 1), economic indicators of triticale production per 3 ha (Table 2)

Table 1. Economic indicators of crop production of corn on 3 ha for a pig farm in 2021.

| R.br. | Production 2020.                  | No.of<br>repetitio<br>ns | Quanti<br>ty | JM       | The<br>pric<br>e | JM       | Amoun<br>t € for 3<br>/ ha |
|-------|-----------------------------------|--------------------------|--------------|----------|------------------|----------|----------------------------|
| I     | Income                            |                          |              |          |                  |          |                            |
| 1.    | Corn of 3 ha                      | 3                        | 10,88        | t/h<br>a | 130,<br>0        | €/t      | 4.243,2<br>0               |
| 2.    | Corn of 3 ha                      | 3                        | 5,0          | t/h<br>a | 22,3<br>7        | €/t      | 335,55                     |
| 3     | Incentives for plant<br>product   | 1                        | 3            | ha       | 50,0<br>0        | €/ha     | 150,00                     |
| A)    | Total income (1 to 2) for<br>3 ha |                          |              |          |                  |          | 4.728,7<br>5               |
| 4.    | Costs                             |                          |              |          |                  |          |                            |
| 5.    | Seeds for 3 ha                    | 1                        | 50           | kg       | 1,50             | €/k<br>g | 225,00                     |
| 6.    | Fertilizer                        |                          |              |          |                  |          |                            |
| 7.    | Manure for 3 ha                   |                          | 10           | t        | 4,00             | €/t      | 120,00                     |
| 8.    | KAN (29% N) 50% +<br>Urea 50%     |                          | 1.500        | kg       | 0,30             | €/k<br>g | 450,00                     |
| 9.    | Pesticides                        |                          |              |          |                  |          |                            |
| 10.   | Guardian                          |                          | 6            | L        | 3,00             | €/L      | 18,00                      |

|     |                                       |   |    |       |       |       |          |
|-----|---------------------------------------|---|----|-------|-------|-------|----------|
| 11. | Thesis                                |   | 6  | L     | 2,50  | €/L   | 15,00    |
| 12. | Irrigation                            |   |    |       |       |       |          |
| 13. | Energy for 3 ha                       | 2 | 15 | L     | 1,40  | €/L   | 63,00    |
| 14. | Diesel fuel                           |   | 90 | L     | 1,50  | €/L   | 135,00   |
| 15. | Maintenance of mechanization          |   | 3  | ha    | 15,00 | €/ha  | 45,00    |
| 16. | Paid services                         |   |    |       |       |       |          |
| 17. | Plowing                               |   | 3  | ha    |       | €/ha  | 0        |
| 18. | Soil preparation                      |   | 3  | ha    |       | €/ha  | 0        |
| 19. | Sowing                                |   | 3  | ha    | 35,00 | €/ha  | 105,00   |
| 20. | Harvest                               |   | 3  | ha    | 65,00 | €/ha  | 195,00   |
| 21. | Paid seasonal labor                   |   | 60 | r.sat | 1,50  | €/r.s | 90,00    |
| B)  | Total costs (3 to 23)                 |   |    |       |       |       | 1.461,00 |
| II  | PROFIT                                |   |    |       |       |       |          |
| 22. | Total with incentive (A - B)          |   |    |       |       |       | 3.267,78 |
| 23. | Per ha with incentive (22:18)         |   |    |       |       |       | 1.089,26 |
| 24. | Cost price kg of cereal grains (B: 1) |   |    |       |       |       | 0,0447   |
| 25. | Production efficiency (A: B)          |   |    |       |       |       | 3,24     |
| 26. | Profitability of income (22: A) x 100 |   |    |       |       |       | 69,10 %  |

Source: Author's calculation



Achieved results: The average yield of corn on the surveyed farm was 10.88 t / ha, and ranged from 9.88 t / ha to 11.88 t / ha.

The total realized profit on 3 ha is 3,267.78 EUR, production efficiency 3.24 and profitability of income 69.10%.

Table 2. Economic indicators of triticale plant production on 3 ha for a pig farm in 2021.

| R.br. | Production 2020.                | No.of repetitions | Quantity | J M  | The price | JM   | Amount € for 3 / ha |
|-------|---------------------------------|-------------------|----------|------|-----------|------|---------------------|
| I     | Income                          |                   |          |      |           |      |                     |
| 1.    | Triticale of 3 ha               | 3                 | 7,5      | t/ha | 170,0     | €/t  | 3.825,0             |
| 2.    | Straw of 3 ha                   | 3                 | 4        | t/ha | 8,75      | €/t  | 105,0               |
| 3     | Incentives for plant production |                   | 3        | ha   | 50,00     | €/ha | 150,0               |
| A)    | Total income (1-2) for 3 ha     |                   |          |      |           |      | 4.080,0             |
| 4     | Costs                           |                   |          |      |           |      |                     |
| 5.    | Seeds for 3 ha                  | 1                 | 750      | kg   | 0,20      | kg   | 150,0               |
| 6.    | Fertilizer                      |                   |          |      |           |      |                     |
| 7.    | Manure for 3 ha                 |                   | 15       | t    | 4,00      | t    | 60,0                |
| 8.    | KAN (29% N) 50% + Urea 50%      |                   | 1500     | kg   | 0,30      | kg   | 450,0               |
| 9.    | Foliar feeding                  |                   | 6        | kg   | 3,00      | kg   | 18,0                |
| 10.   | Pesticides                      |                   |          |      |           |      |                     |
| 11.   | Meteor                          |                   | 30       | g    | 0,20      | €/L  | 6,0                 |
| 12.   | Irrigation                      |                   |          |      |           |      |                     |
| 13.   | Energy for 3 ha                 | 3                 | 15       | L    | 1,20      | €/L  | 54,0                |

|     |                                       |  |    |    |       |      |         |
|-----|---------------------------------------|--|----|----|-------|------|---------|
| 14. | Diesel fuel                           |  | 90 | L  | 1,20  | €/L  | 108,0   |
| 15. | Maintenance of mechanization          |  | 3  | ha | 19,00 | €/ha | 57,0    |
| 16. | Paid services                         |  |    | ha |       |      |         |
| 17. | Plowing                               |  | 3  | ha |       | €/ha | 0       |
| 18. | Soil preparation                      |  | 3  | ha |       | €/ha | 0       |
| 19. | Sowing                                |  | 3/ |    | 30,00 | €/ha | 90,0    |
| 20. | Harvest                               |  | 3  | ha | 55,00 | €/ha | 165,0   |
| 21. | Paid seasonal labor                   |  |    | 46 | r.sat | 1,50 | €/h     |
| 22. | Other variable costs                  |  |    |    |       |      |         |
| 23. | Storage cost                          |  |    |    | €/t   |      | €/t     |
| 24. | Transport to the customer             |  |    |    | t     |      | €/t     |
| B)  | Total costs (3 to 24)                 |  |    |    |       |      | 1.227,0 |
| II  | PROFIT                                |  |    |    |       |      |         |
| 25. | Total with incentive (A - B)          |  |    |    |       |      | 2.853,0 |
| 26. | Per ha with incentives (25:17)        |  |    |    |       |      | 951,0   |
| 27. | Cost price kg of cereal grains (B: 1) |  |    |    |       |      | 0,0545  |
| 28. | Production efficiency (A: B)          |  |    |    |       |      | 3,33    |
| 29. | Profitability of income (25: A) x 100 |  |    |    |       |      | 69,92%  |

*Source: Author's calculation*

Achieved results: The average yield of triticale on the examined farm was 7.5 t/ha, and ranged from 7 t/ha to 8 t/ha.

The total realized profit on 3 ha is 2,853.0 EUR, production economy 3.33 and profitability of income 69.92%.

## Results

### a. Direction of production

Mini pig farms are engaged in fattening white mangoes - feeding piglets from three to six months and fattening white mangoes from six to nine months.

### b. Choice of strains - pork lines (red mangulica)

The earliest descriptions of mangulica mention two strains: white and black mangulica, and over time five strains appeared: blue or white, black, lasso, brown or barish and red mangulica (Mičić, 2016), (Mičić et al. 2016, 2017, 2017a, 2020).

Mangulica for this method of cultivation will be chosen from larger strains, it is best to be Blond or White Mangulica, which reach higher body weights. These lines of pigs with an intensive diet at the age of 12 months achieve a net body weight of 100 kg per head.

### c. Farm capacity

For this type of red mango cultivation, the size of the space depends on the number of heads to be bred. In nutrition and health care, the density, according to certain measures, can be from 40 to 80 heads per 1 ha.

In our program we will take an area of 1 ha for the size of the farm. The maximum capacity of 100 red mango pigs would be in the mentioned area. The farm raises 40 females and 40 males, which is divided into four enclosures: two of 0.15 ha for pigs of 25-60 kg and two of 0.25 ha for pigs of 60-100 kg.

## Discussions

### Pig breeding system

On the researched farm, pigs are raised indoors under the open sky.

The area where pigs are raised has the following composition of areas: glades - pastures occupy 50-80% of the area, and the rest of the space is overgrown areas, shrubs and the like.

Enclosed breeding area, the pig is a 1.50 m high wire net. Knit size 5x5 cm, wire thickness 2.8 mm. The wire mesh is stretched and nailed to acacia wooden poles driven into the ground at a distance of 2.5 m from the pole. The pillars are 2.5 m long, of which 100 cm is buried in the ground, and 1.5 m remains above the ground.

The wire mesh is nailed to the poles on the inside with clamps. On the inside of the pillars, they were tightened - and their bark was removed, and at the crossing from the ground - air in the length of 100 cm burned this crossing.

The piglets were released into the enclosure after 3 months. Piglets are females separated from males to avoid mating and disrupt the breeding system. Pigs stay in this area until 6 months of age.

At the age of 6 months, the red pig mango stands out for slaughter. Separately slaughtered and cut into halves, red mangoes were processed, their internal organs (intestines, heart, liver, spleen, etc.) were removed and freshly prepared in the skin, chilled, delivered fresh to customers in Serbia or frozen in refrigerators and prepared for refrigeration. export abroad. markets.

### **Diet of red Mangulica fatteners**

Diet is the basic regulator of the number of mangoes in nature.

Proper nutrition of mangoes in enclosed areas greatly affects the results of cultivation.

The principles that must be taken into account when feeding pigs are as follows:

1. The food given to Mangulica is as natural as possible in its composition.
2. In achieving body weight, the quality of food is a limiting factor on the examined farm.
3. Food is produced in its own fields, and varied in its composition.

In nature, in the summer period, ie during the vegetation period, pigs can use as much food as possible (sown areas, meadows, fruits, etc.).

In any case, pigs are fed intensively during the entire period of rearing, given the large number of heads per unit area. Water is constantly available to pigs for bilge, as well as for drinking, (Rede, i Petrović 1997), (Grujić, 2000), (Petrović, i Manojlović, 1999, 2004), (Radović, 2000), (Tomović, 2008), (Jensen, et al. 2008), (Vidović, et al. 2012), (Živković, i Perunović, 2012),

The economy of pig feed production on the farm from the group of cereals was monitored:

-cost price of the mixture for feeding pigs white mangulica of 25-60 kg (Table 3),

-cost price of the mixture for feeding white mangul pigs from 60-100 kg (Table 4),

-applied economic indicators in pig fattening (Table 5).

*Table 3. The cost of the mixture for feeding white Mangulica of 25-60 kg*

| Nutrients        | Mixture kg | Content of kg of mixture in% | Nutrients kg | Price € / kg | Amount € |
|------------------|------------|------------------------------|--------------|--------------|----------|
| Corn             |            | 33,2                         | 332          | 0,0447       | 14,84    |
| Soybean meal     |            | 14                           | 140          | 0,4266       | 59,72    |
| Soybean semolina |            | 4,5                          | 45           | 0,43         | 19,35    |
| Cattle brush     |            | 3                            | 30           | 0,0545       | 1,64     |
| Turazel          |            | 0,2                          | 2            | 0,1417       | 0,28     |
| Triticale        |            | 40                           | 400          | 0,0545       | 21,80    |
| Livestock chalk  |            | 1,6                          | 16           | 0,035        | 0,56     |
| So               |            | 0,5                          | 5            | 0,43         | 2,15     |
| In total         | 1.000      | 100                          | 1.000        | 0,1204       | 120,35   |

*Source: Author's calculation*

From Table 3 it can be seen that the mixture is produced on the farm in flour form at a producer price of 0.1204 EUR / kg, intended for feeding pigs in the first phase of fattening from 25 to 60 kg body weight. The mixture is produced from its own raw materials and contains 18.45% of crude protein including all the necessary vitamins, micro and macro minerals, with protein and energy nutrients for the highest daily gain.

*Table 4. The cost of the mixture for feeding white Mangulica of 60-100 kg*

| Nutrients        | Mixture kg | Share in% kg of mixture | Nutrients kg | Price € / kg | Amount € |
|------------------|------------|-------------------------|--------------|--------------|----------|
| Corn             |            | 33,2                    | 332          | 0,0447       | 14,84    |
| Soybean meal     |            | 10                      | 100          | 0,4266       | 42,66    |
| Cattle brush     |            | 10                      | 100          | 0,1206       | 12,06    |
| Soybean semolina |            | 2,5                     | 25           | 0,43         | 10,75    |
| Turazel          |            | 0,2                     | 2            | 0,1417       | 0,2834   |

|                 |       |     |       |        |          |
|-----------------|-------|-----|-------|--------|----------|
| Triticale       |       | 40  | 400   | 0,0545 | 21,80    |
| Sunflower meal  |       | 2   | 20    | 0,2236 | 4,472    |
| Livestock chalk |       | 1,6 | 16    | 0,035  | 0,56     |
| So              |       | 0,5 | 5     | 0,43   | 2,15     |
| In total        | 1.000 | 100 | 1.000 | 0,1096 | 109,5754 |

*Source: Author's calculation*

Table 4 shows that the mixture was produced on the farm in the form of flour at a producer price of 0.1096 EUR / kg, intended for pig nutrition, in the second phase of fattening from 60 to 100 kg of body weight (Mičić 2016a).

The mixture is produced from its own raw materials and contains 16.44% of crude protein, with all the necessary vitamins, micro and macro minerals with protein and energy nutrients.

By using this mixture, pigs achieve maximum potential in meat yield, in daily yield.

### **Fat pigs manguice on the farm**

About 80 red manguica pigs are fattened on the farm, which are sold with 100 kg at an average price of € 2.70 / kg.

Table 5. Achieved economic indicators in pig fattening

|    |                                     |          |                             |           |      |           |
|----|-------------------------------------|----------|-----------------------------|-----------|------|-----------|
| 1. | Production year: 2020               | J. M.    | Conversion rate kg of food: |           |      | 1,08      |
| 2. | Period of this: Jan-Decem           | -        | Death rate in fattening:    |           |      | 2,00%     |
| 3. | Number of heads put into fattening: | 82       | throat                      |           |      |           |
| 4. | Average weight of fatteners:        | 100      | kg /throat                  |           |      |           |
| 5. | Fattening length:                   | 180      | dana                        |           |      |           |
| I  | Income                              | Quantity | J.M.                        | The price | J.M. | Total €   |
| 6. | Fattening pigs (4 x 6)              | 80       | throat                      | 2,70      | €/kg | 21.600,00 |
| 7. | Manure                              | 80       | t                           | 2,00      | €/t  | 160,00    |

|         |                                                           |       |           |        |          |           |
|---------|-----------------------------------------------------------|-------|-----------|--------|----------|-----------|
| 8.      | Subsidies per head                                        | 80    | throat    | 8,70   | €/throat | 696,00    |
| A<br>)  | Total revenue (1 to 8)                                    |       |           |        |          | 22.456,00 |
| II      | Costs                                                     |       |           |        |          |           |
| 9.      | Piglets (average head)                                    | 25    | kg/throat |        |          |           |
| 1<br>0. | Piglets (3 x 9)                                           | 2.050 | kg/throat | 2,20   | €/kg     | 4.510,00  |
| 1<br>1. | Its a concentrate mixtur                                  |       |           |        |          |           |
| 1<br>2. | TS1 (od 25-60 kg) (0,9334 kg/d. x 90d.x80 throat) 6.720,5 |       |           | 0,1204 | €/kg     | 809,15    |
| 1<br>3. | TS2 (od 60-100 kg) (1,2266 kg/d.x90d.x80 throat) 8.831,5  |       |           | 0,1096 | €/kg     | 967,93    |
| 1<br>4. | Average daily per throat                                  | 1,08  | kg        |        |          |           |
| 1<br>5. | Corn                                                      |       | €/kg      |        | €/kg     | 0         |
| 1<br>6. | Triticale                                                 |       | €/kg      |        | €/kg     | 0         |
| 1<br>7. | Fodder flour                                              |       | €/kg      |        | €/kg     | 0         |
| 1<br>8. | Livestock chalk                                           |       | €/kg      |        | €/kg     | 0         |
| 1<br>9. | So                                                        |       | €/kg      |        | €/kg     | 0         |
| 2<br>0. | Mechanical work (8 x 20)                                  |       | €/kg      | 2,00   | €/kg     | 160,00    |
| 2<br>1. | Water per throat (21 x 5) x 6: 1,000                      | 10    | L / day   | 1,00   | €/m3     | 10,32     |
| 2<br>2. | Veterinary services and medicines (8 x 22)                |       |           | 1,00   | €/throat | 80,00     |

|         |                                                  |      |      |          |           |
|---------|--------------------------------------------------|------|------|----------|-----------|
| 2<br>3. | Human labor (4 x 6) x 23                         | kg/€ | 0,10 | €/throat | 800,00    |
| 2<br>4. | Indirect costs (6 x 24)                          | kg/€ | 1,40 | €/throat | 112,00    |
| 2<br>5. | Depreciation of buildings and equipment (6 x 25) |      | 2,00 | €/throat | 160,00    |
| B<br>)  | Total costs (9 to 25)                            |      |      |          | 7.609,4   |
| II<br>I | PROFIT                                           |      |      |          |           |
| 2<br>6. | On a farm with an incentive (A - B)              |      |      |          | 14.846,60 |
| 2<br>7. | Per throat on the farm (26: 6)                   |      |      |          | 185,58    |
| 2<br>8. | Cost price kg ž. m. B: (4 x 8)                   |      |      |          | 0,95      |
| 2<br>9. | Production efficiency (A: B)                     |      |      |          | 2,95      |
| 3<br>0. | Profitability of income (26: A) x 100            |      |      |          | 66,11%    |

*Source: Author's calculation*

Achieved results: The average weight of fatteners on the examined farm in Grčanica was 100 kg / head, and ranged from 98 kg/ head to 102 kg/ head. With an average price of 0.95 EUR/ kg, the value of one fattener cost 95.0 EUR/ head. The price of manure, according to the applied estimated market price, was 2.0 euros per head = 160 euros.

Total profit for 80 head of fatteners 14,846,605 euros, production efficiency 2.95 and profitability of income 66.11%.

## Conclusion

In order to examine the economic justification of fatteners, on a farm from Serbia in the Nisava district, tests were conducted in the village of Mekiš in the



municipality of Doljevac, on a farm of red mangoes, which has the best conditions for that production.

The private farm has 80 fatteners, the average input weight of piglets for fattening was 25 kg, and the achieved output weight was 100 kg.

The analysis of economic results is based on the calculation of production after fattening. The prices used during 2021 were used in the calculation.

Due to significant changes in energy prices in the production year, the average prices realized during 2021 are used in the calculation of costs.

Only the income from the sale of pigs is included in the calculation of income, while the potential income from pork is not calculated in half. Slaughter pigs yielded halves of fairly uniform weight, with an average weight of 80.5 kg.

The realized total income in the production of red mangosteen is 14,846.61 euros, + economic indicators of triticale plant production on 3 ha of 2,853.0 euros, + economic indicators of corn production on 3 ha 3,267.78 euros = 20,967.38 euros.

The price of live red mango is 0.95 EUR / kg, pork in half with skin, head, front and back legs and fat at a price of 1.18 EUR / kg.

The production of pigs on the Cvetković Bobana farm in the village of Mekiš in the Nišava district is economically justified for 2021.

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## THE IMPORTANCE OF GASTRONOMY IN THE DEVELOPMENT OF RURAL TOURISM IN SERBIA

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**ABSTRAKT:** Nutritional culture is a reflection of complex living conditions, geographical and climatic specifics, historical and social events in rural areas, and due to the multiplied influence of many factors varies from village to village, and from household to household, among ethnic groups. Serbian gastronomy has developed under the influence of foreign cultures, but has always retained and developed authenticity, thanks to a strong tradition. Thus, our climate has given gastronomic products its own character, which is intensifying every day, regardless of old roots and new influences. Modern trends in Serbian gastronomy not only do not lag behind European ones, but keep pace with them. They are basically colored by the spirit of our national cuisine, always giving and taking what is best. Agriculture is an integral part of gastronomy, and if it is at a higher level of development, gastronomy will be more diverse. From the aspect of healthy food (products with organic status and products with protected geographical origin) and the natural environment, it can be said that tourism is dependent on agriculture, and on the other hand to directly or indirectly encourage the prosperity of agriculture and economy. Traditional farming and cultivation of certain products in certain areas have led to characteristic products that can represent national, regional and even international brands. So far, 54 domestic products with a geographical indication have been protected in Serbia by the Institute for the Protection of Intellectual Property. In order for rural-rural tourism to develop faster and with better quality in Serbia, it is necessary to organize better marketing in the media, to talk more about life in the countryside, because in that way tradition and lifestyle will be preserved.

**Key words:** gastronomy, rural tourism, agriculture

## 1. INTRODUCTION

Rural areas are areas of exceptional value, places of traditional culture and heritage (Todorovic, i Bjeljic, 2007), with a specific environment (Ljesevic et al., 2011).

Although there is some harmonization within Europe regarding the definition of rural areas, there is still no single agreement on a threshold that would mark the border between urban and rural areas, and the fact that rural areas often differ in character (rural areas are also suburban). rural areas, and pastures in the high mountains, but also Mediterranean olive groves). State authorities use criteria to define rural areas based on population density and according to the Organization for Economic Cooperation and Development (OECD), the definition of rural areas is that: "at the local level, a population density of 150 people per square kilometer is preferred, while at the regional level, geographical units are grouped into three types: predominantly rural (> 50%), significantly rural (15-50%) and predominantly urbanized regions (<15%)" (OECD, 1994, p. 10). Although there is no single definition, it can be observed that rural settlements can be of different sizes, but they are usually small, with less than 10,000 inhabitants and a lower population density.

Also, the dominant activity is agriculture, and the influence of tradition and the past is strong. According to the authors Ashley and Maxwell (2001), common characteristics of rural areas are:

- Areas where the settlement (houses, auxiliary and other facilities) and the infrastructure of the village occupy a smaller part of the landscape, and are dominated by fields and pastures, forests, water bodies, mountains and deserts;
- Areas where most people spend their working time on farms;
- Areas rich in arable agricultural land, which is characterized by a relatively low cost;
- Spaces with high transport costs that are at a greater distance from urban areas and inadequate infrastructure.

In the last three decades, rural areas have been given a new role. Preservation of rural nature and landscape has become extremely important, and historical buildings and traditional way of life attract the attention of tourists. Rural areas are often characterized by special customs and identity, and if they have mountains, reserves, rivers, lakes and more, they become attractive for tourism development. In this way, rural tourism is seen as one of the ways to revitalize rural areas.

## **2. CONCEPTUAL DEFINITION OF RURAL TOURISM**

The professional literature offers different definitions of rural tourism, from those that observe it through the prism of space, to those that include a wide range of activities and elements of the overall product of rural tourism. Defining rural tourism as a type of tourism that takes place in a rural area cannot be satisfactory or adequate due to the complexity of this phenomenon.

One of the most comprehensive definitions of rural tourism was given by the Pan-European NGO (EuroTer) according to which rural tourism, within the tourism economy, is "tourist valorization of agricultural areas, natural resources, cultural heritage, rural settlements, local traditional customs and products through specially designed tourism products, reflect the identity of the area and meet the needs of guests in terms of accommodation, food and beverage services, recreation and activities, animation and other services, with the aim of sustainable local development, but also providing adequate responses to today's guest needs within newly established urban-rural relations."(Horwath Consulting Zagreb, 2009, p. 10).

The problem of defining rural tourism has become even more difficult, because very often in the professional literature, but also in practice, the term rural tourism is identified with the term agritourism. Some authors (Di Muzio, 2000; Hall et al., 2003) argue that the terms agritourism and rural tourism are identical, while another group of authors (Kusen, 2007; Sznajder et al., 2009; Adams, 2008; Sirgy, 2001) argue that agrotourism is only a part of the segmentation of rural tourism (besides hunting, eco, health, cultural, wine, gastronomic and others). Even greater confusion arises when the term rural tourism is added.

However, these three terms can be distinguished if it is accepted that agritourism is "a set of tourist products, services and activities in agrotourism accommodation units such as rural tourist households", and that rural tourism includes tourist activities in rural settlements, which is certainly a broader term than agritourism. The broadest term is rural tourism which is treated as a set of tourist activities in rural areas.

## **3. CONCEPT AND ELEMENTS OF RURAL TOURISM**

Rural tourism is based on natural resources, rural heritage, rural lifestyle and rural activities, that is, activities in rural areas.

Natural resources include: rivers, lakes, mountains, forests ... Rural heritage includes traditional architecture, history, castles, churches, villages ...

Rural lifestyle includes local events, gastronomy, agritourism, traditional music ... Rural activities are horseback riding, cycling, fishing, walking, water sports ...

The basis of rural tourism are three elements (Dragulanescu, Drutu, 2012)

- space, people and products because:

- A space without people cannot be sustainable;
- People without space or products have very limited opportunities for success;
- Products that are not based on the characteristics of space and people's experiences, can not ensure the development of the local community.

Rural tourism is an increasingly popular tool used by nationally developed agencies to increase the profits of the rural population (Fun et al., 2014). The introduction of innovative and indigenous products in rural tourism makes sense if there are economic, environmental and social benefits. The answer to this question is possible by systematic information, identifying the needs of modern consumers (Jakovic et al., 2015). Creating an authentic tourism product, based on the recognizable competitive advantages of the destination, is considered a key factor.

A rural destination needs to retain a unique identity, an authentic sense of place for tourism to be effective in the long run (Mihailovic and Moric, 2012). Local community initiatives for rural development can contribute to this (Belij et al., 2014). By combining physical-geographical and socio-geographical principles with socio-psychological attitudes of the local population about identity, the individuality of a certain whole in space is confirmed (Vujadinovic et al., 2010).

#### **4. FORMS OF RURAL TOURISM**

Rural tourism is a complex type of tourism because it is composed of different types of tourism that occur in rural areas, and from the aspect of tourist offer or product, the following forms stand out (Demonja and Ruzic, 2010; Todorovic and Stetic, 2009):

- Sports and recreational tourism reflects the need of tourists to spend time in nature and at the same time practice various sports activities such as walking, cycling, running, hiking, horseback riding, swimming and the like.
- Adventure (adrenaline) tourism basically has recreation, but tourists are motivated to experience adventure with a lot of risk and excitement. These

include alpine climbing, mountain biking, acrobatic skiing, kite flying, and for this form of tourism canyons, steep slopes and dangerous mountain trails are used.

- Camping tourism means accommodation and stay of tourists in camps that are most often located within an agritourism farm or a catering facility, but also near natural and cultural attractions. For some tourists, camping is a lifestyle that implies that a person is free, environmentally conscious, loves nature and socializes with people, and is always on the move.

- Gastronomic tourism implies the production of specialties in the traditional way, and very often thematic gastro-trips are organized (cheese roads, wine roads). Also, food is the reason for organizing events in rural areas (cabbage festival, sausage festival, pumpkin days, etc.).

- Wine tourism is associated with gastronomic tourism because wine is a "friend" of food. Vineyards can be important tourist resources, so they are used in the promotion of tourist destinations (Tuscany, Provence).

- Health tourism is a form of rest associated with staying in health resorts in order to preserve and improve health, treat certain diseases, and for that purpose thermo-mineral springs, healing muds and numerous therapeutic methods are used.

- Cultural tourism is a trip to rural areas to visit cultural monuments, museums, galleries and cultural events. This form of tourism puts culture, education, experiences and experiences in the foreground, which is the basis of tourism development and survival.

- Religious tourism means a trip motivated very often by religious motives to visit religious shrines or religious events.

- Hunting and fishing tourism are typical of rural areas and take place on its forest, agricultural and water land.

- Ecotourism is characterized by naturally preserved areas, the use of simple types of accommodation, strict adherence to environmental protection, including a limited number of visitors. Ecotourism means attitude, ethics and behavior. This form of tourism involves environmentally conscious tourists who try to reduce the negative effects on the environment.

- Educational tourism is based on introducing tourists to the characteristics of rural areas, and gaining experience during their stay in nature. Tourists are children of preschool age, students of primary and secondary schools (tourism or agriculture), and these educational trips have pedagogical and teaching value.

- Nautical tourism is a form of tourism in which tourists use a boat or other vessel for accommodation and stay, and they rest in a rural area on lakes, rivers and canals.
- Residential tourism means secondary housing of the city population during weekends, holidays or vacations in their own facilities. These facilities are located near cities, and the owners rent them to other people.
- Nostalgic tourism is based on a special connection of an individual with a certain area. These are people who used to live in villages, but for certain reasons (business, family) moved to cities, and by visiting those villages they do not want to lose contact with people they know, nor to lose memories from their childhood.

#### **4.1. GASTRONOMIC TOURISM**

The popularity of food tourism has been known around the world for several decades (Santich, 2004). New trends are slowly shifting attention from mass tourism to alternative forms and so food and drink, which are an integral part of all forms of tourism, may be the primary motive for coming to a particular destination (Baum, 2011; Kalenjuk et al., 2012a). An increasingly popular activity of tourists is the preparation of meals in various households or schools of authentic regional cooking (Hall, Mitchell, 2006). Taking into account the basic characteristics of gastronomic tourism as an increasingly popular form of tourist movement, the focus of interest in addition to various offers from large metropolises are villages and rural households where food comes from (Baum, 2011; Kalenjuk et al., 2014; Vuksanovic et al., 2016). Rural areas represent areas with potentially the best preserved gastronomic tradition and authenticity (Banjac et al., 2016).

From this aspect, tourism should be viewed as a branch of the economy that has a significant social and economic function of a particular area and as such contributes to highlighting and better valuing the agricultural environment (Henderson, 2004; Quan and Wang, 2004; Gagic et al., 2014). Through the gastronomy preserved in the villages, tourists get to know the culture and customs of the visited area, but also the characteristic tastes and smells that bring with them an authentic experience. Numerous tourism organizations have recognized the potential of gastronomy in the regions in which they operate and included them in their offer of rural areas, developing a selective form of tourism. which is known worldwide as food tourism (Boniface, 2003) or gastronomic (Hjalanger



and Richards, 2002), culinary (Wolf, 2006; Ignatov and Smith, 2006), tasting (Boniface, 2003) and gourmet tourism.

Nutritional culture is a reflection of complex living conditions, geographical and climatic features, historical and social events in rural areas, and due to the multiplied influence of many factors varies from village to village, and from household to household (Stojanovic and Cerovic, 2008). and among ethnic groups. Food, and especially biologically valuable food, is an integral part of every tourist product, regardless of the reason for visiting a certain tourist destination. In the chain of successful marketing of agricultural, food and gastronomic products in tourism, the key segments are not always determined by tourism policy, but by national, economic, agricultural and food policies (Hjalager and Corigliano, 2000).

Local food can be viewed as (Hall and Mitchel, 2001; Kalenjuk et al., 2012b):

- Part of the local culture that tourists spend;
  - An important part of tourist promotion;
  - Potential component of local agricultural and economic development,
- and
- Regional factor influenced by consumption patterns and observed tourist preferences.

In contrast to many other forms of tourism, gastronomic destinations are usually available throughout the year, at any time of day and in all weather conditions (Richards, 2002), which gives priority to the development of this form of tourism (Kalenjuk et al., 2012c; Kalenjuk and Tesanovic, 2013c), provided that these are traffic easily accessible sites.

## **5. DEVELOPMENT OF GASTRONOMY IN SERBIA**

Gastronomy is an existential need, it is an activity, profession and science that is compared to art, and it concerns the preparation, serving, serving and enjoying quality food. Gastronomy takes place in time and space, with the application of modern technologies, managed by modern management.

Our gastronomy, like many others, has experienced many foreign influences and changes. Our rulers tied the lives of their sons to some of the neighboring courts, in order to secure the borders and strengthen the friendship. These foreign ladies, in addition to general culture, brought gastronomic novelties and customs to the court, and spontaneously incorporated them into general

etiquette. But we should not forget that in the 12th century, people ate with a golden spoon and fork at the Serbian court, while Europe ate with their fingers. There was a certain order at the table, which remained the same for years. On the roads in Serbia, passengers could often be seen visiting the restaurants of that time and expressing positive impressions about the food they would receive.

Serbian gastronomy has developed under the influence of foreign cultures, but has always retained and developed authenticity, thanks to a strong tradition. Thus, our climate with dalastronomic products is its own feature, which is getting stronger every day, regardless of old roots and new influences.

Serbian national cuisine is passed down from generation to generation in our families, gaining new forms, but retaining its essence and uniqueness.

Modern trends in Serbian gastronomy not only do not lag behind European ones, but keep pace with them. They are basically colored by the spirit of our national cuisine, always giving and taking what is best.

Our cuisine is known as delicious, but also as overly greasy and too spicy, although we Serbs do not mind. Our people attach great importance to what they eat, but also to how it is prepared, and all for the benefit of their health. That is why our fasting dishes are also known, which are not only a feature of Orthodox but also of all others, in the name of a healthier life in general. The fasting dishes that were prepared during the fast were: prebranac beans, peppers stuffed with fasting filling, tomato or potato soup.

In order to be able to consume as diverse a food as possible, the inhabitants of Serbia had to engage in agriculture. It is an integral part of gastronomy, and if it is at a higher level of development, gastronomy will be more diverse. From the aspect of healthy food and natural environment, it can be said that tourism is dependent on agriculture, and on the other hand that it directly or indirectly encourages the prosperity of agriculture, ie the economy (Vujovic, 2007).

The gastronomic offer in rural tourism should be based on the original, inherited wealth, to use agricultural products of the households themselves, and to prepare from them products that can be offered to take away (sweets, honey, jams, brandy, dried meat, flour ground to watercress, mushrooms and the like). The gastronomic offer has specific physical, material products, defined shape, color, structure, taste and smell. Gastro products can be stored and stored, unlike services that do not have physical attributes, are elusive and intangible (Tesanovic, 2009; Skrinjar et al., 2010) and which tourists cannot take with them.

Gastro products contain a strong reference to the local, because at the same time they represent a certain geographical area, tradition, cultural heritage,

the identity of the local community, but also their identity. These are the so-called domestic products, if they characterize the state - national products, and if they characterize a nation - ethno products.

Traditional farming and cultivation of certain products in certain areas have led to characteristic products that can represent national, regional and even international brands. So far, 54 domestic products with a geographical indication have been protected in Serbia by the Institute for the Protection of Intellectual Property.<sup>11</sup>

Table 1. Domestic products with a geographical indication  
(Source)<sup>12</sup>

| Domestic products with a geographical indication                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <ul style="list-style-type: none"> <li>- Beef Uzice prosciutto</li> <li>- Pork Uzice prosciutto</li> <li>- Uzice bacon</li> <li>- Srem kulen</li> <li>- Srem homemade sausage</li> <li>- Srem salami</li> <li>- Pozarevac sausage</li> <li>- Rtanj tea</li> <li>- Krivovir cheese</li> <li>- Homolj sheep cheese</li> <li>- Homolj goat cheese</li> <li>- Homolj cow's cheese</li> <li>- Banat Riesling</li> <li>- Jagodina rose</li> <li>- Vrsac champion beer</li> </ul> | <ul style="list-style-type: none"> <li>- Kladovo Caviar (Caviar of Kladovo)</li> <li>- Water Vrnjci</li> <li>- Apatin deer beer</li> <li>- Bujanovac mineral water AQUA HEBA</li> <li>- Mineral water Duboka</li> <li>- Pearl Island, Muscat Crocan</li> <li>- Prince Milos Bukovicka Banja</li> <li>- Petrovska Klobasa (Petrovac sausage)</li> <li>- Leskovac barbecue meat (for burgers and kebabs)</li> </ul> | <ul style="list-style-type: none"> <li>- Svrljig belmuz</li> <li>- Bezdan damask</li> <li>- Stara Planina cheese</li> <li>- Homemade ajvar from Leskovac</li> <li>- Frushkogorski linden honey</li> <li>- Duck honey</li> <li>- Sjenica sheep cheese</li> <li>- Sjenica lamb</li> <li>- Pirot cheese made from cow's milk</li> <li>- Sombor cheese</li> <li>- Ecani Carp</li> <li>- Zlatar cheese</li> <li>- Sjenica cow's cheese</li> </ul> |

<sup>11</sup> (<http://www.zis.gov.rs/prava-intelektualne-svojine/oznake-geografskog-porekla/spisak-ogp.953.html>)

<sup>12</sup> (<http://www.zis.gov.rs/prava-intelektualne-svojine/oznake-geografskog-porekla/spisak-ogp.953.html>)

|                                                                        |                              |                           |
|------------------------------------------------------------------------|------------------------------|---------------------------|
| - Field of Kosovo (Amselfeld, Field of the black bird, Champ de merle) | - Valjevo tobacco greaves    | - Lemeski kulen           |
| - Kosovo wine (Amselfelder)                                            | - Bermet                     | - Vrsac ham               |
|                                                                        | - Svrlijig cheese            | - Vrbicki garlic          |
|                                                                        | - Karlovac Riesling          | - Djerdap honey           |
|                                                                        | - Futog fresh and sauerkraut | - Oblacinka from Oblacina |
|                                                                        | - Homolj honey               | - Svrlijig cow's cheese   |
|                                                                        | - Arilje raspberry           |                           |

"Products with a geographical indication guarantee that their specificity and quality are the result of a combination of natural resources (climate, soil, special breeds, varieties) and traditions, knowledge and skills that are passed from generation to generation", according to the Lisbon Convention, it is not subject to customs barriers, there are no prescribed quotas for its export to the European Union market, and on the domestic and foreign markets it is sold more expensive than products that do not have that feature.<sup>13</sup> However, the Lisbon Agreement on International Protection of Names of Origin of Products, internationally protected only three Serbian products.<sup>14</sup>

Gastro tourism has a stimulating effect on local development, because it can:

- Extend the tourist season;
- Diversify the rural economy;
- Stimulate agriculture;
- Create new jobs;
- Contribute to regional attractiveness;
- Sustainability of the local environment;
- Promotion of cultural heritage, ie strengthening local identity and sense of belonging to a certain community. All this generally does not require major investments (World Tourism Organization, 2012).

<sup>13</sup> (<http://www.novosti.rs/vesti/naslovna/drustvo/aktuelno.290.html:473928-Zasticeno-je-49-srpskih-proizvoda-samo-tri-i-u-inostranstvu>)

<sup>14</sup> (<http://www.novosti.rs/vesti/naslovna/ekonomija/aktuelno.239.html:521405-Zasticena-samo-tri-srpska-brenda>)

## **6. SIGNIFICANCE AND PLACE OF GASTRONOMY IN RURAL TOURISM OF SERBIA**

Every village in Serbia is a story for itself, especially, specifically, in terms of customs, habits and food culture. This diversity of ways of preparation and variety of dishes is a good precondition for creating a gastronomic rural almanac, promoting the best dishes and households and creating a database of folk dishes and customs.

The gastronomic offer of rural areas of Serbia is part of the general material culture through which we can best get acquainted with the customs and traditions of the village (Portic, 2011). Residents of large cities have a desire to spend their vacation in nature - the village, where, in addition to all the benefits, from clean air and silence, they will also enjoy agricultural products used to prepare healthy food. Households that provide such food services are often chosen. Every region in Serbia has something that is attributed to that climate and that is the main trump card - the marketing brand of each region. It should not be forgotten that business people from nearby urban areas can organize their business lunches and certain ceremonies in rural households and thus significantly help the economy of rural farms and villages.

In urban areas, in addition to fast food, you should set up small houses in the style of folk architecture made of wood with domestic products such as: smoked meat, cheese, cream, sour milk, *wurda*, whey, bread, so-called. high breads from the oven that need to be protected, domestic winter food, wreaths of onions and peppers, domestic poultry (if it ran around the yard), and special attention should be paid to pies and *pitierias*, as a new national object (Portic, 2011).

One of the activities that could bring material resources to households is food preparation, ie. local specialties, respecting the authenticity of the hearth, as well as the protocol of hospitality. These specific additional contents can only be partially realized within one household, so it is necessary to cultivate good neighborly relations with neighbors who are often referred to each other (Portic, 2011).

In order for rural-rural tourism to develop faster and with better quality in Serbia, it is necessary to organize better marketing in the media, to talk more about life in the countryside, because in that way tradition and lifestyle will be preserved. Ecologically healthy environment should be observed in certain rural

areas or entire regions through the production of herbs, teas, forests and other products. The production of nettle as an extremely healthy and medicinal plant, meadow cabbage, sremus and other edible plant species, can be the basis for the gastronomic offer of healthy organic food, produced in an organic way.

## 7. CONCLUSION

Serbian gastronomic products can have a significant place in the promotion of rural tourism. It is necessary to attract consumers with the best possible promotion and, whenever possible, offer their tasting, because only the experience of the taste and smell of food and drinks in them can cause a motive to visit some of the regions and enjoy gastronomic products. One of the important segments in the gastronomy sector can certainly be agricultural products that are produced in an organic way and products with protected geographical origin. Such products occupy an enviable percentage of participation in human nutrition in Europe and the world. Therefore, it is necessary to raise the current situation in the promotion of Serbian gastronomy to a higher level and include the highest state institutions dealing with the development of tourism in Serbia.

Agriculture is an integral part of gastronomy, and if it is at a higher level of development, gastronomy will be more diverse. From the aspect of healthy food and natural environment, it can be said that tourism is dependent on agriculture, and on the other hand, it directly or indirectly encourages the prosperity of agriculture - the economy.

Traditional farming and cultivation of certain products in certain areas have led to characteristic products that can represent national, regional and even international brands. So far, 54 domestic products with a geographical indication have been protected in Serbia by the Institute for the Protection of Intellectual Property.

Traditional segments of importance for attracting gastronomic tourists have been preserved to a sufficient extent that they can be implemented through various offers in rural tourism. The production and preparation of authentic food in households would thus contribute to the development of gastronomic tourism in the area of our country and the entire economy.

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## COMPARATIVE OVERVIEW OF THE ESTABLISHMENT OF COOPERATIVES IN THE REPUBLIC OF SERBIA AND THE REPUBLIC OF CROATIA

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**Abstract:** *Cooperatives are slowly but surely returning to various areas of social life, since the adoption of the Law of Associated Labor in 1976, the existence of cooperatives was abolished. Today, there are over 600 newly established cooperatives in the Republic of Serbia, thanks to the Law of Cooperatives from 2015. The Law of Cooperatives in the Republic of Croatia was brought in 2011 and amended several times in 2013, 2014, 2018 and 2019. We can say that the cooperatives are the economic backbone of staying and surviving in the countryside. Since the cooperative knows no borders, our country will, in the future, continue to cooperate with many countries from the world, the European Union and the region, in order to use their decades of experience. Therefore, the authors of the paper made a comparative review and analysis of the establishment and existence of cooperatives in the Republic of Serbia and the Republic of Croatia.*

**Key words:** *cooperatives, establishment, Law of Cooperatives, Republic of Serbia, Republic of Croatia.*

### INTRODUCTORY CONSIDERATIONS

Vuk Stefanović Karadžić in the „Srbski riječnik“ from 1818 <sup>15</sup>states that among the South Slavs there is a so-called "Cooperative", which was popularly referred to as the "big house"<sup>16</sup> and which was based on love, mutual cooperation,

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<sup>15</sup> Karadžić, V., *Srbski riječnik*, Prosveta, Beograd, 1969.

<sup>16</sup> In addition to this term, the name "strong house", "big family" and the like were also used. See more: Novaković, N., *Teorije o nastanku i strukturi porodičnih zadruga*, Stanovništvo 1-4/2005, p. 105-106.

helping and, above all, respect for the elderly by the younger, parents by children, husband by wife and, especially, elders of the family cooperatives (and that was the oldest male member) by everyone else. The word cooperative originates from ancient Rome, from the Latin expression "*Plures familiae in eadem domo (more Serbico)*" - "Many families in the same house (several Serbs)"<sup>17</sup>.

Cooperatives have been and are of great importance for the overall social and economic life, especially for the life in the countryside.

However, in the last few decades, both in the Republic of Serbia and in the Republic of Croatia (Slavonia), villages are becoming more and more empty, dying out and disappearing due to the fact that rural areas are finding it harder to live and young people don't want to stay, even though they have much better living and working conditions today than their ancestors did. Young people, in search of a safer job and a better existence, are leaving their country and moving on. It is of special importance that rural areas with appropriate infrastructure, cooperative homes, a clinic and appropriate staff for the treatment of the population, become attractive to young married couples, who see their future in such environments. The construction and renovation of cooperative homes, the opening of post offices in villages, the provision of services to agricultural producers by cooperatives contributes to young people to stay in their homes and not to leave their country in search of a better existence. Today, more and more, when a great economic crisis is on the horizon (both in our country and in the whole world), there are talks that every country should strive for self-sustainability, i.e. to have such economic and social development, which will enable it's complete (which is very difficult) or greater independence from the rest of the world. It is certain that cooperatives can, to a large extent, contribute to such sustainable development, because they have extremely great potential. Namely, previous experiences at the international level have clearly shown that cooperative organization has numerous advantages over other forms of organization, i.e. Business and that these advantages are very different in nature. The fact is that cooperatives are something that is positive and that we should strive for in the future. Namely, when we mention *cooperatives*, we all immediately think of the agricultural sector, which is certainly wrong, because there should be cooperatives in almost all areas of social life. In the Republic of Croatia, agricultural cooperatives (engaged in plant breeding, animal husbandry, forestry or hunting), workers' cooperatives, fishing cooperatives, housing cooperatives, construction cooperatives, social cooperatives, consumer

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<sup>17</sup> Kostić, C., *Tipologija porodica V.St.Karadžića i V. Bogišića*, <https://hrcak.srce.hr/file/176866>, (30.05.2022.)

cooperatives, craft cooperatives and secondary cooperatives may be established as cooperatives with certain special features. In modern agriculture, cooperatives are a prerequisite for (self) sustainability of farms on small land, given that those farms are most in number. Namely, the association enables small producers to procure seeds, fertilizers, planting material, chemicals, and fuel at a cheaper price, but also to place their goods on the market easier and faster, at previously known and guaranteed prices.

The fact is that when individuals are united in one team, which is based on emotion, trust, help and readiness to solve problems together, then the chances of success are much higher, which can be applied to cooperatives. The term "co-operate" means mutual trust, the absence of unfair competition, putting common interests above individual interests, doing cooperative business in which everyone wins (and no one loses), of course, in proportion to their work and investment.

## **THE ROLE OF COOPERATIVES**

All members of the cooperative have equal rights in management and participate equally in it. Cooperatives have, above all, influenced the solution of social and economic problems and enabled fast employment. The main role of the cooperative is to ensure that the members of the cooperative meet their needs through the cooperative, which are of different character. When people are united, they can more easily withstand the competition on the market, provide a better offer of services and have the opportunity to create better products, get the necessary raw materials and other resources cheaper. It should be pointed out that cooperatives establish a direct link between agricultural producers and the market itself and, in that way, enable much easier placement of their products, which on the one hand reduces their costs and on the other hand increases their income. The importance of rural areas was seen during the Covid-19 pandemic, when people turned en masse to work in their gardens, fields and cultivating the land. They realized that when they have healthy, organic food, clean air, unpolluted land and drinking water, they can solve all problems much easier. In the member states of the European Union, the issue of cooperatives is regulated differently in the constitutions of European countries, so we distinguish three categories of countries: 1) countries whose constitutions have recognized the role and value of cooperatives and mention them as such; 2) countries that have not recognized this and do not recognize their existence, ie. Freedom of association of citizens and 3) countries whose constitutions guarantee freedom of association of citizens, but

they do not mention in their provisions the cooperatives as institutions<sup>18</sup>. When it comes to the Lisbon Treaty, which is a replacement for the failed Constitution of the European Union, cooperatives are not mentioned, but only the right of citizens to associate, is emphasized. On the other hand, there are EU member states, which in their constitutional text mention cooperatives and recognize their certain social role (Bulgaria, Italy, Spain, Portugal, Hungary, Malta), there are also constitutions of certain countries where cooperative organization is mentioned, but does not explicitly emphasize the existence and activities of cooperatives (Greece, Cyprus, Poland)<sup>19</sup>. [4] As for the current Constitution of the Republic of Serbia from 2006, it should be said that it does not mention the village in any place, organization (except in Article 86, act 1, which states that it "guarantees private, cooperative and public property"). The situation is the same with the Constitution of the Republic of Croatia, with Article 43, act 1 guaranteeing everyone the right to free association in order to protect their interests or advocate for social, economic, political, national, cultural or other beliefs and goals<sup>20</sup>. Important cooperative values are manifested through the principles of self-responsibility (members have multiple responsibilities towards their cooperative); democracy (the right of members to participate in the work of the cooperative, to be informed and involved in the decision-making process); equality (members of the cooperative have the same rights and opportunities); fairness (all co-operatives have equal treatment); solidarity (cooperatives are a manifestation of collective strength and shared responsibility).

In order to reduce business costs, enable easier product sales and provide better services, generate higher income, cooperative unions are created, which represent independent, interesting, business and professional organizations. Cooperative unions establish cooperatives and other cooperative unions in order to realize, harmonize and improve their business, protect common interests, enable business connections and the like. Such an association has the status of a legal entity, which it acquires by registering in the Register, and at least 10 cooperatives are needed for its establishment. The Cooperative Union performs numerous tasks, among which the most important are the improvement of the above-mentioned cooperative principles, encouraging cooperatives, providing professional and other assistance to cooperatives and cooperatives and representing their interests before state bodies, local governments and territorial autonomy and other

<sup>18</sup> See more: Bataveljić, D., Usluge zadruga i reafirmacija zadružnog pokreta, u: MIĆOVIĆ, Miodrag (urednik), XXI vek - vek usluga i uslužnog prava, Knjiga 7, Institut za pravne i društvene nauke, Pravni fakultet Univerziteta u Kragujevcu, Kragujevac, 2016, p. 3-14.

<sup>19</sup> See Ustav Republike Srbije, "Sl. glasnik RS", no. 98/2006 i 115/2021.

<sup>20</sup> See Ustav Republike Hrvatske, Narodne Novine no. 56/90, 135/97, 08/98, 113/00, 124/00, 28/01, 41/01, 55/01, 76/10, 85/10, 05/14

institutions and organizations. Abroad and cooperation with other cooperative associations outside the borders of our country and the like. Also, the cooperative union for a certain territory or a particular type of cooperative, organizes and encourages professional development, scientific research, information, publishing and marketing activities, which is of interest for the improvement of cooperatives, keeps records of cooperatives and cooperative statistics, performs mediation and other affairs in accordance with the Law of Cooperatives<sup>21</sup>

## COOPERATIVES IN THE REPUBLIC OF SERBIA

The Republic of Serbia passed the Law of Cooperatives in 1996, which was amended and harmonized with modern trends in cooperatives and norms valid in the European Union and developed countries, by passing a new, now valid Law from 2015. The provisions of the said Law, which legalized on January 7, 2016, regulate: the legal status of cooperatives, their establishment, management and bodies of cooperatives, acquisition and termination of cooperative status, book of cooperatives, cooperatives, cooperative unions, complex cooperatives, registration of cooperatives, cooperative audit, as well as other issues important for the position and work of the cooperative. Today, there are slightly less than three million cooperatives in the world, with more than one billion cooperative members.<sup>22</sup> The cooperative is an independent and autonomous organization, while membership in the cooperative is voluntary, which means that members can join, but also leave the cooperative of their own free will. Cooperatives operate in a democratic manner, decisions are made freely, and cooperatives are not under the control of government institutions. What is important to emphasize is that the individual share of cooperatives is not independent and unchanging, but on the contrary, it depends on the success of the cooperative, and, therefore, increases or decreases. Thus, the distribution of the realized income is done according to the contribution to the joint work, as well as according to the size of the share in the property of the cooperative, and the constant control is performed by the cooperative members themselves. In the management of the cooperative, one of the basic principles of democracy is applied, according to which each cooperative member has the same right to vote, in accordance with the principle of "one cooperative member, one vote". A cooperative is a legal entity, which is a special form of organization of individuals who realize their economic, social,

<sup>21</sup> Zakon o zadrugama RS, "Sl. glasnik RS", no. 112/2015, article 70.

<sup>22</sup> Knežević, M., *Zadruga u savremenom privrednom okruženju*, Naučne publikacije državnog univerziteta u Novom Pazaru, Serija B: Društvene i humanističke nauke, April 2021, Volumen 4, no 1, p.53-63.

cultural and other interests by operating on cooperative principles and who manage and control the business of the cooperative.<sup>23</sup>

The cooperative acquires the status of a legal entity by registration in the register kept by the body responsible for registration of economic entities, but it should be emphasized that the cooperative cannot be organized as a company, nor can it merge or merge with a company or other legal entity other than the cooperative, as well as change the form into a company or other legal entity.<sup>24</sup> The cooperative is responsible for its obligations with its property, provided that the cooperative members are responsible for the obligations of the cooperative only up to the amount of the given contribution, but not with personal property. In legal transactions, the cooperative may act: in its own name and for its own account, in its own name and on behalf of the cooperative, as well as in the name and on behalf of the cooperative.

In the Republic of Serbia, it is allowed to establish agricultural housing, consumer, craft, workers, student-youth, social, health cooperatives. The cooperative can be founded by at least five able-bodied individuals at the founding assembly organs.

A cooperative can be founded by at least five able-bodied individuals at the founding assembly, by concluding a founding agreement, adopting cooperative rules and electing bodies.<sup>25</sup> The minimal founding price of the cooperative is 100RSD.<sup>26</sup>

The cooperative is managed by the cooperative members in such a way that "one cooperative member has one vote" in the cooperative assembly, while the cooperative bodies are: the assembly, the board of directors, the supervisory board and the director.<sup>27</sup>

## COOPERATIVES IN THE REPUBLIC OF CROATIA

The first cooperative in the Republic of Croatia, called "*Pitomačka zanatnička zadružnica*", was founded in 1862 in Pitomača and to this day has not ceased its activities, the only thing that today has a new name "*Prva obrtna štedno-kreditna zadruha*".<sup>28</sup> At the beginning of the 20th century, cooperatives became an

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<sup>23</sup> Zakon o zadrugama RS, Ibid. par.2.

<sup>24</sup> Ibid, art. 5.

<sup>25</sup> Ibid, art.14.,15.

<sup>26</sup> Ibid, art.20. par.4.

<sup>27</sup> Ibid, art. 33. par. 1., art. 34. par. 1.

<sup>28</sup> Hrvatski poljoprivredni zadružni savez, Povijest zadrugarstva u Hrvatskoj, [https://www.hzs.hr/o\\_zadruzi.htm](https://www.hzs.hr/o_zadruzi.htm), (01.06.2020.)



extremely strong economic system, so that over 1,500 cooperatives with about 250,000 members operated in today's Croatia.<sup>29</sup> A co-operative member is a physical or legal person who invests a membership fee in a co-operative, whether in money, property or rights, and who operates on the basis of his co-operative, which means purchasing, using or selling his services and products. Cooperative members contribute the same financially to a cooperative with a common interest and a certain degree of community. Starting from the concept of cooperatives, we can say that every man lives from his work and creativity by which he receives products that he associates in the cooperative with other members of the cooperative.<sup>30</sup> The cooperative is a legal entity whose establishment is prescribed by the Law of Cooperatives,<sup>31</sup> established with the intention of gaining profit, in which the members are obliged to enter the membership fee, and are responsible for the obligations of the cooperative up to the amount of money they invested in the cooperative. Fully able-bodied natural and legal persons may establish a cooperative for which, in accordance with the Law on Cooperatives, at least seven founders are required. The founder of the cooperative becomes a member at the time of its establishment and is entered in the directory of members of the cooperative. All the founders of the cooperative form the founding assembly, which adopts the rules of the cooperative, which are the founding and basic general act of the cooperative. The rules of the cooperative must have information on the company, headquarters and business, internal structure, conditions and manner of acquiring membership, form and amount, entry and return of member roles, rights, obligations and responsibilities of members, conditions and manner of termination of membership and other issues related to membership in the cooperative, bodies of the cooperative: their competencies, rights and obligations, election and recall procedure, mandate of members, manner of decision making and other issues related to the work of cooperative bodies, representation and representation of the cooperative and rights and powers of managers, assets of the cooperative and the manner of disposing of assets, use of profit or surplus income, coverage of losses or deficit in business, part of the profit or surplus income allocated to reserve requirements, status changes and termination of the cooperative, informing members and business secrets, manner and procedure amendments to the rules and other issues important for the work and operations of the cooperative.<sup>32</sup> It is considered that the cooperative was founded at the time

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<sup>29</sup> *Ibid.*

<sup>30</sup> See more: Bataveljić, D., Bataveljić, B., Zadruga - poseban oblik organizovanja fizičkih lica, u: NEŠKOVIĆ, Slobodan (urednik): Zbornik radova Zlatno zрно Šumadije, Edicija - Bezbednost u postmodernom ambijentu, Centar za strateška istraživanja nacionalne bezbednosti - Cesna B, Beograd, Knjiga 12, 2012, p. 370-379.

<sup>31</sup> Zakon o zadrugama RH, Narodne novine no. 4/11, 125/13, 76/14, 114/18, 98/19.

<sup>32</sup> Art.8.par.2. *Ibid.*

of signing the statement of the founder on the acceptance of the rules of the cooperative at the founding assembly. The cooperative acquires the status of a legal entity by entering it in the court register of the locally competent commercial court, which states: company, seat, OIB and subject of business of the cooperative, day of the founding assembly, name and surname of the manager and OIB, powers to represent the cooperative, name and OIB and OIB members of the supervisory board, if the cooperative has a supervisory board, the responsibility of the members of the cooperative for the obligations of the cooperative, if prescribed by the rules of the cooperative.<sup>33</sup> In addition to the Law of Cooperatives, the legal framework of cooperatives in the Republic of Croatia includes three bylaws and the Law on the Introduction of the European Cooperative.<sup>34</sup> According to the Accounting Act, cooperatives are obliged to keep double-entry books. Cooperatives, as well as companies, i.e. legal entities, issue R-1 invoices and pay VAT on the issued invoice, regardless of whether it has been paid or not.<sup>35</sup> Cooperative principles in the cooperative are selling goods for cash, selling goods in the cooperative store at market price, returning the difference between the price for which the cooperative bought goods and the market price at which it sells goods to its members, , in proportion to the amount of purchases of individual members, before paying the difference in price to the members of the cooperative, part of the salary must be set aside for cooperative funds, each cooperative member must pay a certain amount to the cooperative as a cooperative share, which serves to form its own cooperative capital, the cooperative is an association of people one vote, regardless of the amount of the paid share, the cooperative is not tied to a limited number of members, so entry is open to an unlimited number of new potential members, the cooperative accepts new members asking them for moral qualities, the cooperative is founded on political and religious neutrality and is open to all people, regardless of differences in political and religious beliefs.<sup>36</sup>

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<sup>33</sup> Art. 9.st.par. 1. *Ibid.*

<sup>34</sup> Zakon o uvođenju Europske zadruga - Societas Cooperativa Europaea (SCE), Narodne novine no. 63/08, 110/15.

<sup>35</sup> See more: Božić, V., Osnivanje zadruga u Republici Hrvatskoj - *pro et contra*. U: BATAVELJIĆ, Dragan (ur.), RAPAJIĆ, Milan (ur.). Zbornik radova. Knić. 2020. p. 247-259.

<sup>36</sup> Mataga Ž, Poljoprivredno zadrugarstvo Hrvatske – Zagreb, Zadružni savez Hrvatske, Centar za informiranje u poljoprivredi, str.91. Vidii: Mataga Ž, Poljoprivredno zadrugarstvo u Hrvatskoj: razvoj i temeljn i problemi, Hrvatski poljoprivredni združni savez, Zagreb, Hrvatska, Sociologija i prostor: časopis za istraživanje prostornoga I sociokulturnog razvoja, Vol. 43 No. 1 (167), 2005., p. 17-42.

Below is an overview of the essential elements in establishing a cooperative.

|                                                                  |                                                                                                                                                               |
|------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                  | COOPERATIVE                                                                                                                                                   |
| Regulated by law                                                 | Law of Cooper                                                                                                                                                 |
| legal status                                                     | Legal entity, has its own OIB and MB                                                                                                                          |
| Name / „Tvrka“                                                   | It must contain the "cooperative" in the name, and an indication of the main activity performed by the cooperative                                            |
| Founder Type                                                     | At least 7 able-bodied natural or legal persons                                                                                                               |
| Type of registration number                                      | MBS                                                                                                                                                           |
| Tax Number                                                       | MB DZS                                                                                                                                                        |
| Enrolls in...                                                    | Commercial Court Register                                                                                                                                     |
| Place of registration                                            | Commercial Court                                                                                                                                              |
| Share capital...                                                 | Cooperatives invest equal membership roles that can be in money, things and rights expressed in kuna equivalent, and min. HRK 1,000.00 per cooperative member |
| Establishment costs (excluding membership fees or share capital) | depending on the number of members of the cooperative, approx. 3,000.00 kn                                                                                    |
| Time required for establishment / opening                        | About 30 days                                                                                                                                                 |
| Type of document                                                 | A commercial court decision                                                                                                                                   |
| Activities                                                       | All permitted economic activities, with some exceptions                                                                                                       |

|                                                                           |                                                                                                                                                                                                                                                             |
|---------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Responsibility                                                            | The cooperative in legal transactions is responsible for its obligations with all its assets. The cooperative is not responsible for the obligations of its members. A member of the cooperative is not responsible for the obligations of the cooperative. |
| The basic governing and decision-making body                              | Assembly (democratic and direct management of all members of the cooperative who equally participate in decision-making on business and profit distribution)                                                                                                |
| Mandatory bodies                                                          | Assembly and manager (NO for cooperatives with more than 20 members)                                                                                                                                                                                        |
| The basic document that governs the business                              | Rules of the cooperative                                                                                                                                                                                                                                    |
| Employees                                                                 | There are no restrictions                                                                                                                                                                                                                                   |
| Participation in the work of family members (without employment contract) | No                                                                                                                                                                                                                                                          |
| Profit                                                                    | Part of the profits is retained and invested in the cooperative. The cooperative does not exist for its own benefit, its main goal is to service the needs of its members and achieve the greatest possible benefit for them                                |
| Type of taxation                                                          | Profit tax                                                                                                                                                                                                                                                  |
| Tax Rates                                                                 | 20% on the tax base                                                                                                                                                                                                                                         |
| Bookkeeping                                                               | Double-entry bookkeeping under the Accounting Act                                                                                                                                                                                                           |
| The invoice it issues                                                     | R-1                                                                                                                                                                                                                                                         |
| VAT payer                                                                 | Yes, from the moment of starting the business, on all income except membership fees                                                                                                                                                                         |
| Payment of VAT                                                            | By issuing invoices                                                                                                                                                                                                                                         |

|                                                               |    |
|---------------------------------------------------------------|----|
| Mandatory membership                                          |    |
| Possibility of temporary suspension of work for up to 1 year. | No |

## CONCLUDING REMARKS

The fact is that cooperatives were formed in difficult economic and social circumstances during the 19th century, when farmers realized that they could improve their market position only by joint efforts, mutual solidarity and mutual assistance, by investing their own property in cooperatives. Cooperatives in all European Union countries, as in most other European countries, are today one of the main links in the entire food chain, adapting the products of their members to market demands and improving their market position, guaranteeing the quality of their products. In this way, they significantly contribute to the economic vitality of rural areas, because they represent a significant source of employment, giving a great contribution to economic growth and development of each country individually. The legal position of cooperatives is based on cooperative principles and cooperative principles, and on the concept of economic entity, so the legal solutions have the task to improve and provide a new approach to cooperatives, as special economic entities in today's modern age. Valja pointed out that they tried and managed to find a great balance between cooperative principles and the very elements of companies. In the Republic of Croatia, cooperatives invest equal membership roles that can be in money, things and rights expressed in kuna equivalent, a minimum of 1,000.00 kn (133.33 euros) per cooperative, in order to perform, as a rule, all permitted economic activities, and what as to the responsibilities of the members of the cooperative, they are not liable for the obligations of the cooperative. It is important to note that the cooperative does not exist for its own benefit, but its main goal is to achieve the greatest possible benefit for its members. The same principles apply in the territory of the Republic of Serbia, provided that the minimum share capital of the cooperative is 100RSD. What is very important for the smooth functioning of cooperatives and their survival, development and sustainability, is the fact that cooperative activities should benefit all members, but in accordance with their participation and contribution to the work of cooperatives. Only in this way, cooperatives in the Republic of Serbia and the Republic of Croatia will represent a significant socio-economic process and an important segment of their economy. We will leave the cooperative practice to the improvement of legal solutions.

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## **ENVIRONMENTAL PROTECTION**





## MAINTAINING THE VITALITY OF BACTERIA UNDER VASELINE OIL

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### **Abstract**

*The aim of this research was to determine the survival of bacteria - stimulators of plant growth stored under vaseline oil for three years as well as the ability to accumulate biomass of *Pseudomonas liquefaciens* and *Pseudobacterium lacticum* culture stored under vaseline oil. Both, *Pseudomonas liquefaciens* and *Pseudobacterium lacticum* remain vital after storage for three years under vaseline oil. The *Pseudobacterium lacticum* culture is equally well maintained at both reduced and room temperature. The *Pseudomonas liquefaciens* culture is better maintained at room temperature. From the point of the ability of *Pseudomonas liquefaciens* to accumulate biomass during the storage time period, significant differences ( $p < 0.05$ ) were observed after 24h as well as after 48h, where the higher dry biomass was determined in the sample stored under oil (4.290 g/L). Furthermore, after 96h, both samples had similar biomass. The ability to accumulate biomass of the *Pseudobacterium lacticum* culture were significantly higher ( $p < 0.05$ ) after 48h and still after 96h. However, it can be concluded that in cultures of *Pseudomonas liquefaciens* and *Pseudobacterium lacticum*, stored under vaseline oil, the ability to reproduce and accumulate biomass is weak.*

**Key words:** vaseline oil, accumulation of biomass, bacteria, storage, *Pseudomonas* sp.

## INTRODUCTION

Preservation of micro-organisms by desiccation has been the preferred method for long term storage of cultures for decades. There are extensive culture collections that depend on these drying methods to preserve a huge diversity of cells for future propagation (Morgan et al., 2006).

The problem of preservation of microbial diversity is as global as questions sustaining the environment. In general the object of any kind of preservation process is to enable long term storage of micro-organisms whilst preserving cell viability (Morgan et al., 2006).

One of the important tasks is to develop a collection of the most effective methods for long-term storage of microbial cultures to ensure long-term preservation of viability and biological characteristics (Gavrilova, 2003). Based to scientific achievements of microbiology as well as introduction of new development of genetic engineering began possible to receive hi-tech strains of microorganisms, producers of enzymes, amino acids, antimicrobial metabolites and other biologically active agents (Dzhakibaeva and Kebekbaeva, 2014).

Interest to studying of lactic bacteria is explained by a wide range of their biological activity and harmlessness for the human and animals. Traditional methods of maintaining the lactic acid bacteria are reduced to their growing on rich nutrient media with frequent subculture. Elective medium is skim milk (skim). A bacterium grown in skimmed milk is stored in the form of milk clot within a month. However, frequent periodic subcultures lead to reduced survival of the bacteria, changing the composition of the population, the loss of a series of physiological and biochemical properties.

One of the simplest ways to maintain (store) microorganisms is storage under oil, primarily medical vaseline oil. The oil layer retains water evaporation and even drying of the nutrient medium. Since oxygen penetrates unhindered through the oil layer to the microorganism, they do not go into a state of anabiosis. In microorganisms stored under oil, metabolic processes are retained, but reproduction is not stopped. The physiological properties of microorganisms do not change either.

For example, a culture of nodule lupine bacteria, stored for 6.5 months under oil, gave almost twice the increase in green mass than fresh culture, and a culture of azotobacteria, which was also kept under oil, more actively fixed atmospheric nitrogen than control one (Uzunova-Doneva, Donev, 2004; 2005). In the case of bacteria of 27 genera, stored for several years under oil at room temperature, their diagnostic, morphological, cultivation and physiological-biochemical properties did not change (Nadirova et al. 2002; Hartsell, 1953; Morton, Pulaski, 1937). The screening period of oil-stored crops should be

determined taking into account the individual specifics of different bacteria.

According to some data, the properties of microorganisms stored under oil can be variable. Variability of properties is observed in microscopic fungi (Beljakova, Lebedeva, 2007). In actinomycetes - producers of antibiotics, the activity of biosynthesis is reduced (Kuznetsov, Rodionova, 1997).

Having in mind the divergence of literature sources in relation to this issue, how epiphytic bacteria are maintained under vaseline oil was studied, which have the ability to stimulate the growth and development of plants.

## MATERIAL AND METHOD

Epiphytic bacteria *Pseudomonas liquefaciens* and *Pseudobacterium lacticum* were grown in 12x120 mm tubes on oblique agar nutrient medium in a thermostat, 6-7 days at a temperature of 26 °C. The obtained cultures were filled with sterile vaseline oil. The layer of oil above the upper edge of the agar was 1 cm thick. The cultures were stored in test tubes, closed with scales and gauze swabs, at 5 °C - in the refrigerator and at room temperature for three years.

The first orientation check of crop viability (growth on oblique agar) was done after 13 months. After three years of storage, the growth of these cultures on fresh oblique agar was checked. Bioaccumulative capacity was determined based on the results of growing cultures, which were stored at room temperature, in a liquid nutrient medium on a shaker at 150-160 rpm for 72 hours. 24-hour cultures, which were maintained by periodic screening, served as controls.

The obtained results were statistically processed with Independent Sample t-test ( $p = 0.05$ ) using SPSS 20.

## RESULTS AND DISCUSSION

The verification of cultures after 15 months of storage is confirmed by the literature data that in some microorganisms, stored under oil, growth retention is observed at the beginning of cultivation (Fateeva, 2007b). Both cultures, grown on fresh agar, lagged behind in the first 24 hours compared to the control, after 48 hours the difference was insignificant, and after 6 days the differences completely disappeared. No differences were found between cultures stored at room and reduced temperature. The exception was the culture of *Pseudomonas liquefaciens*, which was kept at a lower temperature; its growth even after 6 days of cultivation was weaker than in control.

After three years of storage under Vaseline oil (Table 1), growth retention at the beginning of cultivation was more pronounced than in cultures stored for

15 months. Certain variants lagged behind the control not only in the first days, but also after 6-10 days. The culture of *Pseudomonas liquefaciens*, kept at room temperature, initially had a slow growth, and after 10 days the lag behind the control was stopped. After storage at a lower temperature, the same culture did not grow at all on the first and second day, and from 6-10 days its growth was quite weak.

The culture of *Pseudobacterium lacticum* is well maintained at low and room temperature: the growth of the control variant was reached after six days of cultivation.

When storing microorganisms under vaseline oil, the temperature factor should be taken into account, which, obviously, can be related to the individual specifics of microorganisms.

Tables 2 and 3 provide data on the ability of bacteria stored under vaseline oil to multiply and accumulate biomass during cultivation under conditions of enhanced aeration. From the point of *Pseudomonas liquefaciens*, can be seen that at the beginning of the cultivation there is no statistically significant differences between control variant (0.465 g/L) and sample under oil (0.435 g/L). However, during the storage time period, significant differences ( $p < 0.05$ ) were observed after 24h as well as after 48h, where the higher dry biomass was determined in the sample stored under oil (4.290 g/L). Furthermore, after 96h, both samples had similar biomass, i.e. 4.675 g/L for the control variant, while 4.685 g/L for the variant stored under oil.

**Table 1. Survival of bacteria - stimulators of plant growth stored under vaseline oil for three years**

| Culture, storage variant        | Duration of cultivation (days) |     |     |     |
|---------------------------------|--------------------------------|-----|-----|-----|
|                                 | 1                              | 2   | 6   | 10  |
| <i>Pseudomonas liquefaciens</i> |                                |     |     |     |
| Control                         | +++                            | +++ | +++ | +++ |
| under oil at 5 °C               | 0                              | 0   | +   | +   |
| under oil, at room temperature  | 0                              | +   | ++  | +++ |
| <i>Pseudobacterium lacticum</i> |                                |     |     |     |
| Control                         | +++                            | +++ | +++ | +++ |

|                                |   |    |     |     |
|--------------------------------|---|----|-----|-----|
| under oil at 5 °C              | + | ++ | +++ | +++ |
| under oil, at room temperature | + | ++ | +++ | +++ |

0 - there was no growth; + - very weak growth; ++ - poor growth; +++ - normal growth.

**Table 2. Ability to accumulate biomass of *Pseudomonas liquefaciens* culture stored under vaseline oil**

| Storage variant | Weight of dry biomass, g/L of medium |                     |                     |                     |                     |                     |
|-----------------|--------------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
|                 | At the beginning of cultivation      | After cultivation   |                     |                     |                     |                     |
|                 |                                      | 5h                  | 24h                 | 48h                 | 72h                 | 96h                 |
| Control         | 0,465 <sup>aA</sup>                  | 0,520 <sup>bA</sup> | 3,360 <sup>cA</sup> | 3,450 <sup>cA</sup> | 4,465 <sup>aA</sup> | 4,675 <sup>aA</sup> |
| Under oil       | 0,435 <sup>aA</sup>                  | 0,560 <sup>bA</sup> | 2,925 <sup>cB</sup> | 4,290 <sup>aB</sup> | 4,700 <sup>dA</sup> | 4,685 <sup>dA</sup> |

a, b, c, d - values of the same storage variant at a different time interval marked with different letters, have a statistically significant difference ( $p < 0.05$ ), T-test.

A, B - values of different storage variant at a same time interval, marked with different letters, have a statistically significant difference ( $p < 0.05$ ), T-test.

**Table 3. Ability to accumulate biomass in the culture of *Pseudobacterium lacticum* stored under vaseline oil**

| Storage variant | Weight of dry biomass, g/L of medium |                     |                     |                     |                     |                     |
|-----------------|--------------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
|                 | At the beginning of cultivation      | After cultivation   |                     |                     |                     |                     |
|                 |                                      | 5h                  | 24h                 | 48h                 | 72h                 | 96h                 |
| Control         | 0,400 <sup>aA</sup>                  | 0,510 <sup>bA</sup> | 3,100 <sup>cA</sup> | 3,360 <sup>cA</sup> | 4,720 <sup>dA</sup> | 4,955 <sup>dA</sup> |
| Under oil       | 0,375 <sup>aB</sup>                  | 0,515 <sup>bA</sup> | 3,075 <sup>cA</sup> | 4,510 <sup>dB</sup> | 4,970 <sup>eB</sup> | 5,080 <sup>eB</sup> |

a, b, c, d, e - values of the same storage variant at a different time interval marked with different letters, have a statistically significant difference ( $p < 0.05$ ), T-test.

A, B - values of different storage variant at a same time interval, marked with different letters, have a statistically significant difference ( $p < 0.05$ ), T-test.

On the other hand, the ability to accumulate biomass of the *Pseudobacterium lacticum* culture, at the beginning of the cultivation was higher ( $p<0.05$ ) in the control variant (0.400 g/L) compared to the variant under oil (0.375 g/L). Although this value was found to be slightly lower at the beginning of the cultivation, they were significantly higher ( $p<0.05$ ) after 48h and still after 96h (4.955 g/L for the control variant, i.e. 5.080 g/L for the variant stored under oil).

In accordance, when stored under vaseline oil, both cultures retained the ability to reproduce and create biomass. Statistically significant differences ( $p<0.05$ ) were found at both cultures between different time intervals of storage. In this respect, they sometimes surpassed the control variants.

Dzhakibaeva and Kebekbaeva (2014) have been investigated for the viability of different lactic acid bacteria stored on a solid medium, under vaseline as well as under glycerol solution (10%, at  $-20^{\circ}\text{C}$ ). Based on their research, the viability of the tested bacteria strains under vaseline oil is as follows: *Lactobacillus plantarum* 53H -  $10 \times 10^7$  KOE/mL, *Lactobacillus plantarum* 22 -  $9 \times 10^6$  KOE/mL, *Lactobacillus plantarum* 2 -  $8 \times 10^7$ , *Lactobacillus salivarius* 8d -  $9 \times 10^7$  KOE/mL and *Lactobacillus casei* 139 -  $7 \times 10^6$  KOE/mL.

On the other hand, Milko et al. (1984) studied the survival rate of *Mycobacterium lacticolum* and the proportion between its R, S and M variants in the course of 12 months under different conditions of storage (freeze-drying, under vaseline oil, in 0.85% NaCl solution and in distilled water). Authors found high survival rate of the variants in cells freeze-dried in a protective medium containing 10% of sucrose +1% of gelatin as well as in a 0.85% solution of NaCl. The survival rate of the variants differed by 2--3 orders of magnitude in cells freeze-dried with sodium glutamate or suspended in distilled water. The proportion between the R, S and M variants in the population noticeably changed after storage under these conditions.

Viability assays may also be based on biochemical reactions with certain cell components, e.g. sugars or amino acids, on metabolic activity or on the turnover of chromogenic or fluorogenic substrates by cellular enzymes (Braissant et al., 2015; Berninger et al., 2018). Biochemical assays can be suitable for estimating the viability in encapsulated formulations as they allow for *in situ* measurements under certain circumstances. This is an advantage over standard plate-counting, which requires dissolution of the encapsulation matrix to release the cells (Wadhawan et al., 2010; Berninger et al., 2018).

Furthermore, viable cells of *Bacillus subtilis* were detected by intracellular phototautomerism (Kort et al., 2010; Berninger et al., 2018). This

method relies on the pH difference in the cytosol of live and damaged cells. A specific, neutral probe, which can penetrate the cell membrane, dissociates into a fluorescent phototautomeric anion only at the neutral pH in live cells. The emitted signal was monitored by microplate reader at a detection limit of  $10^6$  CFU ml<sup>-1</sup>.

The decision on which method to apply for evaluating viability of bacterial inoculants thus depends on the composition of the formulation, available equipment and expertise, expected number of live and dead cells, definition of viability as well as access to strain-specific information (Berninger et al., 2018).

## CONCLUSION

According to the results, can be concluded that cultures of *Pseudomonas liquefaciens* and *Pseudobacterium lacticum* remain vital after storage for three years under vaseline oil. The *Pseudobacterium lacticum* culture is equally well maintained at both reduced and room temperature. The *Pseudomonas liquefaciens* culture is better maintained at room temperature.

From the point of *Pseudomonas liquefaciens*, during the storage time period, significant differences ( $p < 0.05$ ) were observed after 24h as well as after 48h, where the higher dry biomass was determined in the sample stored under oil (4.290 g/L). Furthermore, after 96h, both samples had similar biomass. The ability to accumulate biomass of the *Pseudobacterium lacticum* culture were significantly higher ( $p < 0.05$ ) after 48h and still after 96h.

However, can be concluded that in cultures of *Pseudomonas liquefaciens* and *Pseudobacterium lacticum*, stored under vaseline oil, the ability to reproduce and accumulate biomass is weak.

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## TROPHIC CHAIN YERSINIA PSEUDOTUBERCULOSIS

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**Abstract:** *This paper tries to reproduce two types of trophic chains on the example of an aquatic ecosystem model, with the participation of planktonic and benthic organisms: "yersinia - infusoria - daphnia - fish" and "yersinia - insect larvae - fish".*

*The concentration of Yersinia pseudotuberculosis during primary infection (infusoria culture medium) was  $10^9$ /ml. In the first hours after infection, the concentration of bacteria in the infusoria decreased ( $10^5$ cfu/mass ). In daphnia, which consumed infected infusoria, the concentration of Yersinia pseudotuberculosis decreases in the first 3-5 days (from  $10^5$  to  $10^3$  cfu/10 individuals), then the reproduction of Yersinia pseudotuberculosis in daphnia continues, and on the 11th day it reaches  $10^7$  cfu/10 individuals.*

*The concentration of Yersinia pseudotuberculosis in the intestines of fish fed infected daphnia ( $10^7$ cfu/10 individuals) is continuously decreasing:  $10^5$ cfu (after 24 hours) and  $10^2$ - $10^1$ cfu/individuals (after 15-30 days).*

**Key words:** *daphnia, ecosystem, infusoria, yersinia, food chain, fish*

## INTRODUCTION

Interactions between genus populations in the ecosystems of natural foci of infection are complex and diverse. Unlike aboveground, terrestrial and aquatic ecosystems are poorly studied in this respect: certain papers are devoted to the analysis of interactions between a large number of pathogenic bacteria and specific hosts, primarily protozoa. Modeling of trophic chains, and even bacteria, in the experiment, although it represents a certain simplification, it allows to challenge and analyze the probability of transmission of the infectious agent in the community, to determine specific routes of its circulation, changes in bacterial population at different levels of the trophic pyramid. the potential epidemiological significance of certain links in these chains.

There are almost no special manuscript in the literature on the migration of pathogenic microorganisms along the trophic chains of aquatic and terrestrial communities; only certain assumptions in this regard are known. Thus, for example, Liston (1990) believes that bacteria and toxins reach fish through trophic chains. Shubin (1993) states that the circulation of pseudotuberculous microbes in natural foci is significantly related to water and hydrobionts: infection of fish and fish-eating birds can be done through food and water throughout the year. Đukić (2011) points out that in the population of yersinia found in the community with infusoria in clay-swamp soil extract, after the first day there is a certain increase in the number ( $10^6$ ) of yersinia, after which their concentration decreases to  $10^5$  cfu/cm<sup>3</sup> on the third day and the eighteenth - up to  $10^3$ cfu/cm<sup>3</sup>). According to the authors, water and hydrobionts take second place (after the soil and its inhabitants) as reservoirs of pseudotuberculous microbes in natural hotspots.

The hydrobiotic organisms are potential natural hosts of *Y. pseudotuberculosis*, and fish as the final link of some alimentary chains, could present epidemic danger as a food product for human consumption (Pushkareva et al., 1994). Gengler et al. (2015) state that entomopathogenic nematodes are an efficient reservoir that provides exponential multiplication, maintenance and dissemination of *Y. pseudotuberculosis*.

Yersinia strains are psychrotrophic bacteria that are resistant to many environmental factors (Triantafillidis et al., 2020). *Yersinia pseudotuberculosis* is present in the soil, in fresh farmproduced plants and root vegetables, but also has numerous animal reservoirs and is abundant in wildlife, including birds, rodents, rabbits, deer, dogs, cats, cattle and insects and amoeba in the environment (Brady et al., 2022; Martínez-Chavarría et al., 2015)

This paper is an attempt to reproduce two types of trophic chains on the example of an aquatic ecosystem model - with the participation of planktonic and

benthic animals: "yersinia - infusoria - daphnia (cyclops) - fish" and "yersinia - insect larvae - fish".

## MATERIAL AND METHODS

The work was performed in the public health institutes of Čačak and Kraljevo in 2007 year. Axenic culture of *Tetrahymena pyriformis* infusoria was infected with pseudotuberculous microbe at a concentration of  $10^9$  cells/ml. After incubation for 1-2 hours at 22-25 °C, the infusoria biomass was collected on filters ("Millipore", pore diameter 6 micrometers) and washed from extracellular bacteria; with bacteriological control of the rinsing water. In aquariums with daphnia (cyclops) infusoria with phagocytic yersinia were introduced, in a concentration that is close to natural -  $10^3$ - $10^4$  cells/l of water. In this way, the infected lower shells are cultivated. Cyclops were used to infect fish, which "consumed" infected tetrahymen for one day-up to the peak concentration (in them) of pseudotuberculous microbes ( $10^5$  cfu/100 individuals). Daphnia were kept for 7-11 days after feeding with infected infusoria, after which yersinia reached their maximum concentration ( $10^7$  cfu/10 individuals).

Each fish (guppies) was infected individually in cups (once), giving it 10 infected daphnia or 100 cyclops as food (with magnifying glass control). Bacteriological examinations were performed during the first week once a day, and then twice a week.

Table 1. *Yersinia pseudotuberculosis* abundance at different trophic levels during "infusoria-daphnia-fish" chain transmission

| Trophic chain links | Time limit (day) | Yersinia concentration     |                       |
|---------------------|------------------|----------------------------|-----------------------|
|                     |                  | "At the entrance"          | "At the exit"         |
| Infusoria           | 1                | $10^9$ /ml (in the middle) | $10^5$ (in infusoria) |
| Daphnia             | 1 to 5           | $10^5$ cfu/10 individuals  | $10^3$                |
|                     | 7 to 11          | $10^5$                     | $10^7$                |
| Fish                | 1                | $10^7$ cfu/10 individuals  | $10^5$                |
|                     | 15 to 30         | $10^3$                     | $10^2$ - $10^1$       |

## RESULTS AND DISCUSSION

In our research, in the process of migration of *Yersinia pseudotuberculosis* along food chains, changes in its number at different trophic levels were found (Table 1). In the infusoria culture medium, the yersinia concentration was  $10^9$ /ml during the primary infection. Infusoria phagocytosed a significant number of bacteria from the environment: after two hours, intense phagocytosis was determined, with each tetrachimene unit containing from several to 30-40 digestive vacuoles filled with yersinia. In the first hours after infection, the concentration of bacteria in the infusoria was  $10^5$  cfu/biomass and remained unchanged during the day.

In daphnia, which consumed infected infusoria, in the first 3-5 days, a decrease in the concentration of *Yersinia pseudotuberculosis* from  $10^5$  to  $10^3$  cfu/10 individuals was observed, after which the reproduction of yersinia in daphnia dominated in relation to their digestion, so an increase in numbers was observed up to 11 days ( $10^7$  cfu/10 individuals). Fish that received daphnia as food with the maximum number of yersinia ( $10^7$  cfu/10 individuals), kept them for 30 days (observation period), but the concentration of the pathogen in the fish intestine was continuously decreasing: initial -  $10^7$ , after 24 hours -  $10^5$ , after 15-30 days -  $10^2$ - $10^1$  cfu/units (Table 1).

In the links of the trophic chain "yersinia - infusoria - cyclops - fish" a slow decrease in the concentration of *Yersinia pseudotuberculosis* was observed - in the consumption of infected infusoria by cyclops and infected cyclops by fish. However, as with the consumption of daphnia, the fish retained the pathogen in the intestine for 30 days (observation, monitoring period) at the level of  $10^2$ - $10^1$  cfu/individual.

The diet of fish with chironomid larvae previously infected with pseudotuberculous microbes ( $10^6$  cfu/individuals) also led to infection of fish: the concentration of bacteria in the intestines of aquarium fish stabilized at  $10^4$  cfu/individuals for 15 days, and then to 30-th day decreased to  $10^1$  cfu/individuals.

Therefore, the first attempts to experimentally reproduce the migration of yersinia along the trophic chains of the aquatic ecosystem, with the participation of zooplankton (lower crustaceans), benthos (chironomids) and nekton (fish) showed that pseudotuberculous microbe, along with using various transmissions across different food chains of the community from lower trophic levels to higher ones, with natural trophic networks being complex (Figure 1).

It is obvious that yersinia reach the organism of freshwater and transient fish (even those used for human consumption), from where pseudotuberculous microbial cultures have been isolated many times (Shubin, 1993).

According to study of Santos-Montanez et al. (2015) *Y. pseudotuberculosis* is able to resist the bacterivorous nature of free-living amoeba *Acanthamoeba castellanii* and have an enhanced ability to replicate and persist in coculture with amoeba.

In these experiments, we tried to give indicative numerical estimates of the process of yersinia migration in the community. The ability of pathogenic microorganisms to exist in water and soil and to reach humans through the food chain, indicates that research on the circulation of pathogens in the environment is of great importance for human health.

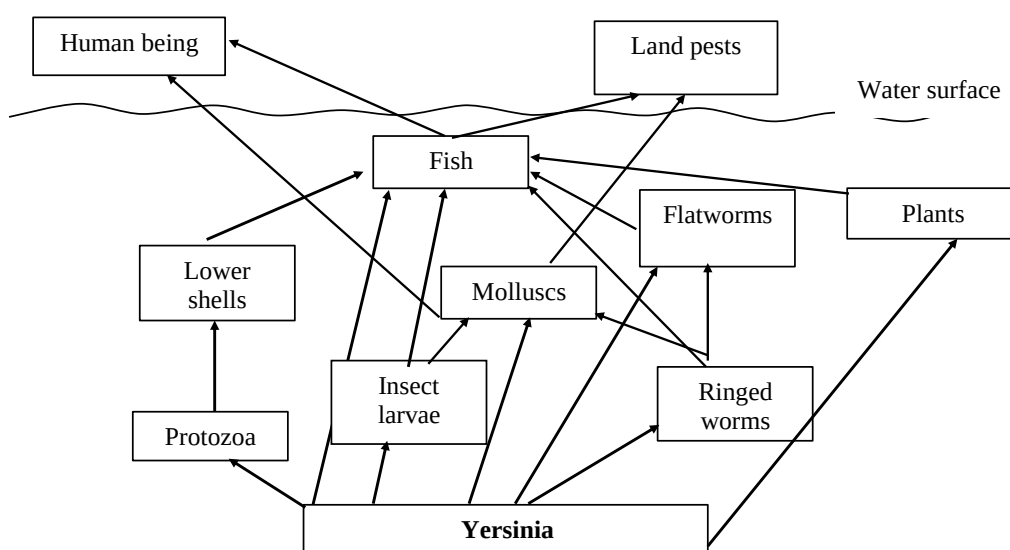


Figure 1. *Y. pseudotuberculosis* migration pathways by trophic chains  
 — experimentally determined pathways  
 — other possible pathways

## CONCLUSION

The patterns and mechanisms of circulation of infectious agents in terrestrial and aquatic ecosystems are just beginning to be studied, because the attention of researchers has traditionally been focused on aboveground ecosystems - natural focus zoonotic infections. Now, the specificity of the natural focus of sapronoses is becoming more and more important, above all - the ecology of the challengers as full members of land and water communities. A wide

perspective of field and experimental research in this relatively new direction of research on the problem of natural focus of infectious diseases opens up. The ability of pathogenic microorganisms to exist in water and soil and to reach humans through the food chain, indicates that research on the circulation of pathogens in the environment is of great importance for human health.

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## MONITORING OF THE ECOLOGICAL CONDITION OF THE ENVIRONMENT

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**Abstract:** *The paper presents the basic tasks and methods of biological monitoring of the ecological condition of the environment, which include bioindication and biotesting. Possibilities of application of living organisms in bioindication research are considered, especially indicator species, which due to their genetic, physiological, anatomical and other characteristics are able to survive in a narrow interval of a certain factor, indicating the presence of that factor in the environment.*

**Key words:** *ecology, monitoring, organism, testing.*

## INTRODUCTION

Ecological quality of the environment means an integral characteristic of the natural environment, which enables the preservation of health and a comfortable human life.

The natural environment in which we live has been formed over hundreds of millions of years. The modern shape of the Earth and the composition of the basic environments of organisms - soil, air, water - are created and maintained thanks to the life activity and interaction of an inconceivable multitude of living beings (Jemcev, Đukić, 2000; Đukić, Đorđević, 2004). It is artificially impossible to create a quality (full-fledged) environment for a human being. Only in the biotope can the quality of the environment be maintained and regulated - parameters that are necessary for life (temperature, humidity, salt composition,

ratio of gases in the atmosphere, climate). Today, science knows at least  $7 \cdot 10^6$  biological species. Scientists believe that this number is only a part of the real diversity of life on Earth.

Since man has adapted<sup>37</sup> and can comfortably survive (live) only in the modern biological environment, in natural ecosystems, the term "ecological quality of the environment" means maintaining ecological balance in nature (relative stability of species composition in the ecosystem and environmental composition), which ensures human health.

It is necessary to distinguish between goals and ways of standardizing and assessing the quality of the human environment based on basic physical and chemical parameters, on the one hand, and ecological forecasts of future changes in ecosystems and human health under anthropogenic stress - on the other.

## ENVIRONMENTAL ASSESSMENT

For the overall assessment of the state of the environment and determining the level of participation of certain sources in its pollution, sanitary-hygienic and toxicological norms are applied (maximum permissible concentrations - MAC - pollutants, maximum permissible levels of impact – MPI. However, in order to predict the results of the impact of anthropogenic factors on both ecosystems and human health, it is necessary to keep in mind many other indicators that characterize the reaction of certain organisms and the whole ecosystem to man-made impact (Đukić i sar., 2008, 2012, 2013, 2015, 2018; Đukić, Mandić, 2016).

Reactions of living systems to various chemical and physical factors and their combination are characterized by such features as the integrity and cumulative nature of many influences, paradoxical effects of low doses on animals and plants, the existence of chain processes and certain consequences of local influences on different "floors" complexly organized ecosystems. It is difficult to predict the reactions of human organisms living in conditions of technogenic artificial ecosystems.

Today, it is already common that one of the obligatory conditions for "stable" socio-economic development is the preservation of the natural environment of man and its restoration after destructive influences (Đukić i sar., 2013, 2015).

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<sup>37</sup> Adaptation - a set of morphophysiological, population and other characteristics of a given biological species, which ensures the possibility of a specific way of life in certain environmental conditions.

It should be noted that living systems (organisms, their communities and entire ecosystems) have the ability to self-regulate, autopurify and adapt. This is, in part, conditioned by the environmental forecast. Ecosystem stability, for example, depends on the diversity of species, on the interrelationships of the abundance of species, which represent different trophic levels, on the reproductive properties of organisms and the regulation of the abundance of each population with intergenous relationships in the community and abiotic factors.

Environmental hazard, or risk, should be assessed not only on the basis of the character and strength of the anthropogenic impact, but also on the basis of the biological properties of the reacting system. Accordingly, there are two groups of methods of ecological monitoring (monitoring the state of the ecosystem): physico-chemical and biological (biomonitoring). Each method of monitoring has its limitations. For the purpose of qualitative assessment and forecast of the state of the natural environment, it is necessary to combine them. Therefore, physico-chemical and biological monitoring do not exclude, but complement each other.

Anthropogenic pollution affects living organisms, including humans, in various combinations and in a very complex way. Their integral effect can be assessed only on the basis of the reaction of living organisms or entire communities. The prognosis of the impact of polluted water, chemical additives in food or polluted air on humans is valid, if the assessment of toxicity includes not only analytical methods, but also the biological diagnosis of the impact of the environment on animals. In addition, many xenobiotics (foreign substances of the biosphere) accumulate in the body. The result of long-term effects of even small concentrations of these substances are pathological changes in the body. Finally, the paradoxical effect of small doses of many biologically active compounds is known, when extremely low doses (less than the MAC) have a stronger effect on the body than their medium doses and concentrations (Mandić i sar., 2010).

The universal indicator of the change in the homeostasis<sup>38</sup> of the test organism is the state of stress at maturity from a "clean" to a "polluted" environment.

The term "stress" is used very differently in many fields of science. As a scientific term, *Selje* first introduced it to medicine in 1936, and it soon entered everyday language as a sign of non-specific mental tension. *Selje* (1979) defines stress as a reaction to the increased load, which is manifested by the syndrome, which consists of all non-specifically caused changes within the biological system.

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<sup>38</sup> Homeostasis - permanence of the internal environment of the organism; homeostatic mechanisms enable adaptation to the environment and preservation of the viability of the organism.

In biology, stress means the reaction of the biological system to extreme environmental factors (stressors), which, depending on the strength, intensity, moment and duration of the impact, can more or less strongly affect the system.

Stress can be divided into two types, which act differently. Eustress is characterized by physiological adaptive reactions, which are caused by bioenergetic processes in the body, when in critical situations the living being must adapt to changed environmental conditions. Distress means pathogenic processes, which usually occur in the case of constant loads or efforts, which the organism is not able to regulate in a short or long period of time. The extent to which a stressor will cause eustress or distress depends on a number of factors, for example, a combination of exogenous stimuli and the internal state of the organism.

The reactivity of an organism to active stressors depends primarily on its genetic constitution. The time factor also plays an important role in the development of stress, which is related to the development of sensitivity to stress, as well as to the duration of the impact of any effective stressor during different periods of life.

The danger of anthropogenic stressors is that biological systems - whether organisms, populations or biocenoses - are insufficiently adapted to them. Anthropogenic stressors are created at such a rate that appropriate adaptation processes often fail to be activated in living systems. Many anthropogenic environmental factors therefore become dangerous stressors. According to the size, intensity, duration and moment of impact, they differ from the "norm" that is usually present in nature, and to which biological systems have adapted. Finally, they often affect the range of tolerance<sup>39</sup>, which often leads to exceeding the allowable load on the organism and the breakdown of the biological system.

It is necessary to pay attention to the fact that in nature, not one stressor acts on the organism, but the whole complex of disruptive factors (complex stressful influence of the environment). In doing so, of course, any particular factor may occasionally or permanently dominate. In this regard, it is understandable that the reactions of organisms to stressors in a laboratory experiment do not always coincide with those in natural conditions. Therefore, the examination of the combined action of the environmental load, i.e. complex stressful influence of the environment, it is extremely important to determine the allowable load and stability of biological systems in the environment that is disturbed due to the action of many anthropogenic stressors.

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<sup>39</sup> Tolerance - tolerance, resilience. The ability of an organism to withstand the adverse effects of an environmental factor.

The stressful influence of the environment leads to the deviation of the basic parameters of the organism from the optimal level.

## **ENVIRONMENTAL HAZARD ASSESSMENT**

The assessment of the degree of ecological danger is traditionally done by determining some potentially harmful substances or influences in the environment and comparing the obtained results with the legally determined (for them) maximum allowed values. At the same time, this method of control has a number of significant shortcomings. Analog methods are, as a rule, demanding, not always fast, they require expensive and sometimes deficient devices and reagents, as well as highly qualified service personnel. Their main disadvantage is that these methods cannot guarantee a real assessment of environmental hazards, if a wide range of analyzed substances is not analyzed, because it is not the levels of pollution and impact that are important, but the biological effects they can cause and cannot provide information and about which even the most accurate chemical or physical analysis cannot provide information.

## **ECOLOGICAL AND SANITARY-HYGIENIC STANDARDIZATION**

Indicators of ecological and sanitary-hygienic standardization (maximum permissible concentrations - MPC, maximum permissible doses - MPD, maximum permissible levels - MPL), which are used in practice, and which are always based on toxicological tests with testing of special biological objects, cannot take into account changes in the toxicity of pollutants due to the effects of synergism or antagonism in the combined action of anthropogenic factors. These norms do not reflect the dependence of the toxic effect of pollution on physical environmental factors, do not take into account the processes of natural transformations of matter in the environment or their disappearance during the detoxification of the environment from specific pollutants. Therefore, along with physico-chemical methods, it is necessary to use methods of biological control and diagnostics - bioindication and biotesting, which provide objective integrated assessments of environmental quality and the basis for forecasting the state of ecosystems (Markert et al., 2003; Wolterbeek, 2002, Mandić i sar., 2021).

Biological methods of environmental quality control do not require prior identification of specific chemical compounds or physical influences. They are

quite easy to implement, many are express, cheap and allow for environmental quality control in a continuous mode. At the same time, after determining the total toxicity of soil or water samples, the application of analytical methods follows in order to determine its causes. Traditional physico-chemical methods also make it possible to assess the participation of certain companies or other sources of pollution in the integrated technogenic effect on nature.

An integrated assessment of the quality of the environment is proposed to: determine the state of bioresources, develop a strategy for rational use of the region, determine the maximum allowable burdens for the region's ecosystem, solve the fate of areas with intensive industrial and agricultural exploitation contaminated with radionuclides, etc.; detection of ecologically poor zones; resolving the issue of construction, commissioning or suspension of a certain company; evaluations of the effectiveness of measures for nature protection, introduction of treatment plants, modernization of production, etc.; applications of new chemicals and devices; raising recreational and protected areas (Đukić i sar., 2008, 2013).

Technical pressure, as a consequence of the scientific and technical revolution, has conditioned the problem of "balancing" the results of anthropogenic impact on the environment as one of the most important tasks of nature protection. Fulfilling this condition is the only way for humanity to survive.

## **ENVIRONMENTAL ASSESSMENT BASED ON BIOLOGICAL MONITORING**

Realization of the basic principles of stable development of civilization in modern conditions is possible only in the case of the existence of appropriate information on the state of the environment, in response to anthropogenic impact, which is obtained during biological monitoring. Environmental quality assessment is a key task of any measures in the field of ecology and rational use of nature. The very term "monitoring" (from the English word monitoring - control) means taking measures of continuous observation (monitoring), measuring and assessing the state of the environment. A complex approach to biological monitoring (combination of bioindication and biotesting methods, use of facilities at different levels of the organization), systematic monitoring, allows to judge the prospects of changing community structure, population productivity and ecosystem stability in relation to anthropogenic factors.

The objects of monitoring are biological systems and the factors that affect them. Simultaneous registration of anthropogenic impact on the ecosystem and biological response to the impact based on the totality of all indicators of living systems is desirable (Holt, Miller, 2010; Najera et al., 2002). Multifactor analysis is necessary, with the inclusion of the most typical anthropogenic influences (for example, chemical substances), as well as changes in natural environmental factors, the level of which changes due to anthropogenic influence. First of all, the biodiversity of species and the composition of coenosis species are taken into account. It is also important to record possible changes in natural populations, for example, disruption of embryonic development and symmetry of adult individuals within the population. It is necessary to discover the rapid "response" of organisms or populations and the results of lasting consequences, because part of the changes can be brought to order by the biosystem.

Examples of the application of bioindication and biotesting methods in the practice of ecological expertise of natural water basins and sources for drinking water supply show that the threshold concentrations of chemical pollutants, which disrupt the vital activity of biotestorganisms, are below accepted MAC values. The constant presence of pollutants, even in low concentrations, leads to a reduction in the diversity of hydrobiont species due to the extinction of species that are most sensitive to water quality. Such changes in biocenoses are determined by bioindication methods - by determining the index and indicators of saprobicity (Mohapatra, Mohamtry, 1992; Đukić, Ristanović, 2005).

A parallel study of health indicators of large groups of people living in polluted areas and using polluted water and agricultural products, reliably shows a reduction (compared to the regional average) in life expectancy, increased overall and premature mortality, and human morbidity, weakening of the immune system, liver and other organs.

The basic principle of biological monitoring is to determine the optimal - control - level, and any deviations from it indicate a stressful impact. Usually, in the case of estimating the optimum of some of the parameters, the question arises as to whether the given conditions will be optimal for other properties of the organism. However, if the examined parameters characterize the basic properties of the organism as a whole, then it is shown that their optimal level is similar. For example, completely different and, so to speak, completely independent parameters, such as asymmetry of morphological properties, blood parameters, oxygen consumption intensity, growth dynamics and frequency of chromosomal aberrations, can change synchronously when the most general baseline changes under a certain stress, feature of the organism - homeostasis of development.

## CONCLUSION

Although the approaches to environmental monitoring are very close, according to the ultimate goal of the research, it should be noted that biotesting is performed at the level of molecules, cells or organisms and indicates possible consequences of environmental pollution for the biotope, and bioindication - at the level of organism, population and community and, as a rule, indicates the result of pollution.

Environmental quality control, with the use of biological facilities, in the last few decades, has been profiled as a scientific and professional field, but which is deficient in terms of teaching literature.

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## PROTECTION OF BIOLOGICAL RESOURCES - LEADING CHALLENGE IN ENVIRONMENTAL PROTECTION

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**Abstract:** *The term biodiversity includes the overall diversity of living organisms within the biosphere. In order for natural processes in ecosystems to take place, it is necessary to preserve all components of biodiversity. The cycles of the circulation of matter and the flow of energy are the basis for the maintenance of life on the planet, and take place in ecosystems thanks to the activity of plants, animals and microorganisms. In addition to enabling the maintenance of all life on the planet, biological resources, that is, living organisms, have an exceptional practical value for humans. Therefore, the protection and conservation of biological resources should be included in the implementation of activities related to environmental protection. In this paper, the importance of preserving the diversity of biological resources, as a group of the most important natural resources on the planet, is considered.*

**Keywords:** *biodiversity, biological resources, plants, animals, microorganisms.*

## 1. INTRODUCTION

The term biodiversity encompasses the overall diversity of living organisms within the biosphere. Given that each biological species has a unique role in the life cycle on the planet, it is necessary to preserve biodiversity, ie ecosystem, genetic and species diversity as the main components (Stevanović and Vasić, 1995; Popović, 2015; Šarčević-Todosijević et al., 2018a; Šarčević-Todosijević and Popović, 2019).

Without biodiversity there are no biogeochemical cycles and oxygen production, no ecosystem functioning, no photosynthesis, no decomposition of organic matter. Preserved biodiversity contributes to climate regulation, reduces the effect of greenhouse gases, maintains air and water quality (Popović, 2015; Šarčević-Todosijević et al., 2018a). In order for natural processes in ecosystems to take place, it is necessary to preserve the integrity of all components of the living community, that is, phyto, zoo- and microbiocenosis.

Plants, as the primary producers of organic matter, form the basis of the food chains of all ecosystems on the planet, but then animal organisms are also included in the food chain, so the importance of zoocenoses in the functioning of ecosystems is indisputable. Further stages of the cycle of matter circulation and energy flow take place in ecosystems thanks to soil microorganisms, which play a primary role in these processes. Through the enzymes they release into the environment, they are involved in almost all biochemical reactions of the soil related to cycles of C, N, P, S and microelements, in which the formation and degradation of soil organic matter occurs, and nutrients become available to plants again. Decomposition of organic compounds of carbon and nitrogen, dissolution of phosphates, oxidation of  $H_2S$ , nitrification, denitrification and nitrogen fixation, transformation of microelements and heavy metals, are just some of the processes that continuously occur in the soil by metabolic activity of microorganisms and enzymes that release (Jemcević and Đukić, 2000; Stevanović and Janković, 2001; Đukić and Đorđević, 2004; Šarčević, 2011; Šarčević-Todosijević et al., 2020). Therefore, microorganisms and other heterotrophic organisms, through their metabolic activity, enable the decomposition of organic compounds of carbon and nitrogen and make them re-available to plants for organic synthesis, which closes the life cycle on the planet. In addition to enabling the maintenance of life on the planet, biological resources, that is, living organisms, have exceptional practical value for humans. In food production at the planetary level, the primary role belongs to plant production, and flowering plants (Magnoliophyta) are of the greatest importance. Flowering plants (Magnoliophyta) include species that are intensively used in pharmacy, medicine

and other areas of human activity (Jančić, 2004; Šarčević-Todosijević et al., 2018a). Providing food for over seven billion people on the planet can only be achieved through agricultural production based on scientific principles. The beginning of the development of agriculture as a human activity, within which living organisms are used to obtain appropriate products, can be considered the beginning of biotechnology. Principles of biotechnology, such as breeding of plants and domestic animals, application of microorganisms and other organisms in the production of food and biologically active substances, genetic engineering, today have enabled the remarkable development of various fields of agriculture, forestry, molecular biology, pharmacy and medicine (Arnold, 2005; Popović, 2010; Popović, 2015; Thieman and Palladino, 2008). Modern development of biotechnology has also opened the possibility of applying biological systems, ie living organisms, in monitoring and bioindication, pollution prevention and bioremediation of polluted ecosystems and the environment (Đukić et al., 2013). Therefore, it is indisputable that the protection and conservation of biological resources should be included in the implementation of activities related to environmental protection. In this paper, the importance of preserving the diversity of biological resources, as a group of the most important natural resources on the planet, is considered.

## **2. SIGNIFICANCE, APPLICATION AND PROTECTION OF BIOLOGICAL RESOURCES**

Plant genetic resources, as biological resources, represent the entire plant material in the world and are of exceptional value to humans (Popović, 2015). Man first used plants that originated from wild flora, and then about 10,000 years ago he began to grow plants. During the history of agriculture, people have cultivated about 7,000 to 10,000 different plant species, and cultivated plants make up only 3-4% of the described plant species (Prodanović et al., 2015). Today, there are about 1300 registered institutions in the world that collect genetic resources, while the state of agrobiodiversity is indicated by the number of newly created varieties and local populations. On the national variety list, there are about 5000 varieties and about 200 plant species, which are grouped into the following groups: small grains and corn, industrial plants, fodder plants, vegetables and fruits and vines, aromatic and medicinal plants (Popović, 2015; Šarčević-Todosijević et al., 2018a). Živanović and Popović (2016) point out the data of the Statistical Office of the Republic of Serbia (2014) that in 2013, field and vegetable production in the Republic of Serbia took place on an area of 3.3 million hectares. The areas under wheat covered 563 000 hectares, and about 2.69 million tons

were produced. Corn, as the most important agricultural product, is sown on an area of about 1.19 million hectares, with an average production of about 5.8 million tons, while industrial plants were grown on an area of about 350 thousand hectares. Površine pod žitima zauzimale su 1,9 miliona hektara. The Republic of Serbia is among the largest producers of oilseeds in Europe, with the production of sunflowers, soybeans and oilseed rape dominating. Successful plant production of soybeans is achieved by choosing high-yielding varieties and appropriate production technology. In the Institute of Field and Vegetable Crops in Novi Sad, a total of 1500 varieties were created and registered, and over 150 NS varieties of soybeans. More than half of the varieties are recognized abroad, and a significant number are on the EU list. Soybean produced in the Republic of Serbia are not genetically modified, which ensures the security of exports to the world market (Živanović and Popović, 2016).

Biological diversity can best be recognized by studying the division Magnoliophyta (flowering plants), one of the most numerous groups within the plant kingdom. Flowering plants include the species on which the diet of all mankind is based, and they represent a significant resource of compounds with healing properties, which is why they are intensively used in pharmacy and medicine (Šarčević-Todosijević et al., 2018a). Numerous scientific studies indicate that the area of the Balkan (Sabovljević, 2003; Radojković et al., 2017; Huseinović et al., 2017; Šarčević-Todosijević et al., 2019; Popović et al., 2019) is extremely rich in medicinal herbs. Šarčević-Todosijević et al. (2018a), at the locality Košutnjak, collected and determined 32 plant species within 24 families of flowering plants: Rosaceae, Brassicaceae, Tiliaceae, Primulaceae, Salicaceae, Aristolochiaceae, Violaceae, Liliaceae, Poaceae, Asteraceae, Geraniaceae, Apiaceae, Araliaceae, Hippocastanaceae, Cornaceae, Fabaceae, Fagaceae, Corylaceae, Betulaceae, Papaveraceae, Ranunculaceae, Sambucaceae, Plantaginaceae, Urticaceae. Determined plant species belonged to the mesophytes, the largest ecological group of terrestrial plants, which grow in temperate climates. Based on the conducted research, Šarčević-Todosijević et al. (2018a) conclude that a significant number of plant species with medicinal properties are present at the Košutnjak locality. The range of therapeutic effects of collected plant species is very wide, including antibacterial, antiviral, anti-inflammatory, antioxidant, anticancer, cytotoxic, antispasmodic, diuretic, sedative and many other effects, based on which the collected plant species are used in traditional and official phytotherapy (Šarčević-Todosijević et al., 2018a). to jest They possess exceptional value as plant genetic resources. Numerous plant species are of great importance in medicine. *Urtica dioica* is a plant whose effectiveness in the treatment of diabetes mellitus has been confirmed by numerous scientific studies (Figure 1).



Figure 1. *Urtica dioica*

(source:

[https://www.sorianatural.com/  
plants/green-nettle-or-stinging-  
nettle-](https://www.sorianatural.com/plants/green-nettle-or-stinging-nettle-)

[i-urtica-dioica-i-l\)](https://www.sorianatural.com/plants/green-nettle-or-stinging-nettle-i-urtica-dioica-i-l)



Figure 2. *Fagopyrum esculentum*

From the same aspect, it is important to note that the Balkan is a natural habitat of plant species, which are traditionally used in the treatment of diabetes mellitus, and whose effectiveness has been confirmed by numerous scientific studies: *Fagopyrum esculentum*, *Linum usitatissimum*, *Vaccinium myrtillus*, *Urtica dioica*, *Taraxacum officinale*, *Allium ampeloprasum* etc. (Matvejev, 1973; Dročić et al., 2020).

Šarčević-Todosijević and Popović (2019) point out that biological diversity can be best known by studying Insect, one of the oldest classes of the animal kingdom, which belongs to the phylum Arthropoda and includes nearly a million species, which have diverse morphology and way of life. Insects are the most numerous and successful group of Arthropoda on land, and since they process huge amounts of plant matter into their own proteins, which are then used through food chains by a number of consumers, they play a significant role in the biosphere. Ephemeroptera represents an ancient order of the class Insecta. The scientific name of Ephemeroptera is due to the short (ephemeral) life of the adults. After swarming and laying eggs, adult individuals fall to the water surface, where they

float like flowers (Janković et al., 1973; Krunić, 1994; Brittain and Sartori, 2003). They are extremely sensitive to changes in the quality of the environment, which occur due to pollution, so they have a significant role in bioindication. According to the key for determining saprobic zones by the percentage share of the most important groups of macrozoobenthos, waters in which the share of Ephemeroptera is higher than the share of larvae of other groups of insects, belong to the oligosaprobic zone, with predominant oxidative processes. The decrease in the number of Ephemeroptera, indicates the transition to the mesosaprobic zone, which is dominated by reduction processes, extremely unfavorable for a given ecosystem and all its cenobionts (Natchev, 1985; Cvijan, 2000).

Table 1. Overview and number of Ephemeroptera and other taxonomic groups in the studied localities of the river Ukrina in the municipality of Derventa (Republika Srpska, BiH) (Šarčević-Todosijević and Popović, 2019)

| Taxonomic groups               | Localities |      |      |     |                                       |
|--------------------------------|------------|------|------|-----|---------------------------------------|
|                                | L1         | L2   | L3   | L4  | Average number (ind./m <sup>2</sup> ) |
| <b>Mollusca</b>                | 7          | 1    | 14   | 2   | <b>6</b>                              |
| <b>Ephemeroptera</b>           | 578        | 1109 | 3254 | 821 | <b>1440</b>                           |
| <b>a) Subordo Schistonota</b>  |            |      |      |     |                                       |
| <b>Familia Baetidae</b>        |            |      |      |     |                                       |
| <i>Baetis</i> sp.              | +          |      | +    | +   |                                       |
| <b>Familia Ephemeridae</b>     |            |      |      |     |                                       |
| <i>Ephemera</i> sp.            | +          | +    | +    | +   |                                       |
| <b>Familia Heptageniidae</b>   |            |      |      |     |                                       |
| <i>Ecdyonurus</i> sp.          | +          | +    |      | +   |                                       |
| <i>Epeorus assimilis</i> Eaton | +          | +    | +    |     |                                       |

|                                       |     |     |     |     |            |
|---------------------------------------|-----|-----|-----|-----|------------|
| <i>Rhithrogena semicolorata</i> Curt. |     |     |     |     |            |
| <i>Heptagenia</i> sp.                 | +   | +   | +   |     |            |
| <b>Familia Leptophlebiidae</b>        |     |     |     |     |            |
| <i>Paraleptophlebia</i> sp.           | +   | +   | +   | +   |            |
| <b>Familia Oligoneuriidae</b>         |     |     |     |     |            |
| <i>Oligoneuriella rhenana</i> Imhoff. | +   | +   | +   | +   |            |
| <b>b) Subordo Pannota</b>             |     |     |     |     |            |
| <b>Familia Ephemerellidae</b>         |     |     |     |     |            |
| <i>Ephemerella ignita</i> Poda        | +   | +   |     | +   |            |
| <b>Familia Caenidae</b>               |     |     |     |     |            |
| <i>Caenis macrura</i> Stephens        | +   | +   | +   |     |            |
| <i>Caenis luctuosa</i> Burmeister     | +   | +   |     | +   |            |
| <b>Plecoptera</b>                     | 34  | 82  | /   | 27  | <b>47</b>  |
| <b>Coleoptera</b>                     | 199 | 205 | 31  | /   | <b>145</b> |
| <b>Diptera</b>                        | 111 | 83  | 79  | 9   | <b>70</b>  |
| <b>Trichoptera</b>                    | 223 | 402 | 301 | 127 | <b>263</b> |

Šarčević-Todosijević and Popović (2019) studied the diversity and possibilities of protection of the Ephemeroptera of the river Ukrina, in the part of the flow that passes through the territory of the municipality of Derventa (Republika Srpska, Bosnia and Herzegovina). They determined a significant taxonomic diversity of the order Ephemeroptera, as well as the number of individuals/m<sup>2</sup>. In the examined localities, Ephemeroptera dominate with an average number of 1440 individuals/m<sup>2</sup>. In addition to Ephemeroptera, in a significantly smaller percentage, Mollusca, Plecoptera, Coleoptera, Diptera and Trichoptera are present. Mollusca with 6 individuals/m<sup>2</sup> has the lowest average number at the



examined localities, followed by Plecoptera with 47 individuals/m<sup>2</sup>, Diptera with 70 individuals/m<sup>2</sup>, Coleoptera with 145 individuals/m<sup>2</sup> and Trichoptera with 263 individuals/m<sup>2</sup> (Table 1). In the territory of the municipality of Derventa, Ephemeroptera significantly contribute to the biological diversity of the ecosystem of the river Ukrina. In food chains, in river ecosystems, larvae are of great importance because they represent food for cenobionts (especially fish). The Ukrina River is characterized by an rich ichthyofauna (Table 2). Fish species from the family Cyprinidae are the most numerous in the river. Most species feed on bottom organisms, most often eggs and larvae of aquatic insects, including representatives of the order Ephemeroptera.

Table 2. Overview of fish species in the river Ukrina (Šarčević-Todosijević and Popović, 2019)

| Redni broj | Vrste                           |
|------------|---------------------------------|
| 1.         | <i>Acipenser ruthenus</i>       |
| 2.         | <i>Ctenopharyngodon idella</i>  |
| 3.         | <i>Acerina cernua</i>           |
| 4.         | <i>Acerina schraetser</i>       |
| 5.         | <i>Rutilus rutilus</i>          |
| 6.         | <i>Noemacheilus barbatulus</i>  |
| 7.         | <i>Chalcalburnus chalcoides</i> |
| 8.         | <i>Misgurnus fossilis</i>       |
| 9.         | <i>Leuciscus cephalus</i>       |
| 10.        | <i>Abramis brama</i>            |
| 11.        | <i>Tinca tinca</i>              |
| 12.        | <i>Cottus gobio</i>             |
| 13.        | <i>Barbus meridionalis</i>      |

In addition to all the above roles, which they have in the ecosystem, the appearance of Ephemeroptera in the summer period of the year is a true natural rarity. However, in order to protect this group of insects, it is necessary to take into account measures to prevent pollution and habitat destruction (Šarčević-Todosijević and Popović, 2019; Šarčević-Todosijević et al., 2021).

Similarly, Šarčević-Todosijević et al. (2017; 2018b) examined the aquatic river invertebrate community, which is necessary for biological and ecological assessment of river water quality. At five localities of the Banjska river (in the area of Vranjska Banja), macrozoobenthos samples were taken seasonally for biological and ecological analysis. The aim of the study was to assess the saprobity index, biological indicator of water status and intensity of organic matter decomposition, ie the level of organic load of the river, based on the community of aquatic invertebrates as bioindicator organisms. The value of the saprobic index in all localities and in all seasons was within the limits of  $\beta$ -mesosaprobic waters or waters (II) class, which corresponds to moderate organic load. The inflow of wastewater significantly affects the composition of the macroinvertebrate community, based on which it is concluded that it is necessary to reduce the load of the river with organic matter and nutrients (Šarčević-Todosijević et al., 2017; 2018b).

In addition to plants and animals, there is no doubt that, given the role they play in ecosystems, microorganisms are their most important component and biological resource that needs to be protected. Different groups of microorganisms live in the soil, whose abundance, microbiological biomass and enzymatic activity are the most important indicators of soil fertility (Jarak et al., 2005; Đukić et al., 2007). Microorganisms involved in the cycle of matter in the soil, mainly include aerobic, anaerobic and facultatively anaerobic bacteria and fungi (Simić, 1989; Đukić and Đorđević, 2004; Đukić et al., 2007).

Determining the number of actinomycetes can serve as an indicator of soil fertility. Phylogenetically, actinomycetes belong to the group of Gram-positive bacteria. Most organisms in this group have common properties; they are rod-shaped, aerobic organotrophs and mostly immobile in the vegetative phase (Madigan et al., 1997). These are soil microorganisms, adapted to grow in relatively dry conditions, with pronounced hydrolysis activity against various polymers, and especially chitinolytic activity on fungal substrate (Đukić and Đorđević, 2004).

In addition to the important role they play in the biogeochemical cycles in the biosphere (Odum, 1972), actinomycetes are the object of intensive research in biotechnology due to the ability to synthesize antibiotics. This ability undoubtedly provides this group of organisms greater competitiveness in nature (Đukić and Đorđević, 2004). Šarčević-Todosijević et al. (2020) studied the influence of ecological factors and nitrogen fertilizers on the number of actinomycetes of chernozem soil, in the climatic conditions of eastern Srem, in different physiological phases of maize development. It was determined that the phenophase of the plant significantly influenced the number of actinomycetes during the research period. The number of actinomycetes was more pronounced

in the phenophase of plant maturation compared to the phenophase of flowering. Although actinomycetes belong to the group of drought-resistant microorganisms, higher precipitation and the availability of water in the soil in the phenophase of plant maturation have conditioned the proliferation of actinomycetes. The number of actinomycetes ranged from  $4.0\text{--}21.0 \times 10^4 \text{g}^{-1}$ . The number of actinomycetes was significantly influenced by the applied fertilizers and mainly increased the number of this group of microorganisms. A similar trend was observed in the phenophase of flowering and in the phenophase of plant maturation. In the phenophase of maize flowering, the highest number of actinomycetes was observed in the variant in which was applied  $\text{N}_{120} \text{ kg ha}^{-1}$ , both on "ugar" ( $14.6 \times 10^4 \text{g}^{-1}$ ) and under plant crops ( $13.5 \times 10^4 \text{g}^{-1}$ ). The amount of nitrogen of  $\text{N}_{180} \text{ kg ha}^{-1}$  significantly reduced the number of this group of microorganisms (Šarčević-Todosijević et al., 2020). This research also showed that soils under cultivated plants are very rich in microorganisms, that microorganisms play a key role in improving soil fertility, increasing crop yields, and that they are very sensitive to environmental factors, especially anthropogenic, in ecosystems.

Mineral fertilizers increase the biological productivity of agroecosystems and the yield of cultivated plants, but their increased use causes environmental pollution, eutrophication and groundwater pollution, accumulation of nitrates and heavy metals in soil and plants and the formation of carcinogenic nitrosamines. Therefore, the rational use of mineral fertilizers is a priority in agricultural production, with the aim of preventing environmental pollution (Jemcev and Đukić, 2000; Živanović et al., 2018; Kolarić et al., 2021).

There are a large number of microbial biopreparations, that is, microbiological fertilizers. Microbiological fertilizers contain live microorganisms, which, when introduced into the rhizosphere, increase the availability of nutrients and promote plant growth. These preparations do not contain chemical additives, but live microorganisms, which have a positive effect on both the soil and the plant; increase the availability of nutrients and promote plant growth, synthesize and decompose humus, ensure the circulation of nutrients, produce a variety of biologically active substances, which positively affect the yield of cultivated plants and control the number of phytopathogenic organisms. The microorganisms in the composition of these preparations are isolated from the ecosystem. They include different ecophysiological groups of microorganisms (Jemcev i Đukić, 2000; Mirecki et al., 2011). Heterotrophic bacteria break down organic compounds of nitrogen and phosphorus and translate them into a form accessible to plants. *Azotobacter* sp. belongs to the group of nitrogen-fixing bacteria. It is a group of microorganisms that absorb elemental nitrogen and reduce it to ammonia, while bacteria of the genera *Rhizobium* and

*Bradyrhizobium* are used to stimulate the formation of nodules and increase symbiotic nitrogen fixation in legumes. All these groups of bacteria in the composition of microbiological biopreparations, intensify the processes of matter circulation in agroecosystems, increase the biological productivity of agroecosystems and the yield of cultivated plants, and have no negative effects on the environment (Đukić et al., 2007).

There is no doubt that the increased and uncontrolled use of pesticides, causes environmental pollution, their accumulation in food chains, and the manifestation of toxic, mutagenic and carcinogenic effects on members of bionosis. Biopesticides are biological preparations for protection of plants from pests, plant pathogens and weeds, do not have a negative impact on the environment and are intensively used in organic production (Đukić et al., 2007). Modern plant protection technologies include the creation of unfavorable conditions for the development of plant diseases through the application of agrotechnical measures, the use of antagonistic organisms and their biological products, superparasites or predators, the introduction of competitive species individually or together, the use of less harmful chemical pesticides (Grgić, 2009). The use of biopreparations encourages mechanisms of environmentally safe protection of plants from pests. Unlike chemical pesticides, which are the group of the most important causes of environmental pollution, biopesticides act only on target organisms, phytophages, phytopathogens and weeds and, through natural processes of regulation, suppress their abundance (Đukić et al., 2007).

### 3. CONCLUSION

Cycles of matter and energy flow are the basis for maintaining life on the planet, and take place in ecosystems thanks to the activity of plants, as primary producers of organic matter, but also the activity of heterotrophic organisms, which decompose organic compounds and make them available to plants for organic synthesis. In addition to enabling the maintenance of all life on the planet through their activities, biological resources, that is, living organisms, have an exceptional practical value for humans. Plant species enable the nutrition of all mankind, but they are also intensively used in medicine, pharmacy and other areas of human activity. The principles of modern biotechnology have ensured the intensive development of agriculture, forestry, molecular biology, medicine and pharmacy, as well as the application of living organisms in the production of food, biologically active substances and environmental protection. Based on that, the leading goals in environmental protection should include the conservation of

natural resources, among which the most important place belongs to the plants, animals and microorganisms.

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