EFFECT OF ORGALIFE FERTILIZER ON SOME QUALITY PROPERTIES OF THE SEED AND GROWTH POTENTIAL OF TWO RICE VARIETIES (ORYZA SATIVA L.) CULTIVATED IN NORTH MACEDONIA

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ABSTRACT

The aim of this study was to examine the effect of Orgalife (liquid organic microbial fertilizer based on manure from californian earthworms) on the seeds of two rice varieties, *San Andrea* and *Opale*, obtained from three production years: 2018, 2019 and 2020. They were treated with three different concentrations (3.3%, 6.7% and 9.9%) compared to control (distilled water). Germination energy, total seed germination, germination index, seedlings growth (root length, shoot length, dry seedling weight) and vigor index (I and II) were examined. One-way analysis of variance (ANOVA) was carried out and separation of means was performed using the LSD test at P=0.05 and P=0.01 significance level. The values of all treatments for all parameters were compared with the control. Germination energy and total germination for *Opale* in 2020 showed statistical significance for the 3.3% concentration at P=0.05, while for *San Andrea* the same significance was noticed only for the germination energy. Germination index for *Opale* gave statistically significant results only for the 2020 production year at P=0.05. For *San Andrea* the situation is slightly different where the treatments affected the germination index only for 2018 and 2020. Orgalife treatments increased the vigor index I and II in 2020 for both varieties and for both levels of significance. The length of the seedlings for *Opale* in 2020 was positively affected by the 3.3% concentration in both levels of significance, while *San Andrea* in the same year had statistical significance for the 9.9% concentration only for P=0.05. The results for the dry matter showed statistical significance for S*an Andrea* for the 6.7% concentration in 2018. As an overall conclusion, we can say that the use of the Orgalife fertilizer showed to be reasonable for the seedling growth and development, better than the effect on the germination parameters.

Key words: organic fertilizer Orgalife, rice seed, growth promotion, germination energy, total germination and seedling growth

Introduction

One of the most extensively cultivated cereals of the world is the rice (*Oryza sativa*) which belongs to the family of *Gramineae* and the genus *Oryza*. The rice is possibly the oldest domesticated grain (10 000 years), and is the staple food for 2.5 billion people (IRRI, 2002). The production of rice depends on a lot of factors. One of the important factors for production of rice is the seed quality which is characterized by the good germination capacity. Quality seed ensures quality seedling (Hasan et al., 2016).

In field conditions, for the purpose of good germination and growth of young seedlings, the seeding material can be treated with materials that affect germination, such as nutrients and growth regulators (Dimitrovski et al., 2020). Direct use of microorganisms to promote plant growth and plant pests/disease control is becoming a rapidly expanding research area. The capacity of specific root-colonizing bacteria, or rhizobacteria, to increase growth and yield of crop plants is attracting great attention (Suslow et al., 1979).

One of the most important nutrients for plant growth is nitrogen and hence heavily applied in agricultural systems via fertilization. Approximately 50% of the N fertilizer applied to plant production is not absorbed by plants, but lost to the environment as ammonia (NH_3), nitrate (NO^{3-}), and nitrous oxide (N_2O, a greenhouse gas with 300 times the heat-trapping capacity of carbon dioxide), raising agricultural production costs and contributing to pollution and climate change. These losses are driven by volatilization of NH_3 and by a matrix of nitrification and denitrification reactions catalysed by soil microorganism. Hence, the crucial player in nitrification is ammonia-oxidizing archaea (Beckman et al. 2018).

Vermicompost is an organic waste rich in nutrients and plant growth-promoting rhizospheric bacteria such as *Pseudomonas, Rhizobium, Bacillus, Azosprillium, Azotobacter,* which is produced from earthworms that is beneficial in enhancing the soil condition (Pathma and Sakthivel, 2012). It has been reported to help in improving the crop yield and quality. Vermicompost can be used as a fertilizer to increase the fruit chemical quality and maintain the soil quality for agricultural sustainability (Mahmud et al.2020). Vermicomposts contain higher humic acid substances (Albanell et al. 1988), compared to the parent material that has been used for composting. Earthworm gut connected microbes supplement vermicomposts with highly water-soluble and light-sensitive plant growth hormones, which gets absorbed on humic acid substances in vermicompost making them extremely stable and helps them persist longer in soils whereby influencing plant growth (Atiyeh et al. 2002; Arancon et al. 2003).

Extracts of humic and humic-like substances positively affected seed germination and growth of seedlings from lettuce and tomato plants (Piccolo et al., 1993), safflower plants (Başalma, 2015), maize fodder (Daur & Bakhashwain, 2013) significantly promote cucumber growth (Xu et al., 2012).

In rice production, effects of liquid extract of microbiological fertilizers were also reported on stimulating growth, germination, productivity, and quality of wheat and rice seedlings. The extract especially influenced the growth of shoot and root system (Wang et al., 2020).

In this study was examined the effect of Orgalife fertilizer on germination and young seedling growth in rice. The fertilizer contains growth promoting microorganisms such as nitrogen-fixation bacteria, nitrification bacteria, aerobic cellulolytic microorganisms and ammonification bacteria. The seeds treated with Orgalife fertilizer tend to emerge faster, and have better rooting capacity, plant growth and development (based on product label information).

Materials and Methods

Materials

Orgalife is commercial organic microbial fertilizer, biostimulator and soil improver prepared from Californian red worms casting base (not to be confused with worm tea). Orgalife - a liquid organic microbial fertilizer, prepared from a solid fertilizer base (casting) of California worms was used during the experiment. This fertilizer is dark-brown colored with a pH of 6.83 containing 48.35% organic matter, of which 2.44% is total N and microorganisms such as nitrogen fixing bacteria - 86% and nitrification bacteria - 79% (based on data from the manufacturer's label). The effect of Orgalife was tested in three different concentrations (3.3%, 6.7% and 9.9%) compared to the control treatment where no fertilizer was added but only distilled water (Table 1). For the purposes of treatment, rice seeds of two varieties, *San Andrea* and *Opale*, produced from three consecutive harvests (2018, 2019 and 2020) were used. The seed from all three production years was kept under controlled conditions.

Table 1. Content of organic matter and nitrogen in the fertilizer and in the prepared solutions

Fertilizer concentration	Orgalife concentration	3.3%	6.7%	9.9%	Control
Organic matter (%)	48.35	1.595	3.239	4.786	0%
Total N content (%)	2.44	0.08	0.163	0.241	0%
Volume of solution per replication of 100 seeds	/	20 ml	20 ml	20 ml	20 ml H_2O

Content of the organic matter and nitrogen is based on product label information

Methods

The examination was performed in the Department of Rice in Kochani at the University "Ss. Cyril and Methodius ”- Skopje. Total seed germination (TG), germination energy (GE), germination index (GI) and seedlings growth (fresh and dry seedlings weight, root length and shoot length), vigor index (I and II) were examined. A total of 12 variants were set up; four replications of 100 grains for all three production years (2018, 2019, 2020) and for all four treatments (33.33 ml / 1L (3.3%), 66.66 ml / 1L (6.7%), 9.99 ml / 1L (9.9%) and control-distilled water). The seeds of the control treatments were soaked in distilled water for 24 hours before transferring them to Petri dishes, while the seeds of the three treatments were submerged with a solution of fertilizer in the three concentrations listed above. Each of the replicates was placed in Petri dishes in an interfilter (IF) germination medium and placed in a germination chamber at a temperature of 25 ° C.

The first counting of germinated grains was performed on the fifth day of immersion of the seeds according to the treatments. The second count was performed on the 14th day after the immersion. The seed was considered germinated when the coleoptile and the root appeared together and the root length was at least 2 mm. Seed germination energy (ER) was determined on the 5th day, and total seed germination (ER) on the

14th day after immersion.These parameters were calculated as percentage from the total number of inoculated seeds in the Petri dish (Official gazette of the Republic of Macedonia No 61/2007).

Germination energy is the ratio between the number of germinated seeds on the fifth day (first counting) and the total number of grains, multiplied by 100.

Percentage of total germination is calculated as the ratio between the number of germinated grains and the total number of seeds, multiplied by 100.

The germination index is calculated by the formula of AOSA (1983), which reads: **Germination index**

$$= \left[\frac{\text{Number of germinated seeds}}{\text{Day of the first count}}\right] + \ldots + \left[\frac{\text{Number of germinated seeds}}{\text{Day of the last count}}\right]$$

In order to estimate the vigor index I and II, seeds from each treatment were germinated in an open Petri dish in the germination cabinet along with the replications. The RL and SL of 40 randomly picked up seedlings in each of the variants developed in dark conditions were measured at the end of the experiment (day 14).

Vigor index I: VI–I = Seedling length (cm) × Germination (%)

Vigor index II: VI–II = Seedling dry weight (mg) × Germination (%)

The 40 seedlings in each variant (treatment-year combination) were dried in air-oven at 100°C to constant weight. The **total dry seedling weight** was measured for each variant.

The results were statistically analyzed by two-way ANOVA with replication (Table 2) and LSD test at 0.05 and 0.01 significance level. Correlation analysis was determined by calculating the Pearson's correlation coefficient. The two way ANOVA was calculated based on the statistical methods for agricultural research provided by Hadživuković (1973).

Results and discussion

The germination energy was slightly affected by the treatments compared to the control. Mean values for *Opale* variety were in a range of 93.92% to 95.00% compared to the control which is 92.67%. The treatment with 3.3% concentration in 2020 gave the best result (99.25%) compared to the control (96.50%) regarding the germination of the seed. In the other variety - *San Andrea*, the mean values for the germination energy were varying from 86.83% to 91.58% compared to the control (91.00%). Also for *San Andrea* positive effect showed only 3.3% treatment in 2020 with 97.50% compared to the control (94.25%). This kind of situation might be dependent on the seed age and the production conditions. According to Andov et al. (2012), the germination energy in three years old rice seed decreases rapidly.

Table 2. Germination energy for *Opale* and *San Andrea* variety

Germination energy (%)							
OPALE	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD**	
						(α 0.05)	**(α 0.01)**
2018	92.25±1.50	91.00±1.63	93.25±2.87	95.25±1.50	**92.94b**	4.66	7.06
2019	91.75±2.50	92.00±1.41	93.25±1.26	86.25±4.35	**90.81a**	5.85	8.86
2020	99.25±1.50	98.75±0.96	98.50±1.00	96.50±1.00	**98.25c**	2.29	3.47
AVG (%)	**94.42ab**	**93.92ab**	**95.00b**	**92.67a**			
SAN ANDREA	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD**	
						(α 0.05)	**(α 0.01)**
2018	82.50±3.11	79.75±3.30	75.00±70.39	85.50±4.04	**80.69a**	11.13	16.85
2019	94.75±1.71	88.75±5.91	89.00±1.41	93.25±3.40	**91.44b**	7.38	11.18
2020	97.50±2.08	97.00±1.41	96.50±1.29	94.25±0.50	**96.31c**	2.74	4.15
AVG (%)	**91.58b**	**88.50ab**	**86.83a**	**91.00b**			

Comparable to the results from the germination energy, also the total germination was driven in the same way for the *Opale*, while for *San Andrea* the control treatment had the highest mean value of 96.83% and the lowest one was the 9.9% concentration with mean value of 93.67%, which can be considered as a negative effect of the maximum concentration treatment on the seed germination.

The germination index for *Opale* showed statistically significant results only for the 2020 production year at P=0.05 and the highest mean value was 26.97 for 3.3% concentration, while for the control it was 26.28. *San Andrea* had a little different situation where the treatments affected the germination index only for 2018 and 2020. In 2018 the 9.9% concentration had the lowest mean value (22.97) compared to the control (23.81) which means it might have some negative effect on the germination. In 2020 the 3.3% concentration had the highest mean value (26.54) compared to the lowest value of the control (25.83).

Table 3. Total germination for *Opale* and *San Andrea* variety

Total germination (%)							
OPALE	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD**	
						(α 0.05)	**(α 0.01)**
2018	93.00±1.41	92.75±0.50	95.25±1.89	96.50±0.58	**94.38b**	2.47	3.75
2019	92.25±2.50	92.75±1.71	94.00±1.41	87.75±3.77	**91.69a**	5.50	8.33
2020	99.75±0.50	99.25±0.96	99.00±0.00	97.75±0.96	**98.94c**	1.42	2.16
AVG (%)	**95.00ab**	**94.92ab**	**96.08b**	**94.00a**			
SAN ANDREA	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD**	
						(α 0.05)	**(α 0.01)**
2018	90.50±1.73	88.25±0.96	87.50±4.51	94.00±1.41	**90.06a**	3.78	5.72
2019	97.50±1.29	97.25±1.26	96.50±3.11	98.75±0.50	**97.50b**	3.96	6.01
2020	98.50±1.00	98.25±1.50	97.00±1.41	97.75±0.96	**97.13b**	2.26	3.43
AVG (%)	**95.50bc**	**94.58ab**	**93.67a**	**96.83c**			

Table 4. Germination index for *Opale* and *San Andrea* variety

Germination index							
OPALE	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG**	**LSD**	
						(α 0.05)	**(α 0.01)**
2018	25.09±0.38	24.82±0.36	25.45±0.70	25.95±0.28	**25.33b**	1.08	1.63
2019	24.94±0.68	25.03±0.39	25.37±0.34	23.52±1.14	**24.71a**	1.55	2.35
2020	26.97±0.34	26.84±0.25	26.77±0.20	26.28±0.24	**26.72c**	0.53	0.80
AVG	**25.67ab**	**25.56ab**	**25.86b**	**25.13a**			
SAN ANDREA	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG**	**LSD**	
						(α 0.05)	**(α 0.01)**
2018	22.97±0.74	22.26±0.64	21.25±2.37	23.81±0.89	**22.57a**	2.42	3.67
2019	25.92±0.41	24.70±1.21	24.69±0.40	25.70±0.66	**25.25b**	1.55	2.35
2020	26.54±0.47	26.42±0.37	26.23±0.34	25.83±0.13	**26.25c**	0.66	1.00
AVG	**25.14b**	**24.46ab**	**24.06a**	**25.11b**			

The seedling vigor index I and II were affected by all Orgalife treatments for both varieties in 2020 for P=0.05 and P=0.01 levels of significance. VI-I for *Opale* showed the highest results for 3.3% concentration (2310) compared to the control (1629.74) which was the lowest value. VI-I for *San Andrea* showed the highest mean value for 9.9% concentration (1848.09) compared to the control (1556.98).

Table 5. Seedling vigor index I for *Oppale* and *San Andrea* variety

Seedling Vigor Index I							
OPALE	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD**	
						(α 0.05)	**(α 0.01)**
2018	2035.77±171.85	2121.80±89.79	1902.14±132.99	1966.68±192.85	**2006.60a**	324.29	491.28
2019	1862.77±158.27	1893.71±161.25	2150.72±80.30	1990.12±90.04	**1974.33a**	241.83	366.35
2020	2310.00±148.24	2048.31±67.64	2224.53±124.96	1629.74±65.20	**2053.15a**	184.84	280.02
AVG (%)	**2069.51b**	**2021.27ab**	**2092.46b**	**1862.18a**			
SAN ANDREA	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD**	
						(α 0.05)	**(α 0.01)**
2018	1502.07±115.48	1289.26±87.57	1576.97±47.94	1580.14±103.52	**1487.11a**	180.76	273.84
2019	1701.13±105.00	1649.72±57.24	1879.82±116.81	1923.14±67.40	**1788.45c**	208.21	315.42
2020	1697.89±61.29	1541.34±65.12	1848.09±44.25	1556.98±92.80	**1661.08b**	83.93	127.15
AVG (%)	**1633.70b**	**1493.44a**	**1768.29c**	**1686.75bc**			

The treatments with 6.7% and 9.9% concentrations gave the highest values for VI-II for *Opale* (19.80) compared to the control (19.60). For *San Andrea* the treatments with 3.3% concentration showed the highest results (24.63) compared to the control (24.45).

Table 6. Seedling vigor index II for *Opale* and *San Andrea* variety

Seedling Vigor Index II							
OPALE	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD**	
						(α 0.05)	**(α 0.01)**
2018	16.28±1.78	16.28±1.20	16.63±1.65	16.98±1.84	**16.54b**	3.72	5.63
2019	16.10±0.53	16.28±2.21	14.10±0.71	15.40±1.14	**15.47a**	3.10	4.70
2020	17.50±1.29	19.80±1.40	19.80±0.81	19.60±1.13	**19.18c**	2.60	3.94
AVG (%)	**16.63a**	**17.45a**	**16.84a**	**17.33a**			
SAN ANDREA	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD**	
						(α 0.05)	**(α 0.01**
2018	20.36±1.17	22.05±0.72	19.69±0.51	21.15±1.21	**20.81a**	1.80	2.72
2019	21.94±1.26	24.33±0.79	21.71±0.56	24.70±1.80	**23.17b**	2.25	3.41
2020	24.63±1.39	24.58±1.79	21.83±1.25	24.45±0.80	**23.87b**	2.80	4.23
AVG (%)	**22.31b**	**23.65c**	**20.90a**	**23.43c**			

The length of the 14 days old seedlings for *Opale* in 2020 was positively affected by the 9.9 % concentration (24.47 cm) in both levels of significance, as well as, *San Andrea* in the same year had statistical significance for the same concentration (18.85 cm) only for P=0.05. Similarly, when bean and pea seedlings were treated with vermicompost aqueous extracts showed positive effect on the growth and development of the seedlings (Ievinsh, G., 2011). In parallel, in a study evaluating the influence of humic acids derived from earthworm-processed organic wastes on plant growth (Atiyeh et al., 2002), the results indicated significantly increased growth of tomato and cucumber seedlings, in terms of plant heights, leaf areas, shoot and root dry weights. According to Atiyeh et al. (2002), increased plant growth happened with increasing the concentrations of humic acids incorporated into the growing medium to a certain proportion, but this differed according to the plant species, the source of the vermicompost, and the nature of the container medium.

Table 7. Seedling length for *Opale* and *San Andrea* variety

Seedling length (cm)							
OPALE	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD**	
						(α 0.05)	**(α 0.01)**
2018	22.82±0.97	20.02±1.40	20.02±1.40	20.28±1.99	**20.78a**	3.28	4.98
2019	20.25±1.72	20.36±1.73	22.88±0.85	22.62±1.02	**21.53a**	2.64	4.00
2020	24.44±1.48	20.69±0.68	22.47±1.26	16.63±0.67	**21.06a**	1.86	2.82
AVG (%)	**22.50c**	**22.36bc**	**21.79ab**	**19.84a**			
SAN ANDREA	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD**	
						(α 0.05)	**(α 0.01)**
2018	16.60±1.28	14.62±0.99	18.02±0.55	16.81±1.10	**16.51a**	1.99	3.01
2019	17.45±1.08	16.96±0.59	19.48±1.29	19.47±0.68	**18.34b**	2.14	3.25
2020	16.53±1.72	16.05±0.91	18.85±0.54	15.34±1.82	**16.69a**	2.44	3.69
AVG (%)	**16.86ab**	**15.88a**	**18.78c**	**17.21b**			

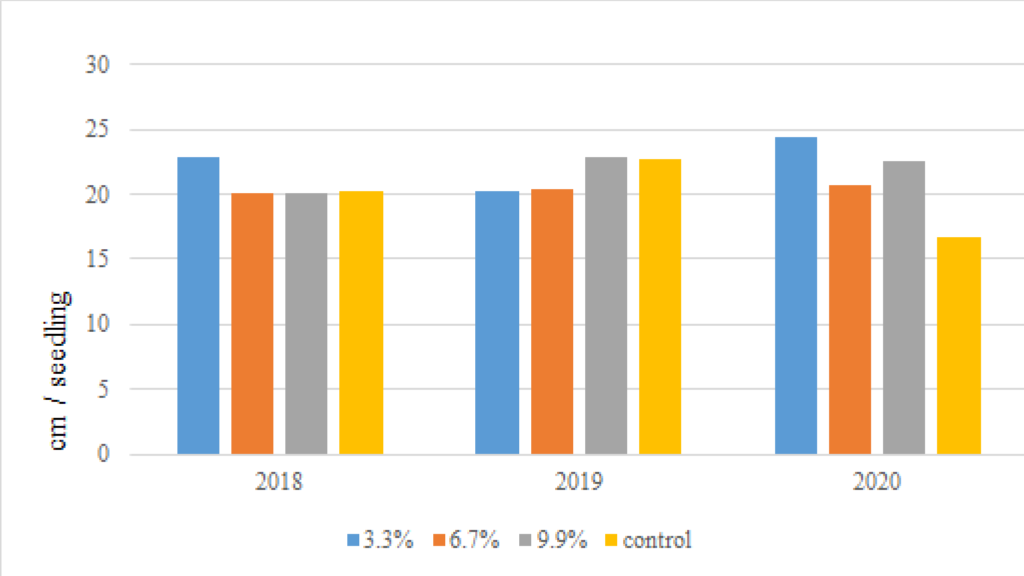

Figure 1. Seedling length for O*pale*

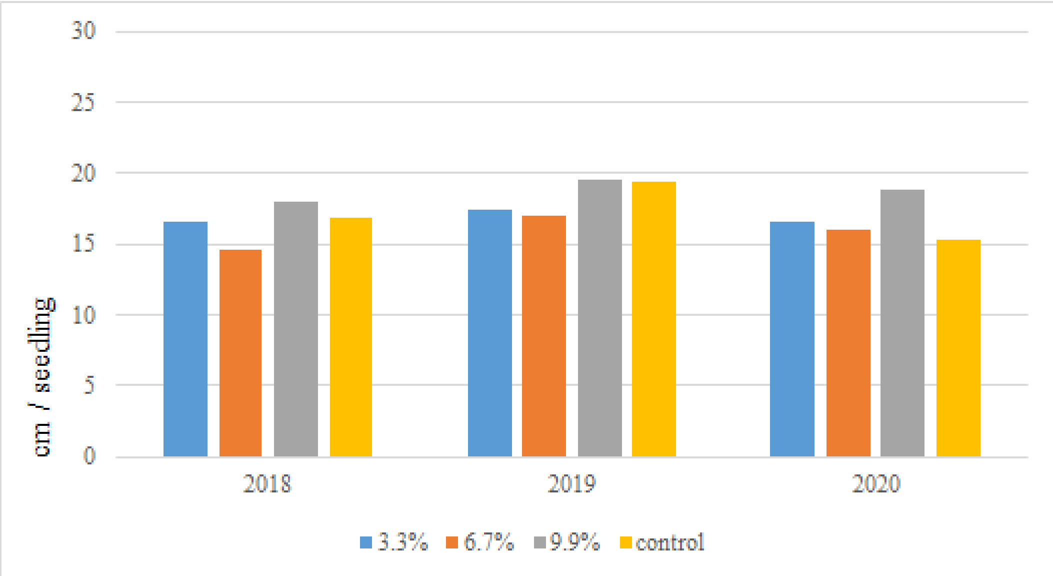

Figure 2. Seedling length for *San Andrea*

The statistical analysis for *Opale* variety did not show any significance among the treatments compared to the control in terms of dry matter. The analysis for dry matter showed statistical significance for *San Andrea* in 2018 for the 6.7% concentration (0.25 g) compared to the control (0.23 g).

Table 8. Dry matter for *Opale* and *San Andrea* variety

Dry matter (g)							
						LSD	
OPALE	**3.3%**	**6.7%**	**9.9%**	**Control**		**(α 0.05)**	**(α 0.01)**

					AVG (%)		
2018	0.18±0.02	0.18±0.01	0.18±0.02	0.18±0.02	**0.18a**	0.04	0.06
2019	0.18±0.01	0.18±0.02	0.15±0.01	0.18±0.01	**0.17a**	0.03	0.05
2020	0.18±0.01	0.20±0.01	0.20±0.01	0.20±0.01	**0.19b**	0.03	0.04
AVG (%)	**0.18a**	**0.19a**	**0.18a**	**0.19a**			
						LSD	
SAN ANDREA	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**(α 0.05)**	**(α 0.01)**
2018	0.23±0.01	0.25±0.01	0.23±0.01	0.23±0.01	**0.24a**	0.02	0.03
2019	0.23±0.01	0.25±0.01	0.23±0.01	0.25±0.02	**0.24ab**	0.02	0.03
2020	0.25±0.01	0.25±0.02	0.23±0.01	0.25±0.01	**0.25b**	0.03	0.04
AVG (%)	**0.24ab**	**0.25c**	**0.23a**	**0.24bc**			

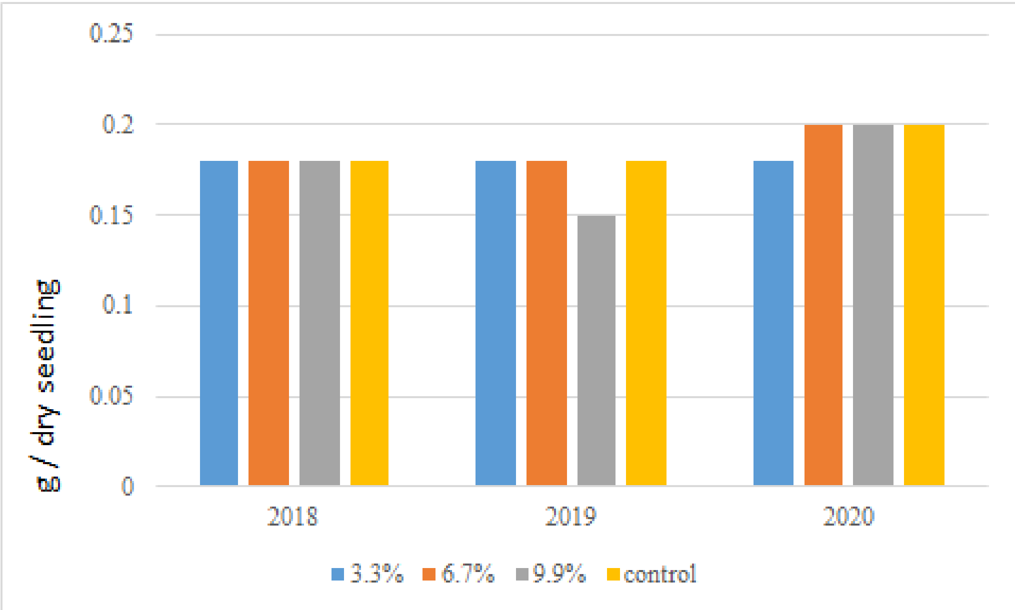

Figure 3. Dry matter for *Opale*

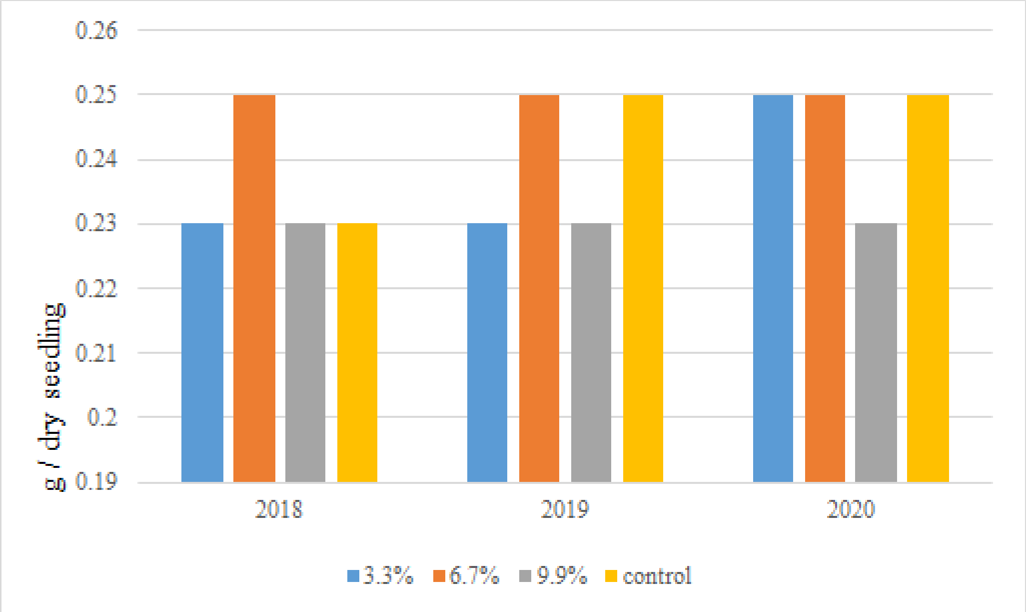

Figure 4. Dry matter for *San Andrea*

Conclusion

According to the results obtained from this study we can conclude that the Orgalife treatments had significant effect on the standard germination test only for the 2020 production year, which can be interpreted as a result of the seed age and the production conditions. Also here is present and visible the negative effect of the maximum concentration dose 9.9% for *San Andrea* variety, which means that different varieties behave differently in the same conditions. The Orgalife treatments did affect the other examined parameters such as the germination index, the early stages of growth and development expressed as seedling vigor index I and II, seedlings length and the total dry matter of the 14 days old seedlings. Based on the obtained results, we cannot recommend only one specific concentration because all of the treatments (3.3%, 6.7% and 9.9%) positively stimulated the germination and the seedling development expressing that effect through the different growing parameters. Although the highest concentration (9.9%) resulted in the highest seedling length and vigor indexes, it is not recommended concentration dose because it causes disbalance in the seedling growth by inhibiting the root development and overstimulating the shoot development. Moreover, this concentration significantly decreased the germination energy and the total germination.

The Orgalife fertilizer in the recommended dosage could be used for encouragement of the rice seedling development, mainly in controlled conditions, for example in seedling production.

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