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Dose-Dependent Effects of Foliar Nano NPK and Zinc on Yield, Antioxidant Capacity, and Metabolic Profile of Sweet Pepper (*Capsicum annuum* L.)

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Abstract

Foliar nanofertilization is increasingly being explored as a strategy to enhance crop nutritional quality; however, dose-dependent physiological and metabolic responses remain insufficiently defined. This study evaluated the effects of conventional NPK (20:20:20) and nano-formulated NPK combined with zinc (3 and 5 g/L) on the mineral composition, bioactive compounds, antioxidant capacity, and metabolic profile of sweet pepper (*Capsicum annuum* L., cv. 'Dora') grown under controlled conditions. Physicochemical characterization of the nanofertilizer by dynamic light scattering and transmission electron microscopy confirmed nanoscale primary particle size and revealed concentration-dependent aggregation behavior at higher Zn levels. Significant differences ($p < 0.05$) were observed among treatments in macro- and microelement content, total phenolics, flavonoids, carotenoids, ascorbic acid, and antioxidant activity. The application of nano NPK combined with 3 g/L Zn resulted in the highest accumulation of total phenolics, flavonoids, and vitamin C, accompanied by enhanced antioxidant capacity, suggesting stimulation of secondary metabolism. In contrast, the higher Zn concentration (5 g/L) further increased carotenoid content but was associated with elevated proline levels, indicating the onset of physiological stress. Multivariate analyses (PCA and ROC) supported dose-dependent metabolic modulation and confirmed that combinations of selected metabolites contributed to clearer differentiation between fertilization regimes. Overall, the results highlight the existence of an optimal nano-zinc application range that enhances fruit functional quality while avoiding stress-related metabolic imbalance, emphasizing the importance of physicochemical stability in nano-enabled fertilization strategies. While this study focused on a single sweet pepper cultivar, future research should explore other pepper species to evaluate whether similar dose-dependent nano Zn effects are observed.

Keywords: sweet pepper; nanofertilizers; antioxidant activity; zinc nanoparticles; functional food quality; metabolomic profiling

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1. Introduction

Fertilization is a key agrotechnical measure in modern agriculture, aimed at optimally supplying crops with essential nutrients, ensuring stable yield and product quality while maintaining or improving soil fertility without negatively impacting the environment [1,2]. Fertilization influences both the quantity and quality of yield by modifying nutrient availability in the rhizosphere. Nutrients can be applied to the soil or directly to above-ground plant parts through foliar application [3]. The process requires balancing biological, technological, ecological, and economic aspects, influenced by climatic, physical, chemical, and pedological factors [4]. Chemical fertilizers, whether synthetic or natural, remain the main method to improve crop growth and productivity [5,6]. However, nutrient use efficiency of conventional fertilizers is often below 50%, with significant losses via leaching, volatilization, or microbial decomposition, contributing to environmental pollution and increased production costs [5–8].

Due to these limitations, nanotechnology has emerged as a promising approach for enhancing fertilization efficiency through controlled nutrient delivery, reducing losses, and minimizing environmental impacts [8,9]. Nanofertilizers, characterized by small particle size, high surface area, and enhanced mobility, improve penetration into plant tissues, nutrient availability, and reduce input requirements, contributing to both economic and environmental sustainability [10–12]. Furthermore, they enable synchronized and gradual release of nutrients like nitrogen and potassium according to plant uptake, mitigating the adverse effects associated with conventional fertilizers, including soil and water contamination [13–15]. Beyond nutrient delivery, nanofertilizers can enhance soil fertility, stimulate beneficial microbial activity, and improve overall soil health [16].

Recent studies show that nano-enabled inputs may also act as biostimulants, modulating plant physiological processes, improving stress tolerance, and stimulating secondary metabolism, thereby enhancing crop quality and yield [17]. These properties position nanofertilizers as a sustainable alternative to conventional agrochemicals, particularly in intensive horticultural crops such as sweet pepper (*Capsicum annuum* L.), a crop of significant economic and nutritional value in North Macedonia and the region [18–23].

Sweet pepper is thermophilic, sensitive to high temperatures, and heavily dependent on nutrient availability for growth and fruit quality [24–29]. Its fruits are rich in vitamin C, carotenoids, phenolics, and other bioactive compounds, which serve as both health-promoting agents and quality indicators [25,30–34]. Optimal macro- (N, P, K) and micronutrient (e.g., Zn) supply is critical for maximizing yield and quality [35–44]. Zinc, in particular, plays key roles in enzymatic activity, photosynthesis, and stress regulation, making foliar application a valuable complement to soil fertilization [39,43,44].

Although nanofertilizers hold great potential, detailed studies on optimal doses and their effects on specific metabolites in sweet pepper are limited. Information on physiological stress mechanisms and risks of overapplication remains scarce, restricting wider adoption. To address this, systematic research is needed to develop safe and effective nanofertilizer strategies that maximize benefits without negative consequences [40].

This study is the first to evaluate the combined foliar application of nano NPK and nano Zn at defined doses in sweet pepper, assessing growth, yield, bioactive compounds, antioxidant capacity, and metabolomic profiles. The work explores dose-dependent effects, highlighting the balance between enhancing fruit quality and avoiding physiological stress. By integrating biochemical, nutritional, and metabolomic analyses, this research provides a comprehensive understanding of the mechanisms through which nanofertilizers modulate plant metabolism and improve fruit quality.

The main objective of this study was to investigate the impact of integrative foliar application of conventional and nanofertilizers on chemical composition, bioactive com-

pounds, phytonutrients, and metabolomic profiles of sweet pepper (*Capsicum annuum* L., cultivar 'Dora') fruits. This multidisciplinary approach enables a better understanding of how nanofertilizers improve fruit quality and stress resistance, guiding the development of sustainable, nanotechnology-based agronomic practices.

2. Materials and Methods

2.1. Experimental Design

The field experiment was conducted in the Strumica region, North Macedonia, within a protected area of 300 m^2. The experimental setup included one sweet pepper variety and four fertilization treatments, including an untreated control. The design comprised 18 rows, with four treatment variants and three replications. Seedlings were planted at 60 cm between rows and 40 cm between plants. Irrigation was applied throughout the growing season, and standard agrotechnical practices were followed.

Before planting, soil fertilization was performed using mineral fertilizer NPK 6-10-30 + 2% MgO at a rate of 12 kg per 300 m^2.

The treatment groups were as follows:

1. Control (untreated);
2. Conventional mineral NPK (20:20:20) soil fertilization (9 kg in the hall with an area of 300 m^2 was applied);
3. Nano NPK (20:20:20) + Nano Zn (3 g/L) foliar application, prepared as an aqueous solution and applied by foliar spraying;
4. Nano NPK (20:20:20) + Nano Zn (5 g/L) foliar application, prepared as an aqueous solution and applied by foliar spraying.

All treatments were established on a common baseline fertilization regime to avoid nutrient limitation and to ensure that observed differences reflect treatment-induced effects rather than deficiencies. The concentrations of nano Zn (3 g/L and 5 g/L) were selected based on previous preliminary studies, in which a broader range of doses was tested and these concentrations were found to provide measurable effects on the quality and metabolic profiles of sweet pepper without causing visible phytotoxicity.

Foliar applications were performed seven times during the growing season. These seven foliar applications were conducted at the following phenological stages: pre-flowering (10–15 days before flowering), early flowering, full flowering, post-flowering (15–20 days after flowering), early fruit set, mid-fruit development, and at the berry stage of fruit development. Each foliar treatment applied nano NPK (20:20:20) combined with nano Zn at 3 g/L or 5 g/L per application. Each treatment and replication included 50 plants, totaling 600 plants in the experiment. Fertilizers were applied as aqueous solutions using a hand-held sprayer to ensure uniform foliar coverage. Foliar application was chosen over soil-based (root) fertilization due to the limited mobility of certain nutrients, particularly zinc, in the soil. Foliar spraying allows for more rapid and efficient uptake of nutrients directly through the leaves, which can lead to faster physiological responses and improved nutrient use efficiency in sweet pepper plants under the applied experimental conditions. The first harvest took place on 23 May and the last harvest on 12 July.

2.2. Material

The study utilized the sweet pepper variety 'Dora', known for its high yield and fruit quality. 'Dora' is a medium-early variety characterized by long, large fruits resembling the "Kurtovska kapija" type. The fruits are light yellow at technological maturity and bright red at botanical maturity. They are fleshy and bilaterally flattened, which facilitates thermal processing. This variety is suitable for fresh consumption as well as various types

of processing. Under favorable cultivation conditions, yields can reach approximately 50 t/ha [45].

2.3. Harvesting Procedures

Harvesting was performed when fruits reached a length of 18 cm, with separation by variety, treatment variant, and replication. Five harvests were conducted throughout the growing season. Fruits were collected and classified according to size and type. All fruits from a given harvest were collected on the same day. Samples were placed in plastic boxes and immediately transported to the laboratory for further analysis [46].

2.4. Soil Analysis

Soil fertility was assessed through sampling conducted before the experiment setup, at depths of 0–20 cm and 20–40 cm [46]. Samples were air-dried in laboratory conditions and prepared for agrochemical analysis. The following parameters were measured [29]:

- pH value—determined potentiometrically using a pH meter (Mettler Toledo, Columbus, OH, USA);
- Content of easily available nitrogen—determined by the method of Tjurin and Kononova;
- Content of easily available phosphorus—determined by AL method and spectrophotometer reading;
- Content of easily available potassium—determined by AL method and spectrophotometer reading;
- Content of humus—determined by the permanganate method of Kotzman;
- Content of carbonates—determined using Scheibler Calcimeter (Gabbrielli Technology, Calenzano, Italy).

2.5. Morphological Characteristics of Fruits

Fruit morphological characteristics, including size (length and width), weight, and thickness, were determined by randomly selecting twenty fruits from each treatment variant and replication [47]. Measurements of three linear fruit dimensions—length (L), width (W), and thickness (T)—were performed using an electronic caliper gauge (Starrett 727 Series, The L.S. Starrett Company, Athol, MA, USA). Fruit weight (FW, g) was measured with a digital balance (ET-1111, Tehnica, Iskra, Slovenia) [29].

2.6. Chemical Analysis of the Pepper Fruits

For optimal fertilization and proper nutrition management, chemical analysis of pepper fruits was conducted to identify possible nutrient deficiencies or excesses [46]. Samples for analysis were collected during the last harvest, with 10 fruits taken separately from each variant and repetition.

Some chemical analyses were performed on fresh samples, while others required drying the fruits to an air-dry state. For this purpose, the fruits were finely chopped and dried at 60 °C until reaching a constant weight, and then ground into powder using an electric mill [46].

The following parameters were determined as follows [29,48]:

- Content of total moisture—by determining the content of free water (by drying the total moisture content, assessed by measuring free water through drying at room temperature and hygroscopic moisture by drying at 105 °C until constant mass;
- Total dry matter, calculated by subtracting total moisture percentage from 100;
- Mineral substances (ash) content, determined by incinerating samples in a muffle furnace at 500 °C;
- Organic matter content, calculated by subtracting ash content from 100;

- Vitamin C content, measured using the Muri method;
- Nitrogen (N) content, determined by the Kjeldahl method;
- Phosphorus (P_2O_5) content, measured by atomic emission spectroscopy with inductively coupled plasma (ICP-AES);
- Potassium (K_2O) content, determined by incineration with concentrated H_2SO_4 and flame photometry;
- Content of calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), boron (B), zinc (Zn), and molybdenum (Mo), determined by ICP-AES.

2.7. Total Phenolic Analysis

The total phenolic content of sweet pepper samples was determined using the Folin–Ciocalteu assay, with minor modifications. Briefly, 0.5 mL of the homogenized extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. The mixture was incubated in the dark for 30 min at 25 °C. Absorbance was measured at 765 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). Results are expressed as milligrams of gallic acid equivalents (mg GAE) per gram of fresh weight (FW) [49].

2.8. Determination of Flavonoids

The flavonoid content was measured according to a method based on the reaction with aluminum chloride ($AlCl_3$). The extract (0.5 mL) was mixed with 1.5 mL methanol, 0.1 mL 10% $AlCl_3$, 0.1 mL 1 M sodium acetate, and 2.8 mL distilled water. After incubation for 30 min at room temperature, the absorbance was measured at 415 nm. The results are expressed as milligrams of quercetin equivalent (mg QE) per gram of fresh sample [49].

2.9. Determination of Carotenoids

The carotenoid content of sweet pepper fruit was analyzed by a method based on extraction in acetone, followed by measurement of absorbance at 450 nm. For extraction, 5 g of homogenized mass was treated with 20 mL of acetone, the mixture was centrifuged at 4000 rpm for 10 min, and the supernatant was used for measurement. The total carotenoid content was calculated according to the formula and expressed as mg/100 g fresh sample.

2.10. Antioxidant Activity (DPPH)

The antioxidant activity was determined by the radial free radical scavenging method using DPPH (2,2-diphenyl-1-picrylhydrazyl). In a 96-well plate, 100 µL of extract was mixed with 3.9 mL of DPPH solution (60 µM in methanol). The mixture was incubated in the dark for 30 min, after which the absorbance at 517 nm was measured. The antioxidant activity is expressed as the percentage (%) inhibition of the DPPH radical [50].

2.11. Ferric Reduction Antioxidant Potential (FRAP)

The FRAP assay for assessing antioxidant capacity was performed according to the method of Benzie and Stewart. The FRAP reagent (300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$) was heated to 37 °C. The extract (0.1 mL) was mixed with 3 mL FRAP reagent and incubated for 30 min. The absorbance was measured at 593 nm. The results are expressed as µmol Trolox equivalent (TE) per gram of fresh weight (FW) [50].

2.12. LC-MS Metabolomic Analysis of Pepper Fruit

2.12.1. Sample Preparation

Fresh sweet pepper fruits (cultivar 'Dora') from each treatment were harvested at the stage of technological maturity. A total of 10 g of pulp (without seeds and stem) were

separated from each biological replicate (n = 3), which were immediately frozen with liquid nitrogen and stored at −80 °C until analysis. Before extraction, the samples were homogenized with liquid nitrogen in porcelain mortars.

2.12.2. Extraction of Metabolites

A total of 1.0 g of the homogenized mass was taken, to which 10 mL of methanol: water (80:20, *v*/*v*) with 0.1% formic acid was added. The mixture was vigorously vortexed for 2 min, then sonicated for 20 min in an ultrasonic bath at 25 °C and centrifuged for 15 min at 14,000 rpm (4 °C). The supernatant was filtered through a 0.22 µm PTFE filter and transferred to LC-MS vials.

2.12.3. Chromatographic Conditions (UHPLC)

The analysis was performed on a UHPLC system (Thermo Scientific Vanquish, Germering, Germany) connected to an electrospray ionization mass spectrometer. Separation was performed on a C18 column (2.1 × 100 mm, 1.7 µm), with the following gradient elution: Phase A—Water with 0.1% formic acid; Phase B—Acetonitrile with 0.1% formic acid.

Time (min)	%A	%B
0.0	95	5
2.0	90	10
8.0	70	30
12.0	50	50
15.0	10	90
18.0	95	5
20.0	95	5

The flow rate was maintained at 0.3 mL/min under isocratic conditions, the column temperature was set at 40 °C, and the injection volume was 5 µL.

2.12.4. Mass Spectrometry (ESI-MS)

The analysis was performed with a Q-TOF mass spectrometer (SCIEX TripleTOF, SCIEX, Framingham, MA, USA) in the positive and negative ESI modes.

The parameters were as follows:

- Scan range: *m*/*z* 50–1200.
- Ion source temperature: 350 °C.
- Capillary voltage: 3.5 kV.
- Nebulizer gas: Nitrogen.
- Acquisition mode: Full scan + Auto MS/MS (data-dependent acquisition).
- Collision energy: Dynamic (20–40 eV).

2.13. Physicochemical Characterization of Nanofertilizers

The hydrodynamic diameter, size distribution, and surface charge of the nano-formulated NPK and nano Zn suspensions were determined by dynamic light scattering (DLS) and electrophoretic light scattering using a Zetasizer Nano ZS instrument (Malvern Panalytical, Malvern, UK). Measurements were performed at 25 °C with a detection angle of 173°.

Nanofertilizer suspensions were analyzed at the working concentrations applied in the field experiment (3 g/L and 5 g/L Zn in combination with nano NPK 20:20:20). Prior to measurement, samples were dispersed in deionized water and sonicated for 10 min to minimize aggregation. Each sample was measured in triplicate, and the results are presented as mean ± standard deviation.

Hydrodynamic diameter (Z-average), polydispersity index (PDI), and zeta potential (mV) were recorded. Zeta potential values were calculated from electrophoretic mobility using the Smoluchowski model. The measurements were used to assess suspension stability, aggregation tendency, and potential interaction behavior with plant leaf surfaces.

2.14. Transmission Electron Microscopy (TEM) Analysis

The morphology and primary particle size of the synthesized nanofertilizer were examined using transmission electron microscopy (TEM). A drop of freshly prepared nanoparticle suspension was placed onto a carbon-coated copper grid and allowed to dry under ambient conditions. Excess liquid was removed using filter paper to prevent aggregation artifacts.

TEM imaging was performed using a JEOL JEM-2100 transmission electron microscope (JEOL Ltd., Tokyo, Japan) operated at an accelerating voltage of 200 kV. Representative micrographs were recorded at different magnifications. Particle size distribution was determined by measuring at least 150 individual particles using the ImageJ software (version 1.54k). The results are expressed as mean particle diameter ± standard deviation.

The analysis was conducted on both working concentrations (3 g/L and 5 g/L Zn in combination with nano NPK 20:20:20) to evaluate potential concentration-dependent morphological differences.

2.15. Statistical Analysis

Analysis of variance (ANOVA) was used to process the data in order to determine statistically significant differences between the different treatments. For a detailed comparison between individual groups, the post hoc Tukey's HSD test was applied with a significance level of $p < 0.05$. For all morphological and biochemical analyses, n = 10 represents ten individual fruits sampled per treatment, and the reported values are the mean ± standard deviation (SD) calculated across these biological replicates, ensuring that the SD reflects natural variability among fruits rather than technical replicate variability. All statistical analyses were performed using the SPSS software (version 29). In addition, for a better visual and multivariate understanding of the differences in the metabolite profile, principal component analysis (PCA) was applied to identify key metabolites and their contribution to the classification of the nanofertilizer treatments. Furthermore, Multivariate Exploratory Receiver Operating Characteristic (ROC) analysis, performed in the MetaboAnalyst platform, was applied descriptively to explore minimal metabolic clusters associated with treatment effects. This approach provides integrative insight into coordinated metabolite shifts rather than serving as a predictive model for treatment classification. The analysis included: (i) generation of individual ROC curves, (ii) calculation of the selection frequency of metabolites as biomarkers, and (iii) assessment of prediction accuracy depending on the number of selected variables.

3. Results

3.1. Physicochemical Properties of Nanofertilizer Suspensions

Dynamic light scattering (DLS) analysis revealed concentration-dependent differences in particle behavior (Table 1). The Nano NPK + 3 g/L Zn suspension exhibited a mean hydrodynamic diameter of 84.65 ± 3.23 nm with a polydispersity index (PDI) of

0.21 ± 0.02, while the 5 g/L Zn suspension showed a diameter of 162.81 ± 5.78 nm and PDI of 0.34 ± 0.04. Zeta potential measurements were −28.4 ± 1.31 mV and −17.6 ± 1.87 mV for the 3 g/L and 5 g/L suspensions, respectively.

Table 1. Physicochemical characterization of nanofertilizer suspensions at working concentrations.

Treatment	Hydrodynamic Diameter (nm)	PDI	Zeta Potential (mV)
Nano NPK + 3 g/L Zn	84.65 ± 3.23	0.21 ± 0.02	−28.4 ± 1.31
Nano NPK + 5 g/L Zn	162.81 ± 5.78	0.34 ± 0.04	−17.6 ± 1.87

Transmission electron microscopy (TEM) confirmed that the primary particle sizes were within the nanoscale range (Table 2, Figure 1). The 3 g/L treatment had a mean particle diameter of 52.37 ± 11.42 nm (range 28–78 nm), predominantly spherical with low aggregation, whereas the 5 g/L treatment had a mean diameter of 55.83 ± 14.70 nm (range 30–92 nm), spherical with moderate clustering.

Table 2. TEM-derived primary particle size distribution.

Treatment	Mean Particle Diameter (nm)	Size Range (nm)	Morphology	Aggregation Level
Nano NPK + 3 g/L Zn	52.37 ± 11.42	28–78	Predominantly spherical	Low
Nano NPK + 5 g/L Zn	55.83 ± 14.70	30–92	Spherical with partial clustering	Moderate

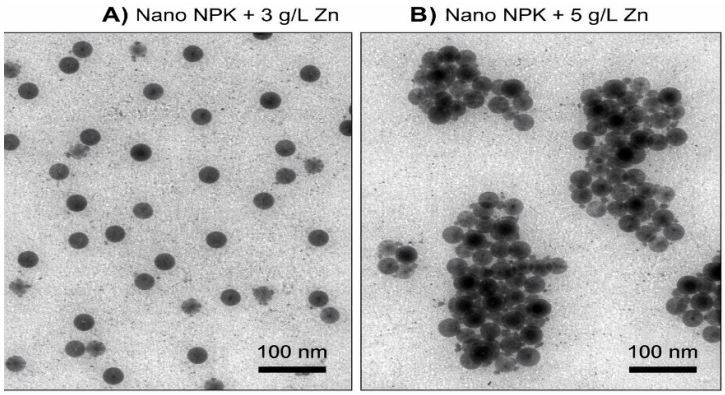

Figure 1. Transmission electron microscopy (TEM) micrographs of nanofertilizer suspensions at working concentrations: (**A**) Nano NPK + 3 g/L Zn and (**B**) Nano NPK + 5 g/L Zn. The 3 g/L treatment shows predominantly well-dispersed, spherical nanoparticles, while partial clustering is visible at 5 g/L. Scale bars represent 100 nm.

3.2. Soil Agrochemical Analysis

The soil used for cultivating sweet green pepper 'Dora' was neutral with an average pH of 7.41 in water and 6.65 in KCl. Humus content averaged 2.56%, while available nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O) were 9.30 mg/100 g, 16.12 mg/100 g, and 21.80 mg/100 g, respectively (Table 3). No $CaCO_3$ was detected. These soil characteristics indicated suitable conditions for pepper growth and for the application of nanofertilizers.

Table 3. Agrochemical soil analysis.

No.	Plot	Depth cm	pH		Available Form (mg/100 g Soil)			Humus (%)	$CaCO_3$ (%)
			H_2O	KCl	N	P_2O_5	K_2O		
1	Pepper 'Dora'	0–20	7.43 ± 0.01	6.70 ± 0.02	8.90 ± 0.08	15.25 ± 0.11	23.10 ± 0.05	2.50 ± 0.07	/
2		20–40	7.40 ± 0.05	6.60 ± 0.14	9.70 ± 0.04	17.00 ± 0.09	20.50 ± 0.16	2.62 ± 0.07	/
Average		0–40	7.41 ± 0.09	6.65 ± 0.01	9.30 ± 0.08	16.12 ± 0.01	21.80 ± 0.13	2.56 ± 0.09	/

Morphological analysis showed significant differences between treatments (Table 4). Variant 3 (Nano NPK + 3 g/L Zn) had the highest values for fruit length (108.26 ± 2.71 mm), width (56.34 ± 1.86 mm), weight (129.58 ± 2.17 g), and thickness (5.79 ± 1.63 mm). Variant 4 (Nano NPK + 5 g/L Zn) also showed increased values compared to the control but slightly lower than Variant 3. Statistical significance was determined by ANOVA and Tukey's post hoc test ($p < 0.05$).

Table 4. Morphological parameters.

Variants	Fruit Length (mm)	Fruit Width (mm)	Fruit Weight (g)	Fruit Thickness (mm)
1	85.37 ± 2.15 ^{a}	47.16 ± 2.04 ^{a}	92.43 ± 2.37 ^{a}	4.52 ± 2.81 ^{a}
2	96.84 ± 1.57 ^{b}	52.47 ± 2.31 ^{b}	112.68 ± 2.69 ^{b}	5.13 ± 3.01 ^{b}
3	108.26 ± 2.71 ^{c}	56.34 ± 1.86 ^{c}	129.58 ± 2.17 ^{c}	5.79 ± 1.63 ^{c}
4	105.63 ± 2.24 ^{d}	55.12 ± 1.49 ^{c}	124.36 ± 1.98 ^{d}	5.61 ± 1.79 ^{c}

$^{a, b, c, d}$—values for the same parameter of the different variants marked with different letters have statistically significant differences ($p < 0.05$), ANOVA, post hoc Tukey's test. Values are presented as mean ± SD of n = 10 individual fruits per treatment (biological replicates).

3.3. The Effect of Fertilizing on Pepper Yield

The yield of pepper cultivar 'Dora' increased significantly in all treatments where different concentrations of nanofertilizers were applied, compared to the control (Variant 1) (Table 5). The highest yield, measured as total yield, yield per plant, and yield per hectare, was observed in the treatment with Nano NPK (20:20:20) + Nano Zn (3 g/L) (variant 3). This treatment resulted in a 20.3% higher yield compared to the control, indicating a significant improvement in productivity with the application of nanotechnology. Statistical analysis (ANOVA, post hoc Tukey's test) showed that the differences between all variants are statistically significant ($p < 0.05$), confirming the positive effect of foliar nano-based nutrient supplementation on biomass accumulation and yield under the applied experimental conditions. Since multiple factors were modified simultaneously (fertilizer formulation, nutrient composition, and application route), the present results should be interpreted as a response to the overall fertilization strategy rather than to a single isolated variable.

The treatment with Nano NPK (20:20:20) + Nano Zn (5 g/L) (Variant 4) also showed significant improvement compared to the control, but the yield was slightly lower than that in Variant 3, which may indicate the existence of an optimal concentration of nano Zn for maximum effect. The obtained results highlight the effectiveness of nanotechnology in horticultural production, providing greater availability of nutrients and improving physiological processes in the plant, resulting in higher and better quality yield.

Table 5. Pepper yield for the variety 'Dora'.

Variant	n	Total Yield per Variant (kg)	Yield per Plant (kg)	Yield ($t \cdot ha^{-1}$)
		$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
1	10	292.02 ± 3.31 [a]	1.57 ± 3.55 [a]	58.40 ± 3.26 [a]
2	10	314.34 ± 3.29 [b]	1.69 ± 3.10 [b]	62.87 ± 4.08 [b]
3	10	351.54 ± 3.16 [c]	1.89 ± 3.29 [c]	70.31 ± 3.31 [c]
4	10	325.50 ± 3.45 [d]	1.75 ± 3.14 [d]	65.10 ± 3.86 [b]

[a, b, c, d]—values for the same parameter of the different variants marked with different letters have statistically significant differences ($p < 0.05$), ANOVA, post hoc Tukey's test. Values are presented as mean ± SD of n = 10 individual fruits per treatment (biological replicates).

3.4. The Effect of Fertilizing on the Chemical Content of Pepper Fruits

Total moisture decreased from 90.90 ± 2.32% in the control to 86.50 ± 2.43% in Variant 4, while total dry matter increased correspondingly (Table 6). Organic matter content ranged from 99.17% to 99.45%, and mineral matter from 0.55% to 0.83%. Vitamin C content varied significantly, with the highest value in Variant 3 (1323.56 ± 0.92 mg/100 g).

Table 6. Chemical content of sweet pepper fruits for the variety 'Dora' (% of dry matter).

Variant	n	Total Moisture (%)	Total Dry Matter (%)	Content of Organic Matter (%)	Content of Mineral Matter (%)	Vitamin C (mg/100 g)
		$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
1	10	90.90 ± 2.32 [a]	9.10 ± 1.37 [a]	99.45 ± 2.21 [a]	0.55 ± 2.72 [a]	1043.11 ± 0.95 [a]
2	10	89.80 ± 2.15 [b]	10.20 ± 2.21 [b]	99.33 ± 3.32 [a]	0.67 ± 1.85 [b]	814.82 ± 0.87 [b]
3	10	88.65 ± 2.67 [c]	11.35 ± 2.36 [c]	99.42 ± 2.09 [a]	0.58 ± 1.16 [a]	1323.56 ± 0.92 [c]
4	10	86.50 ± 2.43 [c]	13.50 ± 1.94 [d]	99.17 ± 2.03 [a]	0.83 ± 1.69 [c]	1251.01 ± 1.08 [d]

[a, b, c, d]—values for the same parameter of the different variants marked with different letters have statistically significant differences ($p < 0.05$), ANOVA, post hoc Tukey's test. Values are presented as mean ± SD of n = 10 individual fruits per treatment (biological replicates).

Nitrogen, phosphorus, potassium, calcium, and magnesium concentrations differed significantly between variants (Table 7). Variant 3 showed the highest N (1.49 ± 1.27%), P_2O_5 (0.82 ± 1.91%), and K_2O (3.35 ± 0.89%), while Variant 4 exhibited the highest MgO (1.15 ± 1.31%).

Table 7. Macroelement composition of sweet pepper fruit variety 'Dora' (% of dry matter).

Variant	n	N	P_2O_5	K_2O	CaO	MgO
		$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
1	10	1.33 ± 1.09 [a]	0.61 ± 1.44 [a]	2.75 ± 0.99 [a]	1.87 ± 0.98 [a]	0.89 ± 1.00 [a]
2	10	1.37 ± 1.18 [a]	0.69 ± 1.37 [a]	2.80 ± 1.30 [b]	1.95 ± 0.83 [a]	1.09 ± 1.07 [b]
3	10	1.49 ± 1.27 [b]	0.82 ± 1.91 [b]	3.35 ± 0.89 [c]	2.10 ± 1.19 [b]	1.05 ± 1.28 [b]
4	10	1.41 ± 1.35 [b]	0.73 ± 1.26 [c]	2.98 ± 0.92 [d]	1.02 ± 1.42 [c]	1.15 ± 1.31 [c]

[a, b, c, d]—values for the same parameter of the different variants marked with different letters have statistically significant differences ($p < 0.05$), ANOVA, post hoc Tukey's test. Values are presented as mean ± SD of n = 10 individual fruits per treatment (biological replicates).

Concentrations of Fe, Mn, B, Zn, and Mo increased in all nanofertilizer treatments compared to the control (Table 8). Variant 3 had the highest accumulation for Fe (93 $mg \cdot kg^{-1}$), Mn (37 $mg \cdot kg^{-1}$), B (55 $mg \cdot kg^{-1}$), Zn (68 $mg \cdot kg^{-1}$), and Mo (40 $mg \cdot kg^{-1}$).

Table 8. Microelement composition of sweet pepper fruits for the variety 'Dora' (mg·kg^{-1} of dry matter).

Variant	n	Fe	Mn	B	Zn	Mo
		$\bar{x} \pm$ SD	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD
1	10	65 ± 0.96 ^{a}	23 ± 1.25 ^{a}	43 ± 1.31 ^{a}	59 ± 1.21 ^{a}	35 ± 0.89 ^{a}
2	10	72 ± 0.58 ^{b}	29 ± 1.11 ^{b}	49 ± 1.49 ^{b}	71 ± 1.38 ^{b}	47 ± 1.52 ^{b}
3	10	93 ± 1.10 ^{c}	37 ± 0.95 ^{c}	55 ± 0.79 ^{c}	68 ± 1.54 ^{c}	40 ± 0.99 ^{c}
4	10	86 ± 1.08 ^{d}	34 ± 1.02 ^{c}	51 ± 0.96 ^{d}	62 ± 1.67 ^{d}	43 ± 0.97 ^{c}

$^{a, b, c, d}$—values for the same parameter of the different variants marked with different letters have statistically significant differences ($p < 0.05$), ANOVA, post hoc Tukey's test. Values are presented as mean ± SD of n = 10 individual fruits per treatment (biological replicates).

The content of phenolic compounds, flavonoids, carotenoids, and vitamin C showed significant differences between treatments (Figure 2, Table 9). Variant 3 displayed the highest total phenolics (3.14 ± 0.15 mg GAE/g FW) and flavonoids (2.97 ± 0.14 mg QE/g FW). Carotenoids were highest in Variant 4 (3.99 ± 0.13 mg/100 g FW). Antioxidant activity, assessed by DPPH and FRAP, was highest in Variant 3 (74.21 ± 0.91% DPPH, 461 ± 0.86 µmol TE/g FW).

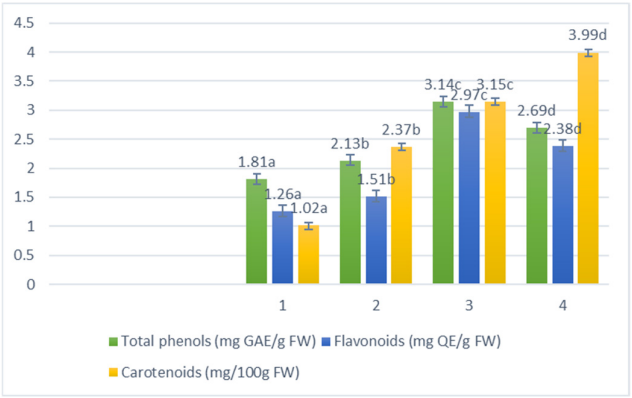

Figure 2. Bioactive compounds of sweet pepper fruits in response to different fertilizer treatments ($\bar{x} \pm$ SD), ANOVA, post hoc Tukey's test. $^{a, b, c, d}$—values for the same parameter of the different variants marked with different letters have statistically significant differences ($p < 0.05$), ANOVA, post hoc Tukey's test.

Table 9. Antioxidative properties of sweet pepper fruits in response to different fertilizer treatments.

Variant	n	DPPH (%)	FRAP (µmol TE/g FW)
		$\bar{x} \pm$ SD	$\bar{x} \pm$ SD
1	10	58.39 ^{a} ± 1.69	323 ^{a} ± 0.95
2	10	54.67 ^{b} ± 1.87	377 ^{b} ± 0.94
3	10	74.21 ^{c} ± 0.91	461 ^{c} ± 0.86
4	10	72.49 ^{c} ± 0.97	455 ^{d} ± 0.98

$^{a, b, c, d}$—values for the same parameter of the different variants marked with different letters have statistically significant differences ($p < 0.05$), ANOVA, post hoc Tukey's test. Values are presented as mean ± SD of n = 10 individual fruits per treatment (biological replicates).

Metabolite profiling indicated statistically significant differences between treatments (Table 10). Chlorogenic acid, rutin, ascorbic acid, lutein, shikimic acid, proline, phenylalanine, citric acid, glucose, ferulic acid, and caffeic acid concentrations varied

across treatments. Variant 3 exhibited the highest concentrations of chlorogenic acid (3.97 ± 1.01 mg/kg), rutin (1.88 ± 0.57 mg/kg), ascorbic acid (142 ± 0.93 mg/kg), lutein (6.79 ± 0.79 mg/kg), and shikimic acid (3.31 ± 1.31 mg/kg). Variant 4 showed higher proline (2.91 ± 0.77 mg/kg). Differences were statistically significant ($p < 0.05$).

Table 10. Concentrations of metabolites in sweet pepper fruits under the influence of different fertilizer treatments (mg/kg fresh weight).

Metabolite	Function/Class	1	2	3	4
Chlorogenic acid	Phenolic acid	2.16 ^{a} ± 0.58	2.53 ^{b} ± 0.88	3.97 ^{c} ± 1.01	3.42 ^{d} ± 0.75
Rutin (Quercetin-3-rutinoside)	Flavonoid	0.85 ^{a} ± 0.69	1.02 ^{b} ± 0.73	1.88 ^{c} ± 0.57	1.63 ^{d} ± 0.93
Ascorbic acid (Vit C)	Antioxidant	95 ^{a} ± 0.78	110 ^{b} ± 0.99	142 ^{c} ± 0.93	135 ^{d} ± 0.94
Lutein	Carotenoid	4.24 ^{a} ± 0.93	5.18 ^{b} ± 1.27	6.79 ^{c} ± 0.79	6.61 ^{c} ± 1.03
Shikimic acid	Phenolic precursor	1.82 ^{a} ± 1.05	2.27 ^{b} ± 1.11	3.31 ^{c} ± 1.31	2.93 ^{d} ± 0.98
Proline	Amino acid/stress marker	1.17 ^{a} ± 0.99	1.45 ^{b} ± 0.75	2.39 ^{c} ± 0.54	2.91 ^{d} ± 0.77
Phenylalanine	Amino acid/precursor	0.93 ^{a} ± 1.26	1.26 ^{b} ± 1.14	2.05 ^{c} ± 0.11	1.70 ^{d} ± 0.09
Citric acid	Organic acid	12.51 ^{a} ± 0.68	14.14 ^{b} ± 0.84	15.36 ^{c} ± 0.23	14.72 ^{d} ± 0.67
Glucose	Sugar	6.83 ^{a} ± 0.97	7.50 ^{b} ± 0.57	8.97 ^{c} ± 0.14	8.46 ^{c} ± 1.00
Ferulic acid	Phenolic acid	0.43 ^{a} ± 1.20	0.52 ^{b} ± 0.98	0.89 ^{c} ± 0.46	0.83 ^{c} ± 0.50
Caffeic acid	Phenolic acid	0.35 ^{a} ± 1.11	0.39 ^{a} ± 1.01	0.72 ^{b} ± 0.29	0.68 ^{c} ± 0.79

$^{a, b, c, d}$—values for the same parameter of the different variants marked with different letters have statistically significant differences ($p < 0.05$), ANOVA, post hoc Tukey's test. Values are presented as mean ± SD of n = 10 individual fruits per treatment (biological replicates).

Principal component analysis (PCA) was performed to explore multivariate patterns in metabolite composition across treatments (Figure 3). The first two principal components explained 52.4% and 21.7% of the total variance, respectively (cumulative 74.1%). PC1 showed strong positive contributions from ascorbic acid, chlorogenic acid, rutin, lutein, and citric acid, while PC2 was mainly influenced by proline and phenylalanine. The PCA score plot indicated separation of treatments, with Nano NPK + 3 g/L Zn positioned along PC1 and Nano NPK + 5 g/L Zn showing partial separation along PC2, whereas control and conventional NPK treatments clustered more closely.

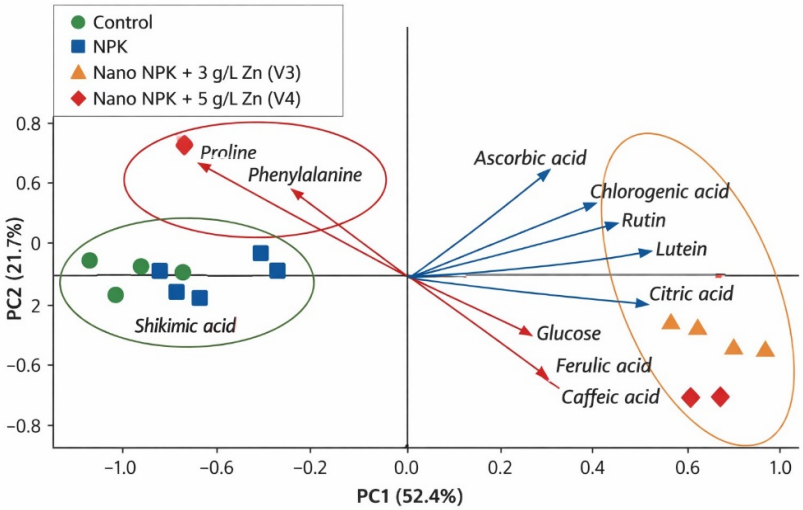

Figure 3. Principal component analysis (PCA) diagram for the metabolite profile of sweet pepper under different fertilization treatments.

ROC analysis of individual metabolites showed limited discriminative power, with AUC values ranging from 0.306 to 0.400 (Figure 4), below the threshold for random classification (AUC = 0.5). This indicates that none of the metabolites alone could serve as a reliable biomarker for differentiating treatments. Such low individual predictive power is expected in complex plant metabolomes, where systemic and coordinated responses occur across multiple metabolites rather than single high-impact compounds.

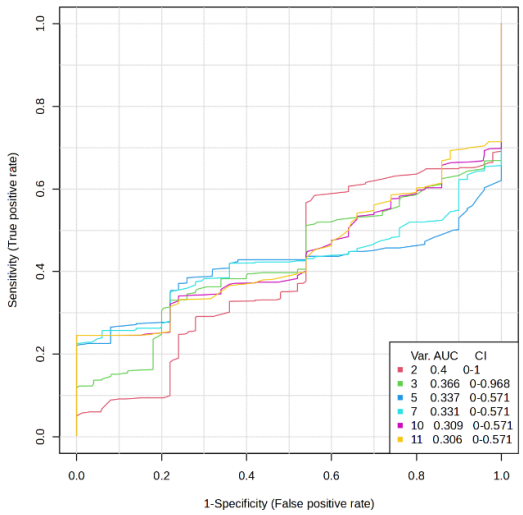

Figure 4. Receiver operating characteristic (ROC) curves for individual metabolites. AUC values are shown, indicating limited discriminatory power of individual metabolites (AUC 0.306–0.400).

Multivariate ROC analysis identified proline, citric acid, caffeic acid, and lutein as recurrently selected variables (Figure 5), revealing a minimal metabolic signature reflecting systemic biochemical adjustments. The maximum predictive accuracy was 41.5%, emphasizing that this analysis serves an exploratory and descriptive purpose rather than a robust predictive model for treatment classification. Despite the limited accuracy, this approach provides integrative insight into coordinated metabolite shifts associated with foliar nanofertilization.

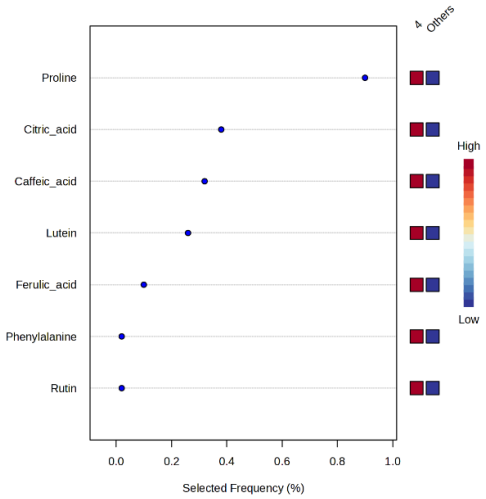

Figure 5. Selection of the most relevant metabolites through multivariate analysis. The most frequently selected compounds are proline, citric acid, caffeic acid, and lutein, which may represent part of a potential metabolic signature.

Analysis of predictive accuracy depending on the number of selected metabolites showed that the highest accuracy is achieved using three metabolites, while including

additional variables reduces predictive performance (Figure 6). This suggests the existence of an optimal minimal set of metabolites that captures the core metabolic response, though their prognostic power is insufficient for practical classification without larger sample sizes or complementary analytical methods.

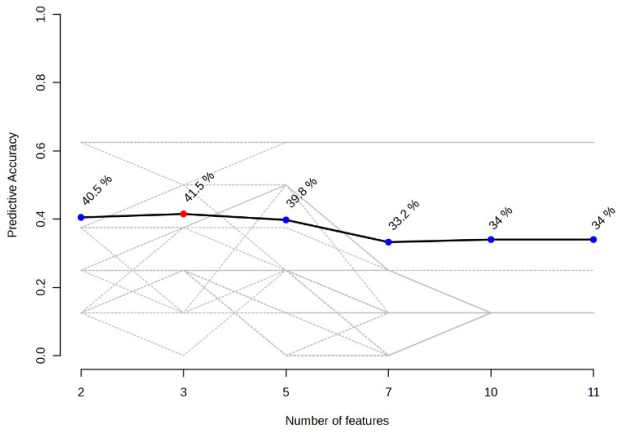

Figure 6. Predictive accuracy depends on the number of selected metabolites. The highest accuracy (41.5%) was achieved with three metabolites, after which the accuracy decreased with the addition of additional variables.

Together, these results demonstrate that, although individual metabolites do not possess strong discriminative power, their combination provides a clearer representation of systemic metabolic adjustments (Figure 5). The presence of proline and organic acids (e.g., citric acid) indicates potential changes in energy metabolism and antioxidant response, whereas phenolic compounds (caffeic acid, ferulic acid, rutin) may reflect protective mechanisms. This is consistent with previous studies showing that coordinated clusters of amino acids and phenolic compounds often emerge under stress or adaptive physiological responses, highlighting the relevance of examining metabolite interactions rather than single compounds.

Overall, these findings confirm that the foliar application of nano NPK combined with optimal Zn supplementation triggers systemic biochemical adjustments in pepper fruits, enhancing both primary and secondary metabolism. The integrative effect improves antioxidant capacity, supports enzymatic pathways dependent on zinc, and potentially increases tolerance to environmental stressors. ROC analysis, while exploratory, complements classical statistical methods and strengthens the interpretation that nano-enabled fertilization does not produce isolated metabolic changes but induces coordinated shifts that enhance nutritional and functional quality. These results underscore the value of integrative metabolomic approaches in precision agriculture for monitoring and optimizing foliar nanonutrient strategies.

4. Discussion

High-quality production of sweet pepper (*Capsicum annuum* L.) depends on balanced mineral nutrition, soil physicochemical properties, and environmental stability [45,51,52]. Due to its rapid vegetative growth and relatively shallow and sensitive root system, pepper is particularly responsive to nutrient availability and fertilization strategy [53]. Within this agronomic framework, nanofertilization has emerged as a precision-oriented approach aimed at improving nutrient use efficiency, modulating plant metabolism, and reducing nutrient losses compared with conventional fertilization systems [8,10,12,54]. This discussion addresses the effects of foliar nanofertilization on yield, biochemical profiles, and

metabolite regulation, and situates these findings within the broader literature on pepper nutrition and nano-enabled agriculture.

The present study demonstrates a clear dose-dependent response of sweet pepper to foliar application of nano NPK (20:20:20) combined with nano Zn. The most pronounced improvements in yield, mineral accumulation, antioxidant capacity, and metabolite profiles were observed at 3 g/L nano Zn, while the higher concentration (5 g/L) induced subtle metabolic shifts indicative of physiological stress. This biphasic response is consistent with the previously reported hormetic effects of nanoparticle-based fertilization, where moderate concentrations stimulate growth and metabolism, whereas excessive levels may disrupt cellular homeostasis [43,55–57]. It should be noted that the four treatments differ simultaneously in formulation type (conventional vs. nano), application route (soil vs. foliar), and nutrient composition (with or without Zn). Therefore, the observed effects cannot be attributed solely to the nano-formulation itself. It is important to emphasize that the experimental design follows standard agronomic practice, where treatments are evaluated against a common nutritional baseline rather than under strictly isolated conditions. In field-based plant nutrition studies, the complete separation of individual factors (e.g., formulation type, nutrient composition, and application route) is often not biologically meaningful, as plant growth inherently depends on balanced nutrient availability. Therefore, the observed responses should be interpreted as the incremental effect of the applied treatment relative to the baseline fertilization regime. This approach reflects realistic cultivation conditions and enables the robust assessment of the practical performance of integrated fertilization strategies. Similar biphasic responses have been observed in other studies applying nano Zn or nano NPK to Solanaceae crops [8,32,43,58].

From a physicochemical perspective, the superior performance of the 3 g/L treatment can be partially explained by the greater colloidal stability observed in DLS analysis. The smaller hydrodynamic diameter and more negative zeta potential (−28.4 mV) indicate enhanced dispersion stability, reduced aggregation, and potentially more uniform foliar deposition. Improved nanoparticle dispersion likely facilitates controlled nutrient release and more homogeneous interaction with the leaf surface, promoting efficient cuticular penetration and translocation. In contrast, the reduced surface charge and increased aggregation observed at 5 g/L may have altered nanoparticle–leaf interactions, leading to localized ion accumulation and mild oxidative imbalance. These findings highlight that not only nominal concentration but also suspension stability governs biological performance, emphasizing the importance of physicochemical optimization in nano-enabled fertilization strategies. These findings corroborate reports that colloidal stability directly influences foliar uptake and nutrient bioavailability [10,12].

The increase in macro- and microelement accumulation in fruits confirms the effectiveness of foliar nano-delivery. Zinc plays a central biochemical role as a structural and catalytic cofactor in numerous enzymes, including superoxide dismutase (SOD), carbonic anhydrase, RNA polymerase, and phenylalanine ammonia-lyase (PAL). Through these enzymatic pathways, Zn influences redox balance, nitrogen metabolism, and phenylpropanoid biosynthesis. The elevated concentrations of nitrogen, phosphorus, potassium, and selected micronutrients in the nano NPK + 3 g/L Zn treatment suggest enhanced nutrient assimilation efficiency rather than simple external enrichment. The observed increases in phenolic and antioxidant compounds align with previous studies, though the magnitude and pattern of response differ depending on nanoparticle formulation and foliar concentration [43,58].

Particularly noteworthy is the stimulation of secondary metabolism. The significant increases in chlorogenic acid, caffeic acid, ferulic acid, rutin, and lutein indicate activation of the phenylpropanoid and isoprenoid pathways. The zinc-dependent acti-

vation of PAL likely contributed to the enhanced phenolic synthesis, while improved redox balance supported the accumulation of antioxidant compounds. Similar increases in phenolics and flavonoids following nano Zn or nano Fe application have been reported previously [8,32,43,58], supporting the hypothesis that nano-formulated micronutrients can act as metabolic modulators rather than solely nutrient suppliers.

The elevation of ascorbic acid in the 3 g/L treatment further strengthens this interpretation. Ascorbic acid functions as a central component of the ascorbate–glutathione cycle and is crucial for detoxifying reactive oxygen species (ROS). Its significant increase suggests improved redox homeostasis and enhanced antioxidative capacity, which is corroborated by higher DPPH and FRAP values. The correlation between vitamin C content and antioxidant assays is consistent with previous findings emphasizing the importance of ascorbic acid and carotenoids as major quality determinants in pepper fruits [30,59–61].

In contrast, the higher nano Zn concentration (5 g/L) led to a noticeable increase in proline accumulation. Proline is widely recognized as a biochemical marker of osmotic and oxidative stress, functioning in osmoprotection, ROS scavenging, and membrane stabilization. Its elevation in the higher-dose treatment indicates activation of stress-responsive pathways, suggesting that the threshold for optimal Zn-induced stimulation was exceeded. This observation aligns with reports showing that excessive ZnO nanoparticle concentrations induce oxidative stress and metabolic adjustments in pepper and other crops [43,55,56]. These findings indicate a narrow optimal nano Zn concentration range that maximizes metabolic stimulation without inducing stress-related responses.

The metabolomic analyses provide additional integrative insight. The PCA results provide further support for the coordinated metabolic responses induced by foliar nanofertilization. The strong contribution of ascorbic acid, chlorogenic acid, rutin, and lutein to PC1 suggests that this component represents enhanced antioxidant capacity and secondary metabolism. The clear positioning of the nano NPK + 3 g/L Zn treatment along this axis indicates a pronounced stimulation of bioactive compound accumulation, consistent with improved nutritional quality.

In contrast, the contribution of proline and phenylalanine to PC2 reflects stress-related metabolic adjustment. The partial separation of the nano NPK + 5 g/L Zn treatment along this component suggests a shift toward stress-associated pathways at higher Zn concentration, supporting the interpretation of dose-dependent metabolic modulation.

Overall, the PCA confirms that foliar nanofertilization induces coordinated but moderate metabolic shifts rather than extreme divergence, aligning with the observed biochemical data and supporting the concept of balanced physiological enhancement under optimized nano-nutrient application. Although individual ROC curves demonstrated limited discriminatory power (AUC 0.306–0.400), multivariate analysis identified proline, citric acid, caffeic acid, and lutein as recurrently selected variables. These metabolites do not represent independent biomarkers but rather a coordinated metabolic cluster associated with redox regulation and energy metabolism. The modest predictive accuracy (maximum 41.5%) indicates that treatment effects are distributed across interconnected metabolic pathways rather than driven by single high-impact compounds.

Importantly, the improvements in fruit morphological traits and yield are physiologically coherent with the observed biochemical enhancements. Increased carbohydrate accumulation (glucose), organic acids (citric acid), and enhanced mineral composition indicate improved primary metabolism and carbon–nitrogen balance. Zinc-mediated activation of enzymatic systems likely improved photosynthetic efficiency and assimilate partitioning, resulting in greater biomass accumulation and fruit development. Comparable improvements in yield and biochemical composition have been documented for nano-micronutrient applications in pepper and related horticultural species [6,8,23,32,43,58,62,63]. Future stud-

ies should expand biological replication, incorporate transcriptomic or enzymatic analyses, and evaluate long-term field performance to further clarify nano-micronutrient–plant interactions and disentangle the contributions of formulation type, zinc addition, and application route.

From an applied perspective, the findings suggest that optimized foliar nano-based fertilization can enhance fruit nutritional quality and antioxidant potential while maintaining high productivity. However, careful dose calibration is essential to avoid stress-related metabolic shifts. Future studies should expand biological replication, incorporate transcriptomic or enzymatic activity analyses, and evaluate long-term field performance and environmental interactions to further clarify nano-micronutrients–plant dynamics and safety considerations [64,65]. This will help to clarify which effects are specifically due to the nano-formulation versus application route or zinc addition. While the study integrates physicochemical, agronomic, and metabolomic data, future research should disentangle the independent contributions of nano-formulation versus micronutrient supplementation, include larger biological replication, and evaluate environmental interactions to confirm applicability.

Overall, the results indicate that nano NPK combined with optimally calibrated nano Zn acts as an effective metabolic regulator, enhancing antioxidant capacity, stimulating secondary metabolism, and improving fruit quality. These findings support the potential of nano-enabled fertilization as a precision approach in sustainable horticulture, while emphasizing the importance of physicochemically informed dose optimization.

5. Conclusions

This study demonstrates that foliar application of nano NPK (20:20:20) combined with nano Zn at 3 g/L significantly enhances the biochemical and functional quality of sweet pepper (*Capsicum annuum* L., cultivar 'Dora') by stimulating antioxidant metabolism and secondary metabolite biosynthesis.

The dose-dependent response highlights the critical importance of precise nutrient management, as higher nano Zn concentrations (5 g/L) may induce metabolic stress, emphasizing the narrow optimal concentration window for maximal benefit.

While the study integrated physicochemical characterization, agronomic performance, and metabolomic analyses, it did not isolate the independent contributions of each nano-formulation component. Future research should expand biological replication, evaluate long-term field performance, and incorporate transcriptomic or enzymatic analyses to fully elucidate the mechanisms and environmental safety of nano-enabled fertilization strategies.

Overall, carefully calibrated foliar nanofertilization represents a scientifically grounded and potentially sustainable approach for improving fruit quality in sweet pepper, linking metabolic modulation with agronomic performance and supporting the rational integration of nanotechnology into modern fertilization practices. The findings should be interpreted within the context of integrated fertilization systems, where multiple interacting factors contribute to plant response under realistic agronomic conditions.

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