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Chemical and biological fertilizers affect the seedling development of bell pepper

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Abstract

Objective: Bell pepper (Capsicum annuum L.) is one of the most important greenhouse vegetables. The use of seedlings is a common production method for bell peppers in most greenhouses; therefore, seedling quality has a significant impact on yield. Today, the use of biological fertilizers to replace chemical fertilizers in the production of agricultural products has become important. The present study was conducted to investigate the effect of chemical fertilizers and bio-fertilizers (Nitrokara and Phosphonitrokara) on some seedling characteristics of the bell pepper.

Methods: The study investigated how different fertilizer combinations and timing affect pepper seedling production. The first experiment tested the 20N:20P:20K fertilizer, the second tested 10N:45P:10K, both at different application timings. The third experiment examined the impact of biological fertilizers (Nitrokara and Phosphonitrokara) combined with 20N:20P:20K on the seedling growth. All experiments were conducted using commercial bell pepper cultivars Inspiration, Arancia, and Tarento.

Results: A fertilization regime of 2 g/L 20N:20P:20K, applied every three days, improved plant height, root and shoot fresh and dry weight, root volume, leaf area, stem diameter, and chlorophyll index across cultivars. The Inspiration cultivar was more responsive to the balanced NPK, showing high shoot biomass gains, while cultivar Arancia showed a clear trade-off; the balanced NPK favored shoots, while the high-phosphorus fertilizer enhanced roots. Similar pattern to Arancia, the Tarento cultivar with balanced NPK boosting canopy/photosynthesis and with high-phosphorus fertilizer improving roots and, somewhat, the plant height. Although the addition of biofertilizers to the chemical NPK improved root traits and chlorophyll index in some cultivars, they did not compensate for the loss in shoot productivity as the main yield component, compared to the use of NPK alone.

Conclusion: The balanced 20:20:20 fertilizer at the rate of 2 g/L was optimal for maximizing the aboveground productivity and photosynthetic efficiency, while the high-phosphorus 10:45:10 shifted the biomass allocation toward roots, potentially useful for stress conditions.

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Introduction

Bell pepper (*Capsicum annuum* L) is notable for its high vitamin C, vitamin A, dietary fiber, and antioxidants such as lutein and zeaxanthin (Olatunji and Afolayan 2018). Economically, bell pepper is a high-value vegetable crop produced worldwide, with major production centers in China, Mexico, and the United States (Hernández-Pérez *et al.* 2020). It is in strong global demand, both fresh and processed, with colored varieties and organic produce fetching premium prices (Khazaei and Estaji 2021). Bell pepper cultivation offers high returns per area, especially in greenhouse or protected environments, making it an important income-generating crop for many small- to medium-scale farmers.

In many greenhouses, seedlings are commonly used to establish mature plants, and proper fertilization is essential for their successful transition to field conditions. Seedling quality is significantly influenced by various environmental and management factors, including light, temperature, CO_2, humidity, irrigation, fertilization, cultivation area, and farming practices (Weston 1988; Ciardi *et al.* 1998; Damato and Trotta 2000; Glowacka 2002; Paul and Metzger 2005; Brazaityte *et al.* 2010; Atherton and Rudich 2012). Among these, nutrition, particularly nitrogen, plays a critical role. As a major macronutrient, nitrogen is a fundamental component of plant proteins and is typically supplied through chemical fertilizers (Gholinezhad *et al.* 2024). Adequate nitrogen is essential for continuous and healthy plant growth (Togun *et al.* 2003; Ghasembaghlou *et al.* 2022). Due to their prolonged and overlapping flowering and fruiting cycles, peppers require considerable nitrogen input to support continuous growth. Zhang *et al.* (2024) demonstrated that nitrogen supplementation has a critical role in boosting both fruit production and crop yield.

Phosphorus is a vital nutrient for plant development, playing a central role in regulating numerous enzymatic processes. Its deficiency can lead to stunted growth and darkening of leaves due to an imbalance between leaf expansion and chlorophyll synthesis, where chlorophyll accumulates faster than leaf tissue growth, resulting in darker foliage (Sieczko *et al.* 2024). As the second most important soil nutrient after nitrogen, phosphorus is crucial for the formation of ATP, carbohydrates, and nucleic acids, all of which are essential for energy transfer and genetic functioning in plants. However, Sudasinghe *et al.* (2023) reported that capsaicinoid content increased with rising phosphorus levels but declined when phosphorus was applied in excess. In contrast, carbohydrate levels showed a slight increase in peppers grown in the phosphorus-enriched soils (Yakasai and Rabiu 2025).

Potassium is another essential macronutrient, comprising approximately 6% of a plant's dry weight. It plays a critical role in plant growth and influences various aspects of yield and quality, both directly and indirectly (Gruda 2005). Studies on the response of different vegetable crops to potassium across various soil types have shown that potassium fertilization enhances vegetative growth, crop performance, and the quality of produce, including an increase in beneficial compounds like vitamin C. Given its significant impact on yield and quality, the use of potassium fertilizer is considered economically beneficial (Xisheng 1999). Enhancing both yield and crop quality can be achieved through the strategic application of potassium fertilizer, as potassium is a vital nutrient for both plant development and soil fertility. Inadequate potassium availability can impair key physiological functions, like photosynthetic processes (Kusumiyati *et al.* 2022), and potassium deficiency may lead to chlorophyll degradation, adversely influencing various physiologic processes like cell development and antioxidant reactions (Sanatombi 2023).

In addition to mineral fertilizers, biological fertilizers are used in agriculture. The application of biological fertilizers has enhanced growth, and physiological and biochemical attributes (Jha *et al*. 2009; Ahmed *et al*. 2011; Nasibi *et al*. 2020). Sharma *et al.* (2024) investigated the effects of integrating biofertilizers with chemical fertilizers in bell pepper cultivation and found notable improvements in both fruit yield and quality, alongside a reduced reliance on chemical inputs. According to Raturi *et al.* (2019), the combination of varying fertilizer levels with organic manures and biofertilizers significantly enhanced plant growth, yield, and overall fruit quality. Among the treatments tested, the integrated approach proved to be the most cost-effective strategy for producing high-quality bell peppers.

Proper fertilization is essential for producing healthy seedlings and ensuring their successful establishment in the field or greenhouse (Babarabie *et al.* 2020). This study aimed to evaluate the effects of selected mineral nutrients and biological fertilizers on the growth and development of bell pepper seedlings. This research is important because healthy and vigorous seedlings are critical for achieving high yields and quality in bell pepper production. Understanding how different mineral and biological fertilizers affect seedling growth can help optimize fertilization practices, improve plant development, and reduce reliance on chemical inputs. Thus, it can guide growers in selecting effective, environmentally friendly fertilization strategies to boost productivity and reduce costs.

Materials and Methods

The seeds of the cultivars Inspiration, Arancia, and Tarento were provided by the Sepahan Royesh Company, Isfahan, Iran. The seeds were disinfected with sodium hypochlorite (5%) for 5 min and then rinsed three times with distilled water before germination. Seeds were sown in seedling trays containing peat moss and perlite (3:7 ratio), with one seed in each compartment. One week after sowing the seeds, after seedling emergence (two-leaf stage), the following experiments were conducted.

Experiment 1

In this experiment, two levels (1 and 2 g/L) of NPK (20:20:20) and two times of fertilizing (once every 3 and 6 days) were examined. This experiment was carried out using a factorial experiment based on a completely randomized design with six replications.

Experiment 2

In this experiment, the effects of NPK (10:45:10) concentration and time of fertilizing were studied. Again, two levels of NPK (10:45:10) (1 and 2 g/L) and two times of fertilizing (once every 3 or every 6 days) were examined. This experiment was conducted using a factorial experiment based on a completely randomized design with six replications.

Experiment 3

In this experiment, the effect of the combinations of biofertilizers and NPK (20:20:20) was studied. This experiment was conducted using a completely randomized design with six replications. The treatments of this experiment are presented in Table 1.

Table 1. The treatments of the combination of biofertilizers and the NPK (20:20:20) chemical fertilizer used on The bell pepper plants.

Treatment
20 mL of the Nitrokara biofertilizer once every 3 days
20 mL of the Nitrokara biofertilizer and 1 g/L of NPK (20:20:20) once every 3 days
20 mL of the Nitrokara biofertilizer and 2 g/L of NPK (20:20:20) once every 3 days
20 mL of the Phosphonitrokara biofertilizer once every 3 days
20 mL of the Phosphonitrokara biofertilizer and 1 g/L of NPK (20:20:20) once every 3 days
20 mL of the Phosphonitrokara biofertilizer and 2 g/L of NPK (20:20:20) once every 3 days

All three experiments were conducted and analyzed separately for each cultivar. Every replication consisted of one seedling tray compartment containing one seed. After eight weeks, plant height, dry and fresh weights of roots and shoots, root volume, leaf area, stem diameter, and chlorophyll index were measured. The dry weight of the shoots and roots was obtained after the tissue was dried in an oven at 40 °C and the weights became stable. Reading of leaf chlorophyll was performed using the SCMR (SPAD Chlorophyll Meter) method and SPAD-502 device (Minolta, Japan) on completely developed leaves between the middle vein and the leaf margins. To measure the whole leaf area, during the harvest of the shoots, all leaf blades were separated from the stem, and after determining fresh weight, their areas were measured with a leaf area meter. Root volumes were measured directly from the change in the water height in the measuring container after putting the washed roots in it.

Data analysis

Analysis of variance (ANOVA) was performed in each experiment for each variety. Means were compared using Duncan's multiple range test. The data were analyzed by the IBM SPSS, ver. 26.0 (George and Mallery 2019).

Results

Experiment 1: Effects of the 20N:20P:20K concentration and time of fertilizing on the bell pepper traits

Cultivar Inspiration: Based on ANOVA (Supplementary Table 1), the effect of 20N:20P:20K concentration was significant for all characteristics except stem diameter. Also, the effect of time of fertilizing was significant for leaf area, fresh and dry weight of shoots, and chlorophyll index. The interaction of 20N:20P:20K concentration with the time of fertilizing was only significant for the fresh and dry weight of shoots. Therefore, for the fresh and dry weight of shoots, means of treatments obtained from the combination of the 20N:20P:20K concentrations with time of fertilizer application were compared, while for other traits, the main effects of the fertilizer concentration and fertilization time were evaluated. The mean comparison for the 20N:20P:20K concentration showed that a 2 g/L dose resulted in the highest plant height (31.69 cm), fresh root weight (2.66 g/plant), dry root weight (0.2 g/plant), root volume (2.08 cm^3/plant), leaf area (233.25 mm^2), and chlorophyll index (53.61). For the fertilization time, applying fertilizer once every three days produced the highest leaf area (223.58 mm^2) and chlorophyll index (54.1). Furthermore, the combination of a 2 g/L 20N:20P:20K concentration with fertilization every three days resulted in the highest fresh (28.8 g) and dry (11.28 g) shoot weights (Table 2).

Cultivar Arancia: According to ANOVA (Supplementary Table 2), the main effect of 20N:20P:20K concentration was significant for all studied characteristics. Also, the main effect of time of fertilizing was significant for plant height, fresh and dry weight of shoots, leaf area, stem diameter, and chlorophyll index, while the 20N:20P:20K concentration × time of fertilizing interaction was not significant for any of the traits. The highest values for plant height (30.21 cm), fresh shoot weight (9.53 g), dry shoot weight (1.01 g), fresh root weight (1.58 g), dry root weight (0.14 g), root volume (1.44 cm^3), leaf area (224.83 mm^2), stem diameter (5 mm), and chlorophyll index (53.01) were observed using a 2 g/L concentration of 20N:20P:20K. Also, fertilizing once every three days resulted in the highest values for plant height (29.84 cm), fresh shoot weight (7.76 g), dry shoot weight (0.92 g), leaf area (216.25 mm^2), stem diameter (4.97 mm), and chlorophyll index (53.33).

Table 2. Effect of the interaction of 20N:20P:20K with the time of fertilizing on the fresh and dry weight of shoots in the Inspiration cultivar of bell pepper.

Treatment		Trait	
Time of fertilizing	**20N:20P:20K**	**Shoot fresh weight (g)**	**Shoot dry weight (g)**
Once every 3 days	1 (g/L)	12.67±1.45b	6.52±0.28b
Once every 3 days	2 (g/L)	28.80±1.83a	11.28±0.20a
Once every 6 days	1 (g/L)	12.60±1.40b	4.89±0.10c
Once every 6 days	2 (g/L)	12.80±1.45b	8.03±0.15b

Means followed by different letter(s) are significantly different at the 0.05 probability level, based on Duncan's multiple range test.

Cultivar Tarento: Based on ANOVA (Supplementary Table 3), the main effect of the 20N:20P:20K concentration was significant for all measured characteristics, except for the plant height, fresh root weight, and root volume. Additionally, the timing of fertilization had a significant impact on the fresh and dry shoot weight, leaf area, stem diameter, and chlorophyll index. Furthermore, the 20N:20P:20K concentration × fertilization time interaction was significant for fresh and dry shoot weight, stem diameter, and chlorophyll index.

The comparison of the means indicated that the highest dry weight of roots (0.15 g) and leaf area (203.08 mm^2) was obtained using a 2 g/L concentration of the 20N:20P:20K fertilizer. The highest value for the leaf area (201.109 mm^2) was observed when fertilizing the peppers once every three days. The results about the interaction of the 20N:20P:20K concentration with time of fertilizer application showed the highest values for fresh (10.86 g) and dry weight (1.22 g) of shoots, stem diameter (5.5 mm), and chlorophyll index (59.5) belonged to the use of a 2 g/Lconcentration of 20N:20P:20K once every three days (Table 3).

Table 3. Effect of the interaction of 20N:20P:20K with the time of fertilizing on the studied traits in the Tarento cultivar of bell pepper.

Treatment		**Trait**			
Time of fertilizing	**20N:20P:20K**	**Shoot fresh weight (g)**	**Shoot dry weight (g)**	**Shoot diameter (mm)**	**Chlorophyll index**
Once every 3 days	1 (g/L)	6.39±0.14b	0.69±0.029bc	4.47±0.59b	50.98±0.79b
Once every 3 days	2 (g/L)	10.86±0.47a	1.22±0.09a	5.50±0.16a	59.50±0.85a
Once every 6 days	1 (g/L)	4.47±0.18c	0.56±0.014c	4.28±0.21b	40.68±0.71c
Once every 6 days	2 (g/L)	7.04±0.16b	0.82±0.023b	4.70±0.089b	44.70±0.53c

Means followed by different letter(s) are significantly different at the 0.05 probability level, based on Duncan's multiple range test.

Experiment 2: Effects of 10N:45P:10K concentration and time of fertilizing on the bell pepper traits

***Cultivar Inspiration*:** According to Supplementary Table 4, the main effect of 10N:45P:10K concentration was significant for the plant height, leaf area, stem diameter, dry weight of shoots, and chlorophyll index. Moreover, the main effect of time of fertilizer application was significant for the plant height, leaf area, dry weight of shoots, and chlorophyll index. The interaction of 10N:45P:10K concentration with the time of fertilizing was also significant for the plant height, fresh weight of the roots, leaf area, dry weight of shoots, and chlorophyll index.

The highest stem diameter (4.8 mm) was achieved with a 2 g/L concentration of the 10N:45P:10K fertilizer. Additionally, the interaction between the 10N:45P:10K concentration and fertilization time revealed that the highest value for plant height (28.4 cm), leaf area (196.16 mm^2), fresh root weight (1.83 g), dry shoot weight (0.83 g), and chlorophyll index (48.06) were obtained using a 2 g/L concentration of the NPK fertilizer with applied once every three days (Table 4).

Cultivar Arancia: The main effect of the 10N:45P:10K concentration (Supplementary Table 5) was significant for plant height, fresh root weight, fresh and dry shoot weight, and chlorophyll index. Also, the time of fertilizer application significantly affected the fresh root weight, fresh and dry shoot weight, stem diameter, and chlorophyll index. The 10N:45P:10K concentration × fertilizer timing interaction was significant for fresh root weight and root volume.

Table 4. Effect of the interaction of 10N:45P:10K with the time of fertilizing on the studied traits in the Inspiration cultivar of bell pepper.

Treatment		Trait				
Time of fertilizing	**10N:45P:10K**	**Plant height (cm)**	**Shoot dry weight (g)**	**Root fresh weight (g)**	**Leaf area (mm²)**	**Chlorophyll index**
Once every 3 days	1 (g/L)	0.44a±27.70	0.0186b±0.50	0.19c±1.23	7.94bc±109.50	1.23c±31.31
Once every 3 days	2 (g/L)	0.57a±28.40	0.034a±0.83	0.087a±1.83	6.60a±196.16	1.75a±48.06
Once every 6 days	1 (g/L)	0.58a±27.86	0.017c±0.43	0.11bc±1.35	4.21c±98.50	1.71c±29.13
Once every 6 days	2 (g/L)	0.81b±24.04	0.022b±0.54	0.061ab±1.65	2.59bc±131.70	1.53b±38.7

Means followed by the different letter(s) are significantly different at the 0.05 probability level, based on Duncan's multiple range test.

The results of the mean comparison for the main effect of the 10N:45P:10K concentration indicated the 2 g/L concentration of 10N:45P:10K had the highest values for plant height (27.1 cm), root fresh (5.34 g) and dry (0.57 g) weight, and chlorophyll index (47.5). Furthermore, the highest values for the shoot fresh weight (5.3 g), shoot dry weight (0.56 g), stem diameter (4.32 mm), and chlorophyll index (45.5) were achieved by applying the fertilizer once every three days. Additionally, at a 2 g/L concentration, when applied every three days, the greatest shoot fresh weight (1.36 g per plant) and root volume (1.13 cm^3 per plant) were obtained (Table 5).

Table 5. Effect of the interaction of 10N:45P:10K with the time of fertilizing on the studied traits in the Arancia cultivar of bell pepper.

Treatment		Trait	
Time of fertilizing	**10N:45P:10K**	**Shoot fresh weight (g)**	**Root volume (cm³)**
Once every 3 days	1 (g/L)	0.60±0.07c	0.53±0.08b
Once every 3 days	2 (g/L)	1.36±0.11a	1.13±0.077a
Once every 6 days	1 (g/L)	1.02±0.08b	1.05±0.12a
Once every 6 days	2 (g/L)	1.30±0.022a	0.65±0.061b

Means followed by the different letter(s) are significantly different at the 0.05 probability level, based on Duncan's multiple range test.

***Cultivar Tarento*:** According to the ANOVA (Supplementary Table 6), the main effect of 10N:45P:10K concentration was significant on the plant height, fresh weight of the shoots, leaf area, and the chlorophyll index. The main effect of time of fertilizer application was also significant for all characteristics studied, except for the plant height. Also, the interaction of the 10N:45P:10K concentration with the time of fertilizer application was significant for fresh and dry root weight, fresh and dry shoot weight, and root volume.

The mean comparison showed the highest plant height (26.5 cm), leaf area (119.8 mm^2), and chlorophyll index (48.4) for the 2 g/L concentration of the 10N:45P:10K fertilizer. The highest values for the leaf area (120.8 mm^2), stem diameter (4.5 mm), and chlorophyll index (45.9) were obtained when the fertilizer was used once every three days. Also, according to Table 6, the 2 g/L concentration of 10N:45P:10K, applied once every three days, showed the highest root fresh (1.55 g) and dry (0.11 g) weight, shoot fresh (5.38 g) and dry (0.58 g) weight, and root volume 1.3 cm^3

Table 6. Effect of the interaction of 10N:45P:10K with the time of fertilizing on the studied traits in the Tarento cultivar of bell pepper.

Treatment		Trait				
Time of fertilizing	**10N:45P:10K**	**Shoot fresh weight (g)**	**Shoot dry weight (g)**	**Root volume (cm^3)**	**Root fresh weight (g)**	**Root dry weight (g)**
Once every 3 days	1 (g/L)	4.90±0.12ab	0.53±0.071a	0.98±0.13b	0.92±0.078b	0.095±0.01ab
Once every 3 days	2 (g/L)	5.38±0.35a	0.58±0.017a	1.30±0.05a	1.55±0.03a	0.11±0.006a
Once every 6 days	1 (g/L)	3.20±0.05c	0.52±0.018a	0.73±0.11bc	1.07±0.11b	0.085±0.004b
Once every 6 days	2 (g/L)	4.60±0.16b	0.39±0.013b	0.45±0.14c	0.46±0.079c	0.056±0.008c

Means followed by the different letter(s) are significantly different at the 0.05 probability level, based on Duncan’s multiple range test.

Comparison of Experiments 1 and 2:

The balanced NPK (Experiment 1) was more effective for overall vegetative growth, photosynthetic capacity, and biomass production in the Inspiration cultivar (Table 2). The high-phosphorus NPK (Experiment 2) was beneficial for stem thickening and some interactive responses but less effective in supporting shoot and root biomass (Table 4). Also, balanced NPK (Experiment 1) was more effective for aboveground growth, photosynthesis, and biomass in cultivar Arancia, while the high-phosphorus treatment (Experiment 2) improved root biomass allocation, suggesting a role in root system strengthening but at the expense of shoot performance and chlorophyll content (Table 5). For the Tarento cultivar, the balanced NPK (Experiment 1) was better for the aboveground vegetative growth, canopy development, stem robustness, and photosynthesis, while Experiment 2 was better for the root system development and plant height, but less effective in biomass accumulation aboveground (Tables 3 and 6). This indicates that balanced NPK supports shoot-dominant growth and productivity in Tarento, while high-phosphorus fertilizer shifts resource allocation toward root enhancement.

Experiment 3*: *Effect of the combinations of biological fertilizers and 20N:20P:20K on the bell pepper traits

***Cultivar Inspiration*:** The ANOVA results (Supplementary Table 7) revealed significant differences between treatments at the 1% probability level for all traits studied. Applying 20 mL of Nitrokara, the biological fertilizer, along with the 2 g/L 20N:20P:20K, once every 3 days, produced the highest plant height (30.9 cm), dry weight of root (0.23 g), and chlorophyll index (58.2) as compared to other treatments. On the other hand, the highest fresh weight of roots (2.48 g), root volume (2.25 cm3), leaf area (208.5 mm2), and dry weight of shoots was obtained by using 20 mL of the Phosphonitrokara biological fertilizer along with 1 g/L of 20N:20P:20K, once every 3 days. The highest fresh weight of shoots (13.25 g) and stem diameter (6.07 mm) belonged to the use of 20 mL Phosphonitrokara along with 2 g/L 20N:20P:20K once every 3 days (Table 7).

Table 7. Effect of the combinations of biological fertilizer and 20N:20P:20K on the studied traits of the Inspiration bell pepper cultivar.

Treatment	**Trait**				
	Plant height (cm)	**Root fresh weight (g)**	**Root dry weight (g)**	**Root volume (cm^3)**	**Leaf area (mm^2)**
T_1	26.2±1.02b	0.72±0.034c	0.06±0.006c	0.64±0.10c	161.8±5.42c
T_2	29.6±0.72a	1.25±0.17bcd	0.13±0.01bc	1.16±0.17bc	229.7±8.90b
T_3	30.9±0.78a	1.82±0.18b	0.23±0.02a	1.76±0.18ab	274.6±17.40ab
T_4	24.9±0.71b	0.99±0.15cd	0.09±0.011c	1.11±0.11bc	143.8±7.45c
T_5	30.4±0.99a	2.48±0.29a	0.22±0.03a	2.25±0.27a	308.5±20.90a
T_6	26.9±0.4 b	1.61±0.30bc	0.16±0.034ab	1.55±0.33b	293.4±26.20a

Table 7 continued

Treatment	**Trait**			
	Shoot fresh weight (g)	**Shoot dry weight (g)**	**Shoot diameter (mm)**	**Chlorophyll index**
T_1	2.49±0.13c	0.32±0.016c	3.60±0.13d	28.3±1.30d
T_2	7.86±0.26b	0.72±0.120b	5.04±0.11c	53.10±0.95b
T_3	11.36±0.32a	1.38±0.082a	5.30±0.26bc	58.20±1.10a
T_4	2.19±0.086c	0.25±0.019c	2.71±0.06e	26.10±1.05d
T_5	11.83±0.40a	1.43±0.120a	5.60±0.21ab	48.80±0.15c
T_6	13.25±1.66a	1.33±0.200a	6.07±0.019a	57.70±0.44a

Means followed by different letter(s) are significantly different at the 0.05 probability level, based on Duncan's multiple range test; Treatments: T_1: 20 mL of Nitrokara every 3 days; T_2: 20 mL of Nitrokara and 1 g/L of 20N:20P:20K every 3 days; T_3: 20 mL of Nitrokara and 2 g/L of 20N:20P:20K every 3 days; T_4: 20 mL of Phosphonitrokara every 3 days; T_5: 20 mL of Phosphonitrokara and 1 g/L of 20N:20P:20K every 3 days; T_6: 20 mL of Phosphonitrokara and 2 g/L of 20N:20P:20K every 3 days.

Cultivar Arancia: Based on ANOVA (Supplementary Table 8), significant differences between treatments were observed at 1% probability level for all studied traits, except the fresh weight of shoots. The comparison of the means showed that applying 20 mL of Phosphonitrokara + 1 g/L 20N:20P:20K once every 3 days produced the highest plant height (28.2 cm), fresh and dry weight of roots (1.69 and 0.15 g, respectively), and root volume (1.56 cm^3). The highest fresh shoot weight (11.18 g), leaf area (268 mm^2), and stem diameter (5.6 mm) belonged to applying 20 mL of Phosphonitrokara combined with a 2 g/L concentration of 20N:20P:20K every three days. Additionally, the highest chlorophyll index (58.2) was recorded when 20 mL of the Nitrokara biological fertilizer was applied alongside the 2 g/L concentration of 20N:20P:20K, every three days (Table 8).

Table 8. Effect of the combinations of biological fertilizer and 20N:20P:20K on the studied traits of the Arancia bell pepper cultivar.

Treatment	**Trait**			
	Plant height (cm)	**Root fresh weight (g)**	**Root dry weight (g)**	**Root volume (cm^3)**
T_1	24.6±0.59b	0.74±0.034c	0.063±0.01b	0.72±0.079c
T_2	25.1±0.38b	0.86±0.09cd	0.093±0.008b	0.83±0.084bc
T_3	28.1±0.55a	1.31±0.09b	0.140±0.015a	1.21±0.180ab
T_4	25.7±0.51b	0.96±0.05bcd	0.083±0.003b	0.88±0.094bc
T_5	28.2±0.58a	1.69±0.13a	0.150±0.030a	1.56±0.170a
T_6	25.9±0.56b	1.19±0.16bc	0.140±0.010a	1.20±0.180ab

Table 8 continued

Treatment	**Trait**			
	Shoot fresh weight (g)	**Leaf area (mm^2)**	**Shoot diameter (mm)**	**Chlorophyll index**
T_1	2.4±0.05d	150.8±5.4d	3.5±0.03d	28.8±1.30c
T_2	6.6±0.26c	187.67±9.9c	4.6±0.07c	49.1±1.95b
T_3	9.3±0.40b	214.6±9.4b	4.7±0.12c	56.9±1.10a
T_4	2.1±0.16d	140.8±7.4c	2.7±0.11e	26.1±0.75c
T_5	9.3±0.34b	217.5±6.9b	5.01±0.10b	49.8±0.73b
T_6	11.2±0.33a	268.4±9.2a	5.6±0.19a	56.2±1.44a

Means followed by different letter(s) are significantly different at the 0.05 probability level, based on Duncan's multiple range test; Treatments: T_1: 20 mL of Nitrokara every 3 days; T_2: 20 mL of Nitrokara and 1 g/L of 20N:20P:20K every 3 days; T_3: 20 mL of Nitrokara and 2 g/L of 20N:20P:20K every 3 days; T_4: 20 mL of Phosphonitrokara every 3 days; T_5: 20 mL of Phosphonitrokara and 1 g/L of 20N:20P:20K every 3 days; T_6: 20 mL of Phosphonitrokara and 2 g/L of 20N:20P:20K every 3 days.

***Cultivar Tarento*: ** There was a significant difference between treatments at the probability level of 1% for all studied traits, except the fresh weight of the shoots (Supplementary Table 9). The mean comparison showed that applying the Phosphonitrokara biological fertilizer once every 3 days, along with 1 g/L 20N:20P:20K, produced the highest plant height (26.1 cm), fresh and dry weight of roots (2.07 and 0.18 g, respectively), and root volume (1.95 cm^3). The highest fresh weight of shoots (10.4 g), leaf area (237.6 mm^2), and stem diameter (5.6 mm) was obtained using 20 mL of Phosphonitrokara once every 3 days combined with a 2 g/L 20N:20P:20K. Also, the highest chlorophyll index (60.2) belonged to the use of 20 mL of the Nitrokara biological fertilizer along with 2 g/L 20N:20P:20K, when applied once every 3 days (Table 9).

Table 9. Effect of the combinations of biological fertilizer and 20N:20P:20K on the studied traits of the Tarento bell pepper cultivar.

Treatment	Trait				
	Plant height (cm)	**Root fresh weight (g)**	**Root dry weight (g)**	**Root volume (cm^3)**	**Leaf area (mm^2)**
T_1	24.4±0.58b	0.75±0.037b	0.060±0.004c	0.74±0.06b	141.8±5.42d
T_2	24.6±0.59ab	0.82±0.095b	0.076±0.009c	0.86±0.11b	157.7±3.90c
T_3	26.01±0.60a	0.72±0.037b	0.160±0.020a	0.71±0.06b	214.6±4.40ab
T_4	22.8 ±0.81b	0.94±0.090b	0.086±0.001c	0.78±0.06b	143.8±7.45d
T_5	26.1±0.75a	2.07±0.090a	0.180±0.030a	1.95±0.10a	180.5±8.90bc
T_6	24.5±0.54ab	0.93±0.110b	0.120±0.004b	0.88±0.08b	237.6±9.20a

Table 9 continued

Treatment	Trait			
	Shoot fresh weight (g)	**Shoot dry weight (g)**	**Shoot diameter (mm)**	**Chlorophyll index**
T_1	2.5±0.13d	0.33±0.016d	3.3±0.05d	29.3±0.83e
T_2	6.6±0.45c	0.72±0.012c	4.6±0.22c	51.1±0.45c
T_3	8.4±0.12b	1.13±0.019a	5.1±0.16b	60.0±1.14a
T_4	2.9±0.066d	0.22±0.011e	2.6±0.06e	22.1±1.05f
T_5	8.8±0.40b	1.03±0.036b	4.9±0.13bc	45.5±1.45d
T_6	10.4±0.66a	1.02±0.200b	5.6±0.16a	56.7±0.44b

Means followed by different letter(s) are significantly different at the 0.05 probability level, based on Duncan's multiple range test; Treatments: T_1: 20 mL of Nitrokara every 3 days; T_2: 20 mL of Nitrokara and 1 g/L of 20N:20P:20K every 3 days; T_3: 20 mL of Nitrokara and 2 g/L of 20N:20P:20K every 3 days; T_4: 20 mL of Phosphonitrokara every 3 days; T_5: 20 mL of Phosphonitrokara and 1 g/L of 20N:20P:20K every 3 days; T_6: 20 mL of Phosphonitrokara and 2 g/L of 20N:20P:20K every 3 days.

Discussion

The successful production of strong, healthy seedlings is fundamental to achieving high yields and better quality in the bell pepper, because it influences establishment success after transplanting, ultimately affecting growth performance and productivity throughout the growing season. Various nutrient regimes during seedling development in controlled environments, such as greenhouse, have been shown to significantly impact the seedling quality, influencing the traits such as plant height, biomass, root development, leaf area, and chlorophyll content in bell pepper (Chatzistathis *et al.* 2022; Yan *et al.* 2025). These findings contribute to the growing body of evidence highlighting the critical role of mineral and biological fertilizers in enhancing the bell pepper seedling growth and development. Nitrogen significantly improves the growth, physiological function, and quality of bell pepper seedlings by enhancing the vegetative growth and root development as well as increasing the chlorophyll content and photosynthetic activity (Yan *et al.* 2024). Also, it supports stronger transplant-ready seedlings and acts synergistically with other nutrients and biological inputs. These effects emphasize the importance of nitrogen management in nursery production systems for the bell pepper to ensure vigorous seedlings and successful crop establishment.

The importance of nitrogen fertilization for bell pepper has been well documented, and several studies (Ferrón-Carrillo *et al.* 2021; Abdelkhalik *et al.* 2023) have reported significant improvements in plant height, biomass, and overall plant quality with increasing the nitrogen fertilization rates. Our results aligned with these studies, demonstrating that applying an appropriate concentration of balanced mineral fertilizers containing nitrogen, phosphorus, and potassium substantially improved key growth characteristics of the bell pepper seedlings. Specifically, a 2 g/L concentration of the 20N:20P:20K fertilizer produced superior plant height, root and shoot fresh and dry weight, root volume, leaf area, stem diameter, and chlorophyll index across different cultivars.

Phosphorus deficiency leads to stunted growth and leaf darkening due to an imbalance in chlorophyll synthesis and leaf expansion (Sieczko *et al.* 2024). Our study confirmed the positive effects of the phosphorus-rich fertilizer formulation (10N:45P:10K) on seedling characteristics, including plant height, leaf area, and chlorophyll content. This is consistent with previous research demonstrating the critical role of phosphorus in promoting vigorous root systems and enhancing leaf development, which are crucial for nutrient and water uptake in young plants.

Potassium deficiency can reduce yield and fruit quality. Some studies have found that potassium boosts yield and growth under controlled conditions (Kusumiyati *et al.* 2022; Halaji *et al.* 2023). However, Bikhish *et al.* (2024) reported a decreased performance with higher potassium levels. This apparent discrepancy may be attributed to the specific concentration ranges or interactions with other nutrients. In this study, the balanced 20N:20P:20K fertilizer provided an optimal nutrient environment, supporting an adequate potassium supply without negative effects, thereby enhancing the seedling vigor.

Beyond mineral fertilization, the integration of biological fertilizers in the seedling production is gaining increased attention due to their environmentally friendly nature and ability to promote sustainable agriculture. Biological fertilizers, such as Nitrokara and Phosphonitrokara, consist of beneficial microorganisms that improve plant growth through several mechanisms. These include biological nitrogen fixation, solubilization and mobilization of nutrients (e.g., phosphorus), production of plant growth-promoting hormones (such as auxins and gibberellins), enhancement of the root nutrient uptake efficiency, and antagonism against soil pathogens (Timofeeva *et al.* 2023).

The comparative analysis of the three bell pepper cultivars (Inspiration, Arancia, and Tarento) under balanced and phosphorus-dominant fertilizers revealed distinct patterns of growth between aboveground and belowground traits. Across all cultivars, the balanced NPK treatment enhanced shoot biomass, leaf area, stem diameter, and chlorophyll index, while the high-phosphorus treatment promoted root development and, to some extent, plant height. The better performance of the balanced fertilizer in terms of shoot fresh and dry weight, leaf area, and chlorophyll index reflects the central role of nitrogen and potassium in supporting canopy expansion and photosynthetic efficiency. Nitrogen is fundamental for chlorophyll synthesis, amino acid metabolism, and photosynthetic capacity (Mu and Chen 2021). Similarly, potassium regulates stomatal conductance, osmotic adjustment, and carbohydrate transport, thereby supporting both growth and yield (Imtiaz *et al.* 2023). This explains why the cultivars Inspiration and Tarento recorded maximum shoot biomass and chlorophyll indices under NPK (20:20:20), consistent with findings in peppers and other Solanaceae crops where balanced NPK favored vegetative vigor and photosynthetic traits (Hemida *et al.* 2023; Muhie *et al.* 2023). By contrast, the phosphorus-enriched fertilizer shifted biomass allocation toward the root system, particularly in Arancia and Tarento, which showed a higher root fresh and dry weight under 10:45:10 concentration. Phosphorus is a key determinant of root architecture, influencing lateral root proliferation and root hair development to improve nutrient acquisition (Liu 2021; Aslam *et al.* 2022). High phosphorus supply may stimulate root growth at the expense of shoot expansion, reflecting a resource allocation trade-off under nutrient-enriched conditions (Wang *et al.* 2021). Although, this enhanced root biomass could be advantageous in stress-prone environments, it coincided with reduced chlorophyll index and smaller leaf area, suggesting limitations in canopy photosynthesis and assimilate supply (Tufa 2022).

Cultivar-specific responses were evident. Inspiration demonstrated the strongest shoot responsiveness to the balanced NPK, producing shoot dry weights >11 g per plant, nearly double that of high phosphorus treatments. Arancia exhibited a distinct trade-off, where balanced fertilizer promoted canopy development while the high-phosphorus fertilizer favored roots. Tarento displayed a similar pattern, with balanced NPK enhancing canopy biomass and chlorophyll content, and the high-phosphorus fertilizer improving root traits and plant height. These patterns suggest differential nutrient use efficiencies and adaptive growth strategies among cultivars, which are critical considerations for cultivar-specific fertilization management (Sandhu *et al.* 2021). Our findings support the fact that the balanced NPK formulations are most effective for maximizing aboveground biomass and photosynthetic potential in bell peppers, whereas phosphorus-enriched formulations can be strategically employed to strengthen root systems, particularly in environments where root development confers adaptive advantages. Integrating cultivar-specific responses with nutrient management strategies can, therefore, optimize growth allocation depending on production goals and environmental constraints.

Although biofertilizers improved root traits and chlorophyll index in some cultivars, they did not compensate for the loss in shoot productivity as the main yield component. Thus, from an economic viewpoint, the addition of biofertilizers in our experiment did not justify the cost for maximizing the yield in these cultivars. In other words, the chemical-only treatment provided maximum yield, and biofertilizers brought minor physiological gains (chlorophyll) but no yield advantage, so the added cost is not justified, unless attention is paid to organic farming and reduced use of chemical fertilizers, and some of the costs will be offset in this way. However, past studies have reported positive effects of biological fertilizers on various crops like wheat (Elnahal *et al.* 2022) and corn (Shahwar *et al.* 2023), which demonstrated yield improvement, via inoculation with beneficial microbes. In the bell pepper specifically, Salma *et al.* (2022) found that organic nitrogen fertilizer application increased plant height, leaf size, and dry weight compared to unfertilized controls. However, the combination of biological and complete mineral fertilizers has been less explored, and it needs to be investigated in future research.

Another important aspect of the study was the evaluation of the timing of the fertilizer application, which significantly influenced seedling growth metrics such as leaf area, chlorophyll index, and shoot fresh and dry weight. Fertilizing once every three days consistently resulted in superior growth compared to the less frequent application, indicating that regular nutrient availability supports optimal seedling development. This aligns with agronomic principles emphasizing the importance of consistent nutrient supply during the critical seedling stage to sustain metabolic activities, photosynthesis, and root growth. Moreover, biological fertilizers may contribute to mitigating abiotic stresses such as water deficit and heavy metal toxicity by enhancing the population of beneficial soil microbes that improve plant tolerance (Wu *et al.* 2005).

Conclusion

Optimal mineral fertilization, particularly 2 g/L of 20N:20P:20K applied every three days, significantly enhanced bell pepper seedling growth, including plant height, biomass, root development, leaf area, and chlorophyll content. Phosphorus and potassium contributed to root vigor and leaf expansion, while biological fertilizers improved root traits and chlorophyll, but did not increase shoot biomass compared to the chemical fertilizer. In contrast, the phosphorus-dominant fertilizer (10N:45P:10K) promoted root development and, in some cases, plant height, although often at the expense of canopy expansion and chlorophyll content.

There were cultivar-specific responses, where the Inspiration cultivar showed the highest shoot response to the balanced NPK, but the Arancia cultivar demonstrated the canopy development promoted by the balanced chemical fertilizer, and the favored root growth by the high-phosphorus fertilizer. Therefore, future studies integrating nutrient use efficiency, yield, and quality characteristics can further refine the cultivar-specific fertilization strategies.

Conflict of Interest

The authors declare no conflict of interest with any organization concerning the subject of the manuscript.

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