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Enhancing Seed Potato Production from *In Vitro* Plantlets and Microtubers through Biofertilizer Application: Investigating Effects on Plant Growth, Tuber Yield, Size, and Quality

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Abstract: Seed potato production often relies on mineral fertilizers. However, biofertilizers offer an eco-friendly, cost-effective means to enhance nutrient uptake, plant growth, yields, and quality while bolstering stress resilience. Two cultivars ('Spunta' and 'Russet'), two *in vitro* materials as microtubers and plantlets, and four bio-fertilizers were used to produce seed minitubers. These bio-fertilizers included mycorrhiza (T2), microalgae (T3), beneficial bacteria (PGPR) (T4), and vermicompost (T5). Treatment T1, which received 100% mineral nutrients, was used as the control, while the bio-fertilizers were given 40% of the mineral nutrition relative to the control. The study clearly demonstrated the effectiveness of the biofertilizers used in improving plant growth parameters, particularly highlighting the efficacy of vermicompost. The highest seed tuber yield of 173.12 g was obtained from the combination of 'Spunta' + microtuber + vermicompost'. In both varieties, *in vitro* microtubers led to a higher seed yield than *in vitro* plantlets. In terms of tuber diameter, tuber weight, and tuber number, the performance of the 'Spunta' cultivar was significantly higher than that of the 'Russet' cultivar. Seed tubers derived from *in vitro* microtubers had a larger diameter and were heavier than those derived from *in vitro* plantlets. However, seed tubers produced from *in vitro* plantlets were of a smaller size but more in number. In *in vitro* potato seed tuber production, we recommend the use of 'Spunta' cultivar and *in vitro* microtuber, supplementing with vermicompost to enhance yield, size, number curbing costs, and eco-friendliness.

Keywords: tissue culture plantlet and microtuber; microbial fertilizers; vermicompost; minituber seed yield and quality; *Solanum tuberosum*

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1. Introduction

Potato (*Solanum tuberosum* L.) is considered one of the vital crops in the world besides wheat, rice, and maize in global human nutrition [1] because of its richness in energy, carbohydrates, minerals, vitamins, and antioxidants. Potato tubers contain high levels of potassium and approximately half the daily adult requirement of vitamin C as well as of vitamins A, B, and E [2]. Potatoes play a pivotal role in addressing food security due to their current cultivation and demand, especially in developing countries where they serve as a vital source of food support [2]. It is one of the most consumed vegetables in Tunisia, after tomatoes. It is included in many local recipes.

Potatoes can maintain their cultivar characteristics through successive generations by vegetative propagation. However, due to their vegetative propagation, they are susceptible to numerous seed-borne diseases. These diseases negatively impact seed quality, leading to the gradual deterioration of seed stocks over consecutive years [3]. High-quality seeds are essential for sustainable potato cultivation and food security. However, a significant issue

of seed scarcity needs to be addressed [4]. The *in vitro* propagation of potato seed tubers through rooted stem cuttings has become one of the simplest and most economical methods. This technique has been extensively employed worldwide in potato seed production [4].

In vitro plantlets and microtubers are used for the rapid multiplication of disease-free material in elite seed potato production [5]. These plantlets are cultivated in greenhouses or screenhouses to yield minitubers, which are subsequently utilized for field planting. Minitubers are harvested from the initial generation of *in vitro* plantlets or derived from microtubers [6]. Microtubers produced from *in vitro*-produced propagules are miniature potato tubers measuring 4–12 mm in size and produced under controlled laboratory conditions. Minitubers are commonly produced in greenhouses using soilless substrate mixtures, beds, containers, or hydroponic systems [7]. The minitubers are planted in seed potato fields, and the offspring from these plants are propagated in the field, typically for an additional 2–5 years before being distributed to farmers growing potatoes [6].

The main objective of initial potato seed production is obtaining medium-sized minitubers with good health status. Radouani et al. [8] and Ozkaynak et al. [9] indicated that the size of minitubers may range from 5 to 25 mm. Nevertheless, the size would be affected by the number of minitubers, planting density [10], growing medium [11], and especially nutrient uptake [8]. However, limited information is available regarding the specific composition of nutrients for enriching the growing medium, and the lack of clear information remains a concern for potato seed start-ups. While general descriptions of fertilizing the growing substrate are standard, detailed information is lacking.

The fertilization needs of potato plants across all stages of their lifecycle, especially during the tuberization phase, are significantly impacted by the nutrient content of the soil, substrate, or nutrient solution of the hydroponic system employed [12,13]. NPK is recognized as the primary group of nutrient elements that influence potato tuberization [14,15]. Additionally, other nutrients, such as calcium (Ca) and magnesium (Mg), have been shown to enhance potato tuber production and quality [16]. Numerous studies have endeavored to optimize the nutrient composition of *in vitro* potato media to enhance plantlet growth and achieve higher yields of micro or minitubers [17].

The widespread utilization of chemical fertilizers directly impacts the environment and, subsequently, indirectly affects human health. Recently, novel agricultural approaches like biofertilizers have emerged to alleviate these adverse impacts, demonstrating environmental sustainability while preserving substrate or soil fertility [18–20]. Biofertilizers, which consist of viable or dormant cells of efficient microorganism strains, are an environmentally friendly and cost-effective approach to improve plant nutrient uptake. They can enhance plant growth, increase yields, and improve quality by building resilience against biotic and abiotic stressors [21,22].

Currently, insufficient data exist regarding the effectiveness of certain biofertilizers for *in vitro* potato seed production, especially in soilless cultures. Studying biofertilizer impacts in controlled environmental conditions *in vitro* would be a worthwhile research topic to pursue [23].

Biofertilizers comprise various natural agents, such as mycorrhiza, beneficial bacteria, algae, and vermicompost [21–23]. Arbuscular mycorrhizal (AM) fungi are present in various soil types and frequently establish symbiotic relationships with the roots of numerous plant species [24,25]. These fungi contribute to plant growth and reproduction by facilitating the nutrient uptake of potatoes [23]. Additionally, they stimulate the production of growth-regulating substances, enhance photosynthesis, bolster stress tolerance, and increase resistance against pests [26–28].

Chlorella vulgaris, a type of green microalgae, is recognized for its rich content of proteins, lipids, carbohydrates, pigments, and metabolites with antioxidant properties [20,22,29]. While its use in conventional agriculture is well-established, its utilization as live algal cells in hydroponic systems remains relatively limited [23,30].

Plant growth-promoting rhizobacteria (PGPR), primarily strains of *Pseudomonas*, *Bacillus*, *Azotobactor*, *Phosphobacteria*, and *Rhizobium* species, have the capacity to enhance the

plant growth and overall yield of potatoes [23]. Furthermore, certain PGPR strains can induce systemic resistance to fungi, bacteria, viruses, and sometimes nematodes [27,31,32].

Vermicompost is a nutrient-rich organic fertilizer abundant in humus, NPK, micronutrients, and beneficial soil microorganisms, including nitrogen-fixing and phosphate-solubilizing bacteria and actinomycetes [33]. It also contains growth hormones such as auxins, gibberellins, and cytokinins. Both vermicompost and its liquid byproduct (vermiwash) have demonstrated their efficacy as growth enhancers of potato plants [23,34,35].

Seed tubers constitute a significant expense in potato propagation, accounting for approximately 40% of production costs. Farmers often use small whole tubers or cut larger ones into pieces for planting to mitigate seed costs. Beyond their environmental benefits, integrating biofertilizers can potentially reduce the costs associated with seed potato production [36].

This research explored the potential benefits of incorporating biofertilizers into *in vitro* potato seed production. The proposed hypothesis suggested that bio-fertilizers had the ability to reduce reliance on chemical fertilizers and facilitate microbial fertilization mechanisms, ultimately resulting in enhanced tuberization and superior minituber potato quality. The study endeavors to promote sustainable and top-notch seed potato production methods *in vitro*.

2. Materials and Methods

2.1. Plant Materials

The *in vitro* plantlets and microtubers of potato varieties named 'Spunta' and 'Russet' were used to obtain minituber potato seed material. The effect of biofertilizers on seed tuber yield, size, and quality was investigated. *In vitro* plantlets and microtubers of these cultivars were sourced from the BioCampus company located in Cukurova University Technopark (37°03′37.21″ N; 35°21′18.03″ E) in October 2020. The research was conducted at the Department of Horticulture, Faculty of Agriculture, Cukurova University, Turkey, spanning from November 2020 to March 2021. The microtubers had an average weight of around 30 mg and a transverse diameter of 6–7 mm. At the same time, *in vitro* plantlets were selected and standardized with an average length of 12 cm. The microtubers were placed in 9 cm diameter glass Petri dishes and pre-sprouted for 4 weeks in a greenhouse environment with temperature and relative humidity maintained at approximately 20 °C and 75%, respectively.

The plant materials were planted in 2 L plastic pots with a diameter of 20 cm, containing a growth medium composed of a peat and perlite mixture at a volume ratio of 2:1 (Table 1). These pots were maintained under greenhouse conditions with natural sunlight conditions. The temperature was maintained at 24 °C during the day and 16 °C at night, while the relative humidity was around 65–70% (Figure 1).

Table 1. The chemical and physical composition of growing media used in the study.

Media Characteristics	Results
Electrical conductivity	white peat (H2–H5) 35 mS/m (+/− 25%)
pH	5.5–6.5
fertilizers (NPK 14:10:18)	1.0 kg/m^3
Available Zn mg/kg	28.7
Available Cu mg/kg	16.0
Available Cd mg/kg	0.53
Available Pb mg/kg	12.8
Available Mo mg/kg	0.11
porosity	80–90%

Figure 1. Images of *in vitro* plantlets (**A**) and microtubers (**B**) used in the experiment, as well as seed potato plants grown in a greenhouse environment (**C**,**D**).

2.2. Treatments

After two weeks of planting, the application of four different bio-fertilizer treatments was initiated. The details of these treatments are provided in Table 2. Treatment T1 (100% mineral nutrients) was used as a control. The biofertilizers used included mycorrhiza (T2), microalgae (T3), beneficial bacteria (PGPR) (T4), and vermicompost (T5). In biofertilizer applications, mineral nutrition was utilized at 40% of the control. In other words, mineral nutrients were reduced by 60% compared to the control and replaced with biofertilizers.

Table 2. Description of the treatments used in this study and applied to *in vitro* plantlets and microtubers of cv. 'Spunta' and 'Russet'.

Fertigation Treatments (T)	Composition
T1:Control	100% mineral nutrition [23] (mg L^{-1}): Nitrogen (N) = 160, phosphorus (P) = 30, potassium (K) = 220, calcium (Ca) = 140, magnesium (Mg) = 40, iron (Fe) = 2.5, manganese (Mn) = 0.25, zinc (Zn) = 0.25, boron (B) = 0.20, copper (Cu) = 0.02, and molybdenum (Mo) = 0.04
T2:Mycorrhiza	40%. mineral nutrition + 1000 spores of mycorrhiza inoculation during plantation for each pot: Mycorrhiza bio-fertilizer under the trade name "Endo Roots Soluble (ERS)®. Different mycorrhiza species as cocktail preparation: *Glomus intraradices*, *Glomus aggregatum*, *Glomus mosseae*, *Glomus clarum*, *Glomus monosporus*, *Glomus deserticola*, *Glomus brasilianum*, *Glomus etunicatum*, *Gigaspora margarita* [20,22].
T3:Microalgae	40% mineral nutrition + microalgae: Microalgae *Chlorella Vulgaris* produced in the Cukurova University using 2×10^6 microalgae in 1 mL. This concentration was diluted 40 times with irrigation solution per 7 days [20,22].
T4:Bacteria	40% mineral nutrition + bacteria: Rhizofill® was liquid bacteria bio-fertilizer used in the experiment. The bacteria fertilizer contained four different bacteria species as *Bacillus subtilis* (1×10^9), *Bacillus megaterium* (1×10^9), and *Pseudomonas fluorescens* (1×10^9). A total of 1 mL of Rhizofill in 1 L of irrigation solution was used per 7 days [20,22].
T5:Vermicompost	40% mineral nutrition + vermicompost: The commercial name of Ekosolfarm® is the liquid vermicompost bio-fertilizer used in the experiment. The vermicompost composition had total organic matter of 10%, total nitrogen of 2%, organic nitrogen of 2%, water-soluble potassium pentaoxide (K_2O) of 0.2%, free amino acids of 10%, and beneficial microorganisms. A total of 3 mL of vermicompost in 1 L of irrigation solution was used per 7 days [20,22].

In vitro plants and microtubers were inoculated with 1000 mycorrhizal spores using the ERS® (Bioglobal, Antalya, Turkey)commercial biofertilizer once during transplantation. The bacteria and vermicompost from commercial products, namely Rhizofill® (liquid) (Next Generation Biotech, Istanbul, Turkey) and Ecosolfarm® (liquid) (Ekosolfarm, Manisa, Turkey), respectively, were applied every 7 days to the roots through irrigation. This involved using 1 mL of Rhizofill in 1 L and 3 mL of vermicompost in 1 L of irrigation solution. The microalgae biofertilizer was produced at Çukurova University. The irrigation dosage was adjustted by diluting the solution containing 2×10^6 live microalgae 40 times.

2.3. Plant Growth Assessment

To evaluate the effect of different bio-fertilizers on potato plant growth, the plant length from the substrate to the tip of the latest bud, plant stem diameter, leaves, and stem number, as well as fresh and dry weights of aerial biomass for the stem, branch, and leaves, was measured 90 days after plantation.

A total of 30 plants per treatment were grown. Five plants of uniform growth were randomly selected for each treatment. Chlorophyll was determined using Minolta-SPAD meter 502 (Japan). The fresh weights were evaluated immediately after sample collection, and the dry weights were determined after oven-drying at 65 °C for 48 h [20,22]. The percentage dry matter of microtubers was calculated as follows:

$$DM(\%) = Dry\ weight\ of\ sample \times 100$$

Fresh weight of sample.

2.4. Microtubers Mineral Composition

The tubers of each treatment and cultivar were harvested at seed maturity (Figure 2), washed, dried with tissue paper, weighed using an electric balance, and diced. Finally, a portion was oven-dried at 65 °C until constant weight. The dehydrated material was ground, passed through a 40 mesh sieve, and subsequently used to determine minerals, as reported by Kusvuran [37] and Altuntas et al. [38]. In this study, four macro mineral

elements N, K, Mg, and Ca were analyzed. Approximately 0.1 g of the oven-dried material was mineralized in a muffle furnace at 550 °C for 6 h. After cooling, the resulting ash was dissolved using hydrochloric acid (HCl). Potassium was determined using flame photometry (model 410 flame photometer, Sherwood Scientific, Cambridge, UK). The nitrogen content was determined through Kjeldahl digestion with a Kjeltec System 1026 (Tecator, Höganas, Sweden) [39]. Phosphorus in the samples was assessed through the Barton method [40]. The other minerals, such as Ca and Mg, were determined by atomic absorption spectrometry (Spectra 220, Varian, Palo Alto, CA, USA). Quantification of individual minerals in the samples was performed using calibration curves.

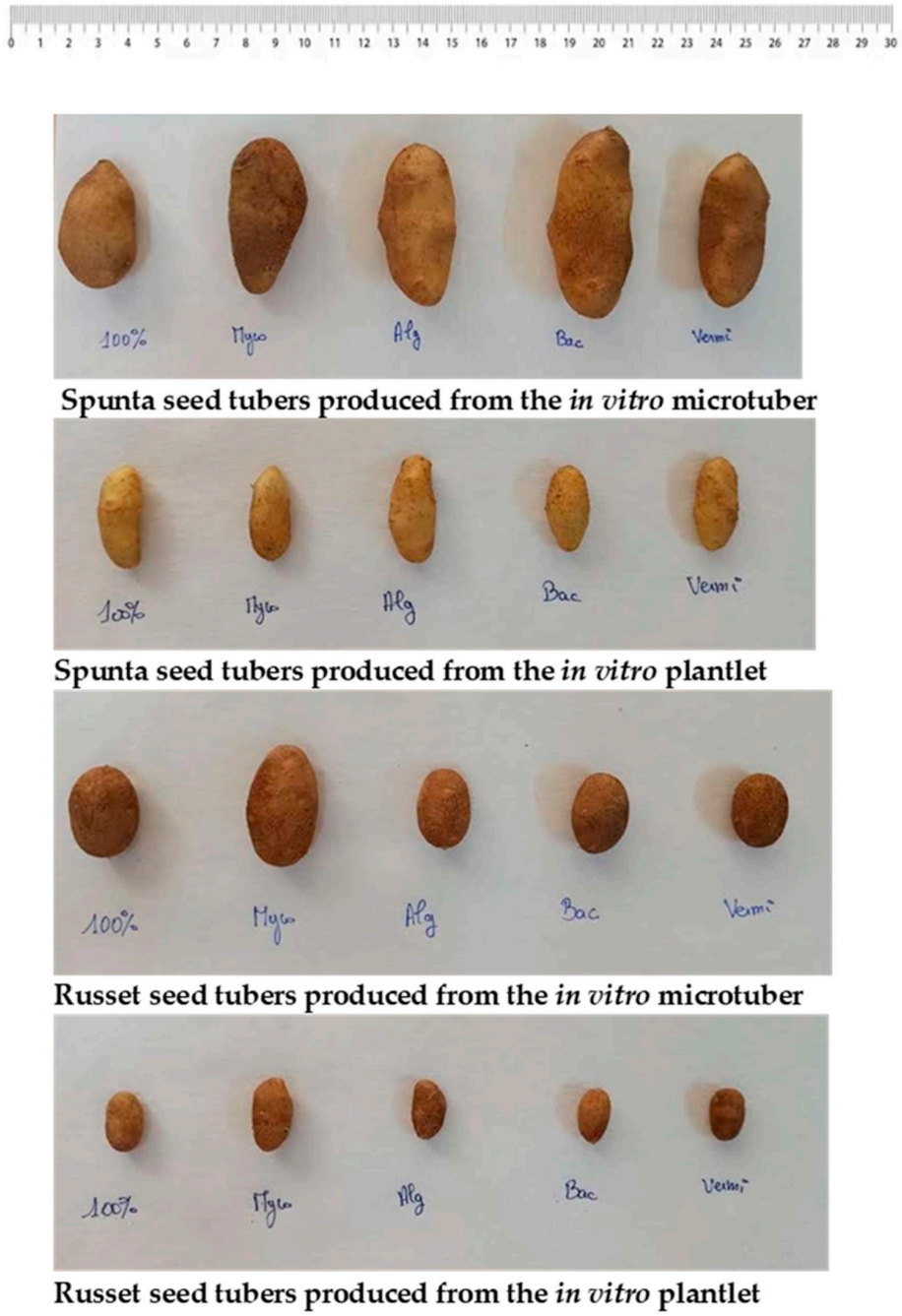

Figure 2. Harvested seed potato tubers from *in vitro* plantlets and *in vitro* microtubers in two cultivars and four different biofertilizers. Tubers lined up in order of T1 control, T2 Mychooriza, T3 Microalgae, T4 Beneficial bacteria (PGPR), T5 Vermicompost.

2.5. Total Sugar and Starch Analysis

The soluble sugar content was analyzed in seed tubers for each treatment and cultivar using high-performance liquid chromatography (HPLC) according to the method applied in [39]. Briefly, samples for sugar determination (glucose, fructose, and sucrose) were prepared from microtubers puree (approximately 10 g), diluted in 50 mL distilled water, and centrifuged at 6000× *g* for 15 min. The extract was filtered through 0.45 µm Millipore filters before analysis. Sugar analyses were performed by injecting 20 µL of the sample extract into an Aminex HPX-87C column held at 85 °C at a flow rate of 0.6 mL/min. Sugar present in each sample was quantified based on a peak of analytical standard obtained from Fluka Chemical (New York, NY, USA).

A 0.1 g fresh sample was homogenized in hot 80% ethanol to determine starch content, followed by centrifugation at 10,000 rpm for 20 min. Afterward, 5 mL of water and 6.5 mL of perchloric acid were added to the residue and refrigerated at 0 °C for 20 min. The resulting mixture was centrifuged, and the supernatant was used for analysis. The final volume was adjusted to 100 mL with distilled water, creating a 1:5 dilution. Then, 4 mL of the anthrone reagent was added to each test tube and heated for approximately 8 min in a boiling water bath. After rapid cooling, the green to dark green intensity was measured at 630 nm using a UV-visible spectrophotometer (Perkin Elmer, USA, model LAMBDA 365 + UV/Vis). Starch content in the fresh potato was determined using a series of glucose working standards solutions (20–100 µg mL^{-1}) [41].

2.6. Statistical Analysis

The statistical model involves a completely randomized design with three factors: 'cultivars,' '*in vitro* material,' and 'biofertilizer'. All plant measurements and yield were carried out in four replications. Starch, sugar, and mineral analyses were investigated in three replications. Each replication consisted of 3–5 plants, and the results represent the mean value. All data were analyzed using the JMP v5.0.1. statistical software. A three-way analysis of variance (ANOVA) was conducted. Treatment means were compared by LSD's significant difference test at $p \leq 0.05$. The significance levels for the three-way ANOVA analyzing the effects of cultivar, *in vitro* material, biofertilizer, and their interactions are presented in Table 3.

Table 3. Significance levels in three-way ANOVA analyzing the effects of cultivar, *in vitro* material, biofertilizer, and their interactions.

	Cultivar (Cv)	***In Vitro* Material (*In Vitro*)**	**Biofertilizer (B)**	**Cv × *In Vitro***	**Cv × B**	***In Vitro* × B**	**Cv × *In Vitro* × B**
Plant length	****	****	****	****	****	*	*
Branch number	****	****	****	**	**	***	****
Leaf number	****	****	ns	****	*	*	*
Stem diameter	****	****	****	****	****	ns	*
Chlorophyll	****	****	ns	****	ns	ns	ns
Shoot fresh w.	****	****	****	****	****	****	**
Shoot dry w.	****	****	****	****	****	****	****
Tuber dry w.	ns	****	ns	**	***	ns	ns
Tuber yield	****	****	****	****	****	****	****
Tuber no	****	****	****	****	****	****	****
Tuber diameter	****	****	****	****	ns	**	ns
Tuber weight	****	****	****	****	**	****	*
Starch	****	****	****	****	****	ns	**
Sugar	****	ns	****	ns	****	****	****
Nitrogen	****	**	****	ns	****	****	****

Table 3. *Cont.*

	Cultivar (Cv)	*In Vitro* Material (*In Vitro*)	Biofertilizer (B)	Cv × *In Vitro*	Cv × B	*In Vitro* × B	Cv × *In Vitro* × B
Potassium	****	****	****	****	****	****	****
Calcium	****	****	****	****	****	****	****
Magnesium	****	****	****	****	****	**	****

*: $p > 0.05$, **: $p \leq 0.05$, ***: $p \leq 0.01$, ****: $p \leq 0.001$, ns: not significant.

3. Results

3.1. Plant Growth Assessment

The experimental study of various biofertilizers was conducted to select the best ones that increase the growth parameters of potato plants from *in vitro* plantlets or from microtubers. There were significant differences regarding plant length, branch number, leaf number, plant stem diameter, SPAD chlorophyll, and shoot fresh and dry weights (Table 4). The effect of the cultivar was highly significant for all growth traits. Spunta performed better than the Russet cultivar in all plant growth parameters. In terms of *in vitro* material, plantlets consistently outperformed microtubers in all plant growth parameters except plant height. The study has clearly demonstrated the effectiveness of biofertilizers used in the experiment on plant growth parameters in showcasing the efficacy of vermicompost. However, no significant difference was observed among the four bio-fertilizers regarding the average chlorophyll content and leaf number compared to the control.

Regarding the 'Cultivar × *in vitro* Material' interaction, the 'Spunta' cultivar with a plantlet resulted in better-developed plants in all growth parameters except plant height. Thus, it is clear that the 'Spunta' cultivar from *in vitro* plantlets showed, in general, the highest performance regarding growth traits compared to the Russet cultivar. Russet exhibited more variable responses for *in vitro* material. The 'Russet × plantlet' combination showed better branch number, leaf number, and chlorophyll results. The 'Russet × microtuber' combination performed better in the plant height, stem diameter, shoot fresh weight, and shoot dry weight parameters.

In the 'Cultivar × Biofertilizer' interaction, the 'Spunta' cultivar achieved robust plant growth across all parameters, with vermicompost biofertilizer being the most effective, followed by mycorrhiza and bacteria, respectively. On the other hand, plants of the 'Russet' cultivar grew slightly better with mycorrhiza than with other biofertilizers. The response of 'Russet' to biofertilizer was not as clear as that of 'Spunta'.

In the '*in vitro* Material × Biofertilizer' interaction, the best responses for the branch number, shoot fresh weight, and shoot dry weight parameters were observed using vermicompost biofertilizer, regardless of whether a microtuber or plantlet was employed. No significant differences were observed in plant length, stem diameter, and chlorophyll parameters within this interaction.

In the triple interaction of 'Cultivar × *in vitro* Material × Biofertilizer', no statistically significant difference was observed in SPAD-chlorophyll. However, branch number, shoot fresh weight, and shoot dry weight were significantly essential and the best (4.25 plants^{-1}, 0.866 plants^{-1}, and 0.866 plants^{-1}, respectively) in the combination of 'Spunta × Plantlet × Vermicompost'. In the same triple interaction, plant length, leaf number, and stem diameter were better than in the other combinations (Table 5).

Table 4. Main effect of cultivar, *in vitro* material, and biofertilizer, as well as the two-way interactions effects of all factors, on plant growth parameters.

Factor		Plant Length (cm)	Branch Number	Leaf Number	Stem Diameter (mm)	SPAD Chlorophyll	Shoot FW (g)	Shoot DW (g)	Tuber DM (%)
Cultivar									
Spunta		81.66 ^{a}	2.35 ^{a}	21.85 ^{a}	6.60 ^{a}	36.70 ^{a}	4.43 ^{a}	0.435 ^{a}	9.67
Russet		22.44 ^{b}	1.38 ^{b}	6.38 ^{b}	2.80 ^{b}	24.65 ^{b}	0.86 ^{b}	0.090 ^{b}	9.41
***In vitro* material**									
Microtuber		54.15 ^{a}	1.50 ^{b}	9.48 ^{b}	4.43 ^{b}	23.88 ^{b}	2.12 ^{b}	0.20 ^{b}	9.08 ^{b}
Plantlet		49.94 ^{b}	2.23 ^{a}	18.75 ^{a}	4.97 ^{a}	37.46 ^{a}	3.16 ^{a}	0.33 ^{a}	10.00 ^{a}
Biofertilizer									
Control		46.19 ^{c}	1.44 ^{b}	14.19	4.76 ^{ab}	30.67	2.40 ^{bc}	0.22 ^{b}	9.36
Mycorrhiza		54.19 ^{ab}	1.56 ^{b}	14.13	4.77 ^{ab}	31.28	2.68 ^{b}	0.25 ^{b}	9.01
Microalgea		52.50 ^{ab}	1.81 ^{b}	14.31	4.30 ^{c}	29.67	2.12 ^{c}	0.24 ^{b}	10.12
Bacteria		51.75 ^{b}	1.38 ^{b}	14.06	4.53 ^{bc}	30.89	2.51 ^{b}	0.26 ^{b}	9.30
Vermicompost		55.61 ^{a}	3.13 ^{a}	13.88	5.14 ^{a}	30.85	3.52 ^{a}	0.35 ^{a}	9.91
Cultivar × *in vitro* Material									
Spunta	Microtuber	85.40 ^{a}	1.80 ^{b}	13.70 ^{b}	5.72 ^{b}	28.63 ^{b}	3.36 ^{b}	0.290 ^{b}	8.89 ^{b}
	Plantles	77.91 ^{b}	2.90 ^{a}	30.00 ^{a}	7.47 ^{a}	44.77 ^{a}	5.50 ^{a}	0.579 ^{a}	10.45 ^{a}
Russet	Microtuber	22.90 ^{c}	1.20 ^{c}	5.25 ^{d}	3.13 ^{c}	19.14 ^{c}	0.89 ^{c}	0.102 ^{c}	9.27 ^{b}
	Plantles	21.98 ^{c}	1.55 ^{bc}	7.50 ^{c}	2.47 ^{d}	30.16 ^{b}	0.82 ^{c}	0.078 ^{c}	9.55 ^{b}
Cultivar × Biofertilizer									
Spunta	Control	69.63 ^{c}	1.50 ^{def}	21.63 a	6.56 ^{b}	36.24	3.88 ^{bc}	0.362 ^{b}	9.34 ^{bc}
	Mycorrhiza	84.38 ^{b}	2.00 ^{bcd}	21.63 a	6.85 ^{ab}	36.04	4.37 ^{b}	0.409 ^{b}	9.28 ^{bc}
	Microalgea	82.6 ^{b}	2.63 ^{b}	22.50 a	5.70 ^{c}	36.30	3.50 ^{c}	0.410 ^{b}	11.39 ^{a}
	Bacteria	82.13 ^{b}	1.75 ^{cde}	21.50 a	6.43 ^{b}	37.46	4.24 ^{b}	0.386 ^{b}	8.93 ^{c}
	Vermicompost	89.65 ^{a}	3.88 ^{a}	22.00 a	7.45 ^{a}	37.45	6.17 ^{a}	0.605 ^{a}	9.41 ^{bc}
Russet	Control	22.75 ^{d}	1.38 ^{def}	6.75 b	2.96 ^{d}	25.10	0.92 ^{d}	0.086 ^{c}	9.37 ^{bc}
	Mycorrhiza	24.00 ^{d}	1.13 ^{ef}	6.63 b	2.69 ^{d}	26.51	0.98 ^{d}	0.086 ^{c}	8.74 ^{c}
	Microalgea	22.50 ^{d}	1.00 ^{f}	6.13 b	2.90 ^{d}	23.04	0.74 ^{d}	0.064 ^{c}	8.85 ^{c}
	Bacteria	21.38 ^{d}	1.00 ^{f}	6.63 b	2.64 ^{d}	24.33	0.77 ^{d}	0.127 ^{c}	9.68 ^{bc}
	Vermicompost	21.56 ^{d}	2.38 ^{bc}	5.75 b	2.84 ^{d}	24.25	0.87 ^{d}	0.089 ^{c}	10.41 ^{ab}

Table 4. *Cont.*

Factor		Plant Length (cm)	Branch Number	Leaf Number	Stem Diameter (mm)	SPAD Chlorophyll	Shoot FW (g)	Shoot DW (g)	Tuber DM (%)
***In vitro* material × Biofertilizer**									
Microtuber	Control	49.63	1.38 ^{d}	10.13 b	4.67	23.60	2.14 ^{d}	0.183 ^{ef}	8.67
	Mycorrhiza	57.25	1.50 ^{d}	9.63 b	4.51	24.66	2.34 ^{cd}	0.207 ^{de}	8.84
	Microalgea	54.88	1.25 ^{d}	9.75 b	4.05	21.68	1.28 ^{e}	0.137 ^{f}	10.29
	Bacteria	53.63	1.13 ^{d}	9.25 b	4.13	24.23	2.06 ^{d}	0.230 ^{cde}	8.94
	Vermicompost	55.38	2.25 ^{bc}	8.63 b	4.78	25.25	2.82 ^{bc}	0.224 ^{cde}	8.65
Plantlet	Control	42.75	1.50 ^{d}	18.25 a	4.85	37.74	2.66 ^{bc}	0.265 ^{cd}	10.04
	Mycorrhiza	51.13	1.63 ^{cd}	18.63 a	5.04	37.89	3.01 ^{b}	0.289 ^{bc}	9.19
	Microalgea	51.12	2.38 ^{b}	18.88 a	4.54	37.66	2.96 ^{b}	0.337 ^{b}	9.95
	Bacteria	49.88	1.63 ^{cd}	18.87 a	4.94	37.56	2.95 ^{b}	0.283 ^{bc}	9.67
	Vermicompost	55.88	4.00 ^{a}	19.13 a	5.51	36.45	4.22 ^{a}	0.470 ^{a}	11.17

Means followed by the same letters within a column are statistically similar based on LSD's significant difference test at $p \leq 0.05$; data are means of four replications.

Table 5. Three-way-interactions effects of cultivar, *in vitro* material, and biofertilizer on plant growth parameters.

Factor										
Cultivar	***In Vitro Material***	***Biofertilizer***	**Plant Length (cm)**	**Branch No per Plant**	**Leaf No per Plant**	**Stem Diameter (mm)**	**SPAD Chlorophyll**	**Shoot FW (g)**	**Shoot DW (g)**	**Tuber DM (%)**
Spunta	Microtuber	Control	73.25 ^{cd}	1.00 ^{d}	14.25 ^{b}	5.88 ^{cd}	27.70	3.35 ^{d}	0.287 ^{ef}	8.73
		Mycorrhiza	90.25 ^{a}	1.75 ^{bcd}	13.25 ^{b}	5.92 ^{cd}	28.90	3.55 ^{d}	0.313 ^{e}	8.83
		Microalgea	89.50 ^{a}	1.50 ^{bcd}	14.75 ^{b}	5.23 ^{d}	27.00	1.93 ^{e}	0.214 ^{fg}	10.84
		Bacteria	84.75 ^{ab}	1.25 ^{cd}	13.25 ^{b}	5.37 ^{cd}	29.30	3.34 ^{d}	0.283 ^{ef}	8.36
		Vermicompost	89.25 ^{a}	3.50 ^{a}	13.00 ^{b}	6.22 ^{c}	30.25	4.65 ^{bc}	0.355 ^{de}	7.67
	Plantlet	Control	66.00 ^{d}	2.00 ^{bc}	29.00 ^{a}	7.24 ^{b}	44.78	4.40 ^{c}	0.438 ^{cd}	9.97
		Mycorrhiza	78.50 ^{bc}	2.25 ^{b}	30.00 ^{a}	7.78 ^{b}	43.18	5.20 ^{b}	0.506 ^{c}	9.74
		Microalgea	75.50 ^{c}	3.75 ^{a}	30.25 ^{a}	6.18 ^{c}	45.60	5.08 ^{bc}	0.606 ^{b}	11.93
		Bacteria	79.50 ^{bc}	2.25 ^{b}	29.75 ^{a}	7.50 ^{b}	45.63	5.14 ^{b}	0.490 ^{c}	9.50
		Vermicompost	90.06 ^{a}	4.25 ^{a}	31.00 ^{a}	8.68 ^{a}	44.65	7.68 ^{a}	0.866 ^{a}	11.14
Russet	Microtuber	Control	26.00 ^{e}	1.75 ^{bcd}	6.00 $^{c-f}$	3.45 ^{e}	19.50	0.92 ^{f}	0.080 ^{i}	8.61
		Mycorrhiza	24.25 ^{e}	1.25 ^{cd}	6.00 $^{c-f}$	3.10 ^{ef}	20.43	1.13 ^{f}	0.101 ^{hi}	8.84
		Microalgea	20.25 ^{e}	1.00 ^{d}	4.75 ^{ef}	2.87 ^{ef}	16.35	0.63 ^{f}	0.060 ^{i}	9.73
		Bacteria	22.50 ^{e}	1.00 ^{d}	5.25 ^{def}	2.90 ^{ef}	19.15	0.76 ^{f}	0.177 ^{gh}	9.52
		Vermicompost	21.50 ^{e}	1.00 ^{d}	4.25 ^{f}	3.34 ^{e}	20.25	0.98 ^{f}	0.094 ^{hi}	9.62
	Plantlet	Control	19.50 ^{e}	1.00 ^{d}	7.50 ^{cd}	2.47 ^{f}	30.70	0.93 ^{f}	0.092 ^{hi}	10.12
		Mycorrhiza	23.75 ^{e}	1.00 ^{d}	7.25 ^{cde}	2.29 ^{f}	32.60	0.83 ^{f}	0.072 ^{i}	8.65
		Microalgea	24.75 ^{e}	1.00 ^{d}	7.50 ^{cd}	2.92 ^{ef}	29.73	0.85 ^{f}	0.068 ^{i}	7.96
		Bacteria	20.25 ^{e}	1.00 ^{d}	8.00 ^{c}	2.37 ^{f}	29.50	0.76 ^{f}	0.076 ^{i}	9.84
		Vermicompost	21.63 ^{e}	3.75 ^{a}	7.25 ^{cde}	2.34 ^{f}	28.25	0.76 ^{f}	0.085 ^{hi}	11.19

Means followed by the same letters within a column are statistically similar based on LSD's significant difference test at $p \leq 0.05$; data are means of four replications. FW: Fresh Weight, DW: Dry Weight, DM: Dry Matter.

There was no difference in tuber dry matter between the varieties (Table 5). The plantlet material provided a higher dry matter content than the microtuber. Regarding the tuber dry matter content, no significant impact of biofertilizers was observed. In the 'Cultivar × *in vitro* Material' interaction, the 'Spunta × Plantlet' combination was recorded as the highest, with 10.45% dry matter content. A variation was observed in the 'Cultivar × Biofertilizer' interaction. However, the 'Spunta × microalgea' and 'Russet × vermicompost' combinations yielded 11.40% and 10.41% higher dry matter, respectively. The *'in vitro* material × Biofertilizer' interaction did not show statistically significant differences. However, the 'Plantlet × vermicompost' interaction recorded the highest dry matter content at 11.17%. The effects of the 'Cultivar × *in vitro* Material × Biofertilizer' interaction were not statistically significant. However, the combination 'Spunta' × Plantlet × Microalgae' showed the highest dry matter content at 11.93%, while 'Russet × Microtuber × Control' exhibited the lowest dry matter content at 8.61%.

3.2. Effect of Bio-Fertilizers on Yield and Yield Components

Figure 3 displays the yield per plant. Highly significant differences were observed among the treatments, cultivars, *in vitro* material, biofertilizer, and their interactions. There was a significant difference in tuber yield between the varieties, with 'Spunta' yielding 93.47 g and 'Russet' yielding 29.63 g per plant. In terms of yield among the *in vitro* materials used, the microtuber outperformed the plantlet significantly with a yield of 79.15 g compared to 43.95 g. The highest seed tuber yield among the biofertilizers was obtained from vermicompost, being 96.70 g. The other biofertilizers performed at a similar significance level to the control and yielded between 47.75 g and 54.94 g.

In the 'Cultivar × *in vitro* material' interaction, the 'Spunta + microtuber' combination produced the highest yield at 124.06 g. The second-highest yield was obtained from the 'Spunta + plantlet' combination at 62.89 g. While lower than Spunta combinations, Russet cultivar yields still produced 34.24 g from microtubers and 25.01 g from plantlets.

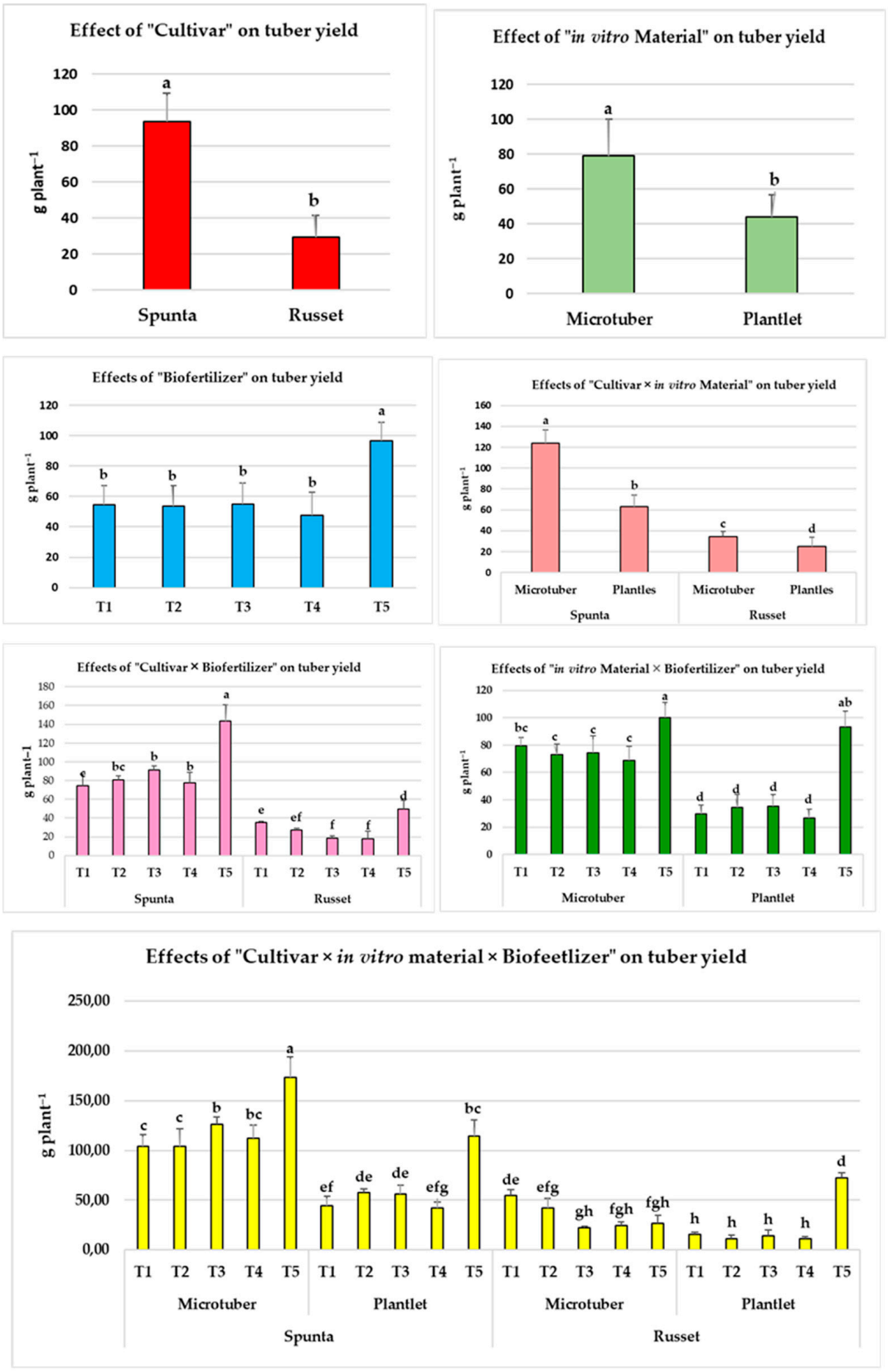

Figure 3. Effects of cultivar, *in vitro* material, biofertilizer, and three-way-interactions on tuber yield per plant. T1 control, T2 Mycorrhiza, T3 Microalgae, T4 Beneficial bacteria (PGPR), T5 Vermicompost. There is no significant difference between means with the same letter in the same color histogram; data are means of four replications.

Regarding tuber yield, in the 'Cultivar × Biofertilizer' interactions, vermicompost had the highest yield in both 'Spunta' and 'Russet' cultivars, with 143.64 g and 49.77 g, respectively. In the case of the 'Spunta' cultivar, the second most productive biofertilizer was microalgae (91.33 g). In contrast, in the 'Russet' cultivar, the second most productive biofertilizer was mycorrhiza (26.88 g), which was lower than the control (34.95 g).

In the '*in vitro* material × biofertilizer' interaction, vermicompost biofertilizer yielded the best results in both *in vitro* materials. Accordingly, 'microtuber + vermicompost' yielded 100.05 g, while 'plantlet + vermicompost' yielded 93.35 g of tubers. When compared to the control, which used 100% mineral fertilizer, 'microtuber × control' yielded 79.34 g, while 'plantlet × control' yielded 29.75 g.

In the 'Cultivar × *in vitro* material × biofertilizer' interaction, the highest seed tuber yield of 173.12 g was obtained from the 'Spunta + microtuber + vermicompost' combination with a significant difference (Figure 3). In this triple combination, the second-highest tuber yield was achieved from the combination 'Spunta + microtuber + microalgae' with 126.55 g. For the Russet cultivar, the highest yield in the triple combination was 72.55 g of tubers obtained from the combination 'Russet + plantlet + vermicompost.' The lowest yield was obtained from the combination 'Russet + plantlet + bacteria' with 11.53 g.

In the study, when the potato varieties were compared regarding tuber diameter, weight, and tuber number, the 'Spunta' cultivar's performance was significantly higher than that of the 'Russet' cultivar (Figure 4). Seed tubers derived from *in vitro* microtubers had a larger diameter and were heavier than those derived from *in vitro* plantlets. Thus, the seed size obtained from microtubers was greater than that from plantlets. The number of tubers produced was significantly lower from microtubers than from plantlets. The effect of biofertilizers on tuber number was highest in vermicompost. The effect of biofertilizers on tuber weight and diameter was slightly lower than that of the control.

In the 'Cultivar × *in vitro* material' interaction, the highest tuber number was obtained from the 'Spunta + plantlet' combination, being 8.25. The heaviest and largest tubers, with 28.26 g and 29.11 mm, respectively, were obtained from the 'Spunta + microtuber' combination.

In the 'Cultivar × Biofertilizer' interaction, the tuber number was highest in vermicompost for both cultivars, with 9.0 for Spunta and 5.8 for Russet. Regarding tuber weight, the impact of biofertilizers was lower than that of the control in both cultivars. The effect of biofertilizers on tuber diameter was not statistically significant on a cultivar basis.

In the '*in vitro* material × Biofertilizer' interaction, the vermicompost produced the highest tuber numbers among the biofertilizers, with 9.75 from plantlets and 5.80 from microtubers. Biofertilizers did not significantly affect the weights of seeds produced from microtubers. However, the weights of seeds produced from plantlets were the highest when treated with vermicompost. The effect of biofertilizers on tuber diameter, whether produced from microtubers or plantlets, was not found to be statistically significant.

The 'Cultivar × *in vitro* material × biofertilizer' interaction was not found to be significant in terms of tuber diameter. In the case of the Spunta cultivar, seeds produced from microtubers had a lower number but higher weight and larger diameter compared to tubers produced from plantlets across all biofertilizers, including the control.

3.3. Effect of Bio-Fertilizers on Mineral Content

The seed tubers' nitrogen, potassium, calcium, and magnesium contents were higher in the Russt variety than in the 'Spunta' cultivar. While the tuber produced from the plantlet material had higher K and Mg contents, higher N and Ca contents were recorded from the microtuber material (Table 6). Vermicompost and bacteria biofertilizers showed a high content of nitrogen and potassium. Bacteria stood out regarding magnesium, and mycorrhiza stood out regarding calcium.

Figure 4. Effects of cultivar, *in vitro* material, biofertilizer three-way-interactions on tuber number per plant, weight (g), and diameter (mm). T1 control, T2 Mycorrhiza, T3 Microalgae, T4 Beneficial bacteria (PGPR), T5 Vermicompost. There is no significant difference between means with the same letter in the same color histogram; data are means of four replications. The parameters that are non-significant are not lettered.

Table 6. Main effect of cultivar, *in vitro* material, biofertilizer, and the two-way interactions on the tubers' sugar, starch, and nutrients.

Factor		Sugar (mg kg $^{-1}$)	Starch (mg kg $^{-1}$)	Nitrogen (%)	Potassium (mg kg $^{-1}$)	Magnesium (mg kg $^{-1}$)	Calcium (mg kg $^{-1}$)
Cultivar							
Spunta		0.262 ^{a}	0.226 ^{b}	3.18 ^{b}	328.28 ^{b}	20.82 ^{a}	9.84 ^{b}
Russet		0.231 ^{b}	0.287 ^{a}	3.41 ^{a}	453.19 ^{a}	17.82 ^{b}	10.86 ^{a}
***In vitro* Material**							
Microtuber		0.244	0.234 ^{b}	3.31 ^{a}	350.54 ^{b}	19.03 ^{b}	10.61 ^{a}
Plantlet		0.249	0.280 ^{a}	3.28 ^{b}	430.94 ^{a}	19.61 ^{a}	10.09 ^{b}
Biofertilizer							
Control		0.258 ^{a}	0.209 ^{b}	3.11 ^{d}	366.83 ^{c}	17.63 ^{d}	9.83 ^{b}
Mycorrhiza		0.248 ^{b}	0.227 ^{b}	3.22 ^{c}	361.23 ^{c}	19.77 ^{b}	10.99 ^{a}
Microalgea		0.247 ^{b}	0.269 ^{a}	3.31 ^{b}	365.54 ^{c}	19.19 ^{c}	10.89 ^{a}
Bacteria		0.250 ^{b}	0.284 ^{a}	3.40 ^{a}	415.20 ^{b}	20.87 ^{a}	10.16 ^{b}
Vermicompost		0.229 ^{c}	0.294 ^{a}	3.43 ^{a}	444.90 ^{a}	19.15 ^{c}	9.89 ^{b}
Cultivar × *in vitro* Material							
Spunta	Microtuber	0.260	0.179 ^{b}	3.17	333.84 ^{c}	20.07 ^{b}	9.80 ^{c}
	Plantles	0.264	0.272 ^{a}	3.19	322.72 ^{c}	21.58 ^{a}	9.88 ^{bc}
Russet	Microtuber	0.228	0.289 ^{a}	3.39	367.23 ^{b}	17.99 ^{c}	11.41 ^{a}
	Plantles	0.233	0.288 ^{a}	3.42	539.17 ^{a}	17.65 ^{c}	10.32 ^{b}
Cultivar × Biofertilizer							
Spunta	Control	0.303 ^{a}	0.125 ^{e}	3.02 ^{e}	340.40 ^{e}	20.00 ^{c}	9.60 ^{cd}
	Mycorrhiza	0.264 ^{b}	0.202 ^{d}	3.03 ^{e}	323.60 ^{ef}	21.88 ^{b}	11.30 ^{a}
	Microalgea	0.248 ^{c}	0.249 ^{c}	3.18 ^{d}	296.13 ^{f}	19.90 ^{c}	10.61 ^{ab}
	Bacteria	0.271 ^{b}	0.250 ^{c}	3.33 ^{c}	351.90 ^{e}	23.00 ^{a}	9.04 ^{de}
	Vermicompost	0.224 ^{e}	0.301 ^{a}	3.34 ^{c}	329.38 ^{ef}	19.34 ^{cd}	8.66 ^{e}
Russet	Control	0.212 ^{f}	0.294 ^{ab}	3.20 ^{d}	393.25 ^{d}	15.26 ^{g}	10.06 ^{bc}
	Mycorrhiza	0.232 ^{de}	0.252 ^{bc}	3.41 ^{b}	398.87 ^{d}	17.67 ^{f}	10.68 ^{ab}
	Microalgea	0.245 ^{c}	0.288 ^{abc}	3.44 ^{b}	434.95 ^{c}	18.48 ^{e}	11.18 ^{a}
	Bacteria	0.230 ^{de}	0.320 ^{a}	3.46 ^{ab}	478.50 ^{b}	18.74 ^{de}	11.28 ^{a}
	Vermicompost	0.235 ^{d}	0.286 ^{abc}	3.52 ^{a}	560.43 ^{a}	18.95 ^{de}	11.12 ^{a}

Table 6. *Cont.*

Factor		Sugar (mg kg $^{-1}$)	Starch (mg kg $^{-1}$)	Nitrogen (%)	Potassium (mg kg $^{-1}$)	Magnesium (mg kg $^{-1}$)	Calcium (mg kg $^{-1}$)
***In vitro* material × Biofertilizer**							
Microtuber	Control	0.253 ^{d}	0.197	3.15 ^{g}	337.93 ^{ef}	17.09 ^{e}	10.78 ^{b}
	Mycorrhiza	0.242 ^{ef}	0.196	3.24 ^{ef}	307.75 ^{fg}	19.66 ^{c}	10.34 ^{b}
	Microalgea	0.212 ^{g}	0.259	3.35 ^{c}	287.60 ^{g}	18.53 ^{d}	11.59 ^{a}
	Bacteria	0.265 ^{b}	0.262	3.34 ^{c}	365.83 ^{de}	21.11 ^{a}	10.88 ^{ab}
	Vermicompost	0.248 ^{de}	0.254	3.32 ^{cd}	453.58 ^{a}	18.75 ^{d}	9.45 ^{cd}
Plantlet	Control	0.263 ^{bc}	0.222	3.08 ^{h}	395.73 ^{cd}	18.17 ^{d}	8.88 ^{d}
	Mycorrhiza	0.253 ^{cd}	0.258	3.20 ^{fg}	414.72 ^{bc}	19.88 ^{bc}	11.64 ^{a}
	Microalgea	0.281 ^{a}	0.279	3.27 ^{de}	443.48 ^{ab}	19.85 ^{c}	10.19 ^{bc}
	Bacteria	0.236 ^{f}	0.307	3.45 ^{b}	464.58 ^{a}	20.62 ^{ab}	9.44 ^{cd}
	Vermicompost	0.211 ^{g}	0.333	3.54 ^{a}	436.23 ^{ab}	19.54 ^{c}	10.33 ^{b}

Means followed by the same letters within a column are statistically similar based on LSD's significant difference test at $p \leq 0.05$; data are means of three replications.

In the interaction of 'Cultivar × *in vitro* Material', the 'Russet' variety's *in vitro* materials exhibited higher nitrogen, potassium, and calcium contents in the seed tubers. In the 'Cultivar × Biofertilizer' interaction, combinations of the 'Russet' cultivar with the biofertilizers provided higher nitrogen, potassium, magnesium, and calcium. In the '*in vitro* Material × Biofertilizer' interaction, it was found that the mineral content stood out in the combination of plantlet and biofertilizers.

In the 'Cultivar × *in vitro* Material × Biofertilizer' interaction, the 'Russet' cultivar generally exhibited a higher mineral content, especially with the use of plantlets and vermicompost, compared to the control (Table 7).

Table 7. Three-way-interactions effects of cultivar, *in vitro* material, and biofertilizer on tubers' sugar, starch, and nutrients.

Factor								
Cultivar	***In Vitro* Material**	**Biofertilizer**	**Sugar (mg kg^{-1})**	**Starch (mg kg^{-1})**	**N (%)**	**K (mg kg^{-1})**	**Mg (mg kg^{-1})**	**Ca (mg kg^{-1})**
Spunta	Microtuber	Control	0.276 ^{bc}	0.109 ^{k}	3.13 ^{fg}	392.35 ^{c}	19.57 ^{ef}	11.39 ^{bcd}
		Mycorrhiza	0.263 ^{cd}	0.156 ^{ijk}	3.05 ^{hg}	316.80 ^{efg}	20.88 ^{cd}	10.18 ^{fghi}
		Microalgea	0.220 ^{h}	0.200 ^{hij}	3.22 ^{def}	261.30 ^{h}	18.12 ^{g}	11.06 ^{cdef}
		Bacteria	0.287 ^{b}	0.219 ^{fgh}	3.22 ^{def}	357.45 $^{c–e}$	23.25 ^{a}	8.82 ^{jk}
		Vermicompost	0.255 ^{def}	0.211 ^{ghi}	3.23 ^{de}	341.30 $^{d–f}$	18.55 ^{fg}	7.58 ^{l}
	Plantlet	Control	0.331 ^{a}	0.142 ^{jk}	2.91 ^{I}	288.45 ^{gh}	20.45 ^{de}	7.81 ^{kl}
		Mycorrhiza	0.265 ^{cd}	0.248 $^{d-h}$	3.02 ^{h}	330.40 ^{efg}	22.88 ^{a}	12.42 ^{ab}
		Microalgea	0.275 ^{bc}	0.301 ^{bcd}	3.15 ^{ef}	330.95 $^{d–g}$	21.68 ^{bc}	10.15 ^{fghi}
		Bacteria	0.256 ^{de}	0.280 ^{bcde}	3.45 ^{bc}	346.35 $^{c–f}$	22.75 ^{ab}	9.27 ^{ij}
		Vermicompost	0.193 ^{j}	0.390 ^{a}	3.45 ^{bc}	317.45 ^{efg}	20.13 ^{de}	9.74 ^{hij}
Russet	Microtuber	Control	0.230 ^{gh}	0.284 ^{bcde}	3.16 ^{def}	283.50 ^{gh}	14.62 ^{I}	10.17 ^{fghi}
		Mycorrhiza	0.222 ^{h}	0.237 ^{efgh}	3.44 ^{bc}	298.70 $^{f–h}$	18.45 ^{g}	10.51 $^{d–h}$
		Microalgea	0.205 ^{ij}	0.318 ^{bc}	3.48 ^{b}	313.90 ^{efg}	18.93 ^{fg}	10.13 ^{abc}
		Bacteria	0.243 ^{efg}	0.304 ^{bcd}	3.46 ^{bc}	374.20 ^{cd}	18.98 ^{fg}	12.94 ^{a}
		Vermicompost	0.242 ^{efg}	0.297 ^{bcde}	3.40 ^{bc}	565.85 ^{a}	18.96 ^{fg}	11.31 ^{cde}
	Plantlet	Control	0.195 ^{j}	0.303 ^{bcd}	3.24 ^{d}	503.00 ^{b}	15.89 ^{h}	9.95 ^{ghi}
		Mycorrhiza	0.241 ^{fg}	0.267 $^{c–g}$	3.38 ^{c}	499.04 ^{b}	16.89 ^{h}	10.86 ^{defg}
		Microalgea	0.285 ^{b}	0.257 $^{d–h}$	3.40 ^{bc}	556.00 ^{a}	18.02 ^{g}	10.23 $^{e–i}$
		Bacteria	0.216 ^{hi}	0.335 ^{ab}	3.47 ^{bc}	582.80 ^{a}	18.50 ^{fg}	9.62 ^{hij}
		Vermicompost	0.229 ^{gh}	0.276 $^{b–f}$	3.63 ^{aP}	550.00 ^{aP}	18.95 ^{fg}	10.93 ^{defg}

Means followed by the same letters within a column are statistically similar based on LSD's significant difference test at $p \leq 0.05$; data are means of three replications.

3.4. Effect of Bio-Fertilizers on Sugar and Starch Contents

While the 'Spunta' cultivar contained high sugar and low starch, the 'Russet' cultivar contained the opposite low sugar and high starch (Table 6). The *in vitro* material tubers showed no difference in sugar, while plantlets was higher in starch than in microtubers. The biofertilizers differentially affected the sugar and starch contents, T1-control induced more sugar than starch, and the average values were 0.255 and 0.209 mg kg^{-1}, respectively. In contrast, the three bio-fertilizers T3-T4-T5 showed a significant increase in starch in tubers, thus reflecting their beneficial impact on yield per plant, especially T5. The late treatment induced the lowest value of sugar compared to all other treatments. Treatments T2–T4 induced similar sugar contents, while plants fertilized by T3–T5 produced tubers containing starch contents that were not significantly different.

In the 'Cultivar × *in vitro* Material' interaction, the difference in sugar content was insignificant. Regarding starch content, the *in vitro* materials of 'Russet' stood out. The interaction between 'Cultivar × Biofertlizer' was significant for sugar and starch contents. As such, when using T1, the highest sugar level was recorded in 'Spunta' from plantlets, while the highest starch content was noted in "Russet + Bacteria'.

In the '*in vitro* material × Biofertilizer' interaction, the plantlet material provided higher sugar and starch contents than the microtuber material. In the 'Cultivar × *in vitro* Material × Biofertilizer' interaction, the highest sugar content was recorded at 0.330 mg.kg^{-1} in the 'Spunta + plantlet + control' combination. In the 'Cultivar × *in vitro* Material × Biofertilizer' interaction, the highest starch content was recorded with 0.390 mg.kg^{-1} in the 'Spunta + plantlet + vermicompost' combination.

4. Discussion

The efficient fertilization of potato seed production, whether from *in vitro* plantlets or microtubers, is considered of utmost importance for the potato industry: seed companies and producers aim to enhance plant growth, yield, and seed quality components. Utilizing beneficial microorganisms, their composition, inoculation procedures, and the ratios with mineral fertilizers is being explored, necessitating the generation of novel information in this domain. Given that the use of biofertilizers is a proprietary technique for those interested in potato seed engaged in the production of potato micro/minitubers, this experiment was designed to assess the impact of various biofertilizers on plant growth traits, yield, and the quality properties of the tubers.

Our results showed that biofertilizers had a significant and differential influence on potato plant growth, such as plant length, stem number, fresh and dry weights per plant, and dry matter in % (Table 4). The positive impacts of these biofertilizers can be linked to their ability for the biological nitrogen fixation of the bacteria and the synthesis of plant hormones such as gibberellins, cytokinin-like compounds, and auxins [42]. These substances stimulate the growth and branching of roots [43], leading to improved water and nutrient absorption efficiency [27,31,32].

Vermicompost, with a composition of 10% total organic matter, 2% organic nitrogen, 0.2% water-soluble potassium pentoxide, 10% free amino acids, and beneficial microorganisms such as PGPRs and mycorrhizas, was found to be more effective in enhancing growth parameters and seed tuber yield. According to Yourtchi et al. [34], the combined effects of varying nitrogen rates and the application of vermicompost has a noteworthy impact. This impact was observed in the significant enhancement of growth parameters, yield, and the NPK content of tubers when compared to treatments involving nitrogen or vermicompost alone.

Vermicompost is efficient on different plant species, such as potatoes [23,44], tomatoes [45,46], strawberries [47,48], and peppers [49]. Manjunath et al. [50] demonstrated that manure and vermicompost led to the prevalence of amino acids, phenolics, and polymer-utilizing microorganisms, resulting in the enhanced metabolic activity of microorganisms.

In addition to plant length and stem number, using T5 resulted in the best shoot weight because of more nutrients made available from the vermicompost and probably the improved physicochemical and microbial conditions of the root environment. This situation possibly resulted in increased leaf number, as we observed when the four biofertilizers were applied in 'Spunta' from plantlets (Table 4), which can lead to an increased photosynthesis rate [51]. Plant growth parameters were significantly different from the main effect of cultivars. As such, 'Spunta' from *in vitro* plantlets showed the highest values of stem and leave numbers per plant, stem diameter, total chlorophyll, and the fresh and dry weights of shoots. This indicates the differential response of potato cultivars and the genetic effect as reported in previous investigations [51,52].

One of the most significant findings is that potato plants, which were supplied with only 40% of the recommended mineral fertilizers and the biofertilizer, exhibited a noteworthy outperformance compared to the control plants. This superiority was observed in terms of tuber yield per plant, the number of tubers per plant, and the dry weight of tubers. These outcomes held true for plantlets and microtubers [32,53]. The positive effects of biofertilizers on crop yield and its components can be attributed to various factors. The stimulating effect of nitrogen-fixing bacteria includes enhanced nitrogen fixation, which leads to increased nitrogen assimilation by plants and subsequently stimulates plant

growth. Additionally, the biofertilizer mycorrhiza contributes to improved plant mineral uptake and promotes better root growth and functionality. The biofertilizers produce phytohormones such as indole acetic acid, gibberellins, and cytokinins while decreasing abscisic acid. Furthermore, the biofertilizer's activities produce amino acids and phenolic compounds, contributing to overall plant health. Another benefit is the improvement in the water status of plants. Nitrate reductase activity is heightened, leading to better nutrient utilization, and the biofertilizers even play a role in producing compounds that act against pathogens [32]. Araújo et al. [54] and Sharma and Pandey [55] reported that applying biofertilizers in conjunction with mineral fertilizers significantly influenced the marketable tuber yield of potatoes compared to untreated plants.

In our experiment, yield and yield components were found to be significantly affected by different biofertilizers and cultivars, where T5 applied in 'Spunta' from microtubers induced the highest values of yield per plant (173.12 g) (Figure 3), indicating the beneficial and positive impact of vermicompost on potato yield. Regarding the number of tubers per plant, treatments T5 (Vermicompost), T2 (Mychorriza), and T4 (Bacteria), applied in 'Spunta' from *in vitro* plantlets, induced the highest number of tubers per plant (10.75, 8.96, and 8.02, respectively) (Figure 4). Ansari et al. [33] reported a significant increase in potato yield and tuber weight with the application of vermicompost. The organic content of vermicompost stimulates humification, enhances microbial activity, and increases enzyme production. This property improves the accessibility of nutrients, consequently fostering plant growth.

In the present study, the 'Spunta' cultivar exhibited high productivity from microtubers and plantlets, while the 'Russet' cv. showed the lowest productivity for microtubers and plantlets regarding yield and the number of tubers per plant.

The 'Spunta' cultivar, having good vegetative development, plant length, leaf and branch numbers, stem diameter, chlorophyll content, and shoot weight, produced the highest yield and number of tubers per plant (Table 4). On the other hand, 'Russet' cv. had lower vegetative growth, plant length, leaf and branch numbers, stem diameter, chlorophyll content, and shoot weight, resulting in the lowest yield and number of tubers per plant. Accordingly, Sharma and Pandey [55] and Felenji et al. [56] reported that the total tuber count was attributed to the maximum number of stems per plant, resulting in a higher yield of tubers. Furthermore, the variation showed a strong correlation with the stem count, a finding consistent with the observations in this study concerning 'Spunta' plantlets.

Alongside evaluating potato plant growth and yield, an indicator commonly used to gauge the influence of biofertilizers on the macro and micronutrient contents was essential for the potato's lifecycle [54]. This assessment revealed significant variations in the levels of these nutrients due to the different biofertilizers examined in this study. This effect was pronounced in tuber mineral contents, where significant differences were observed for N, P, Ca, and Mg. Biofertilizer T5 induced the highest contents of N, P, Ca, and Mg compared to the control, which showed the lowest values, especially for N and Mg. Similar results were reported by Neam et al. [32], Oliveira et al. [57], and Torbian et al. [58].

Araújo et al. [54] conducted a comparative study on the effects of biofertilizers on macronutrient uptake and potato productivity. They concluded that biofertilizers had a positive impact when compared to conventional chemical fertilizers and a combination of conventional fertilizers and biofertilizers. Throughout the potato growth cycle, their findings showed that biofertilizers led to an increase of 30% to 64% in the accumulation of nutrients such as N, P, K, Ca, and Mg in both leaves and tubers.

Our findings demonstrate the advantageous impact of all biofertilizers on potassium levels, which is recognized as a critical nutrient for potato plants during the tuberization stage [58,59]. Consequently, this leads to an augmentation in the tuber yield per plant compared to T1-control. Khan et al. [60] indicated that higher potassium doses increased tuber yield, dry matter, starch, and vitamin C content. Potassium is crucial for the synthesis of sugars and starch and for the translocation of carbohydrates in potatoes. In line with this, the higher level of potassium combined with an elevated nitrogen rate can regulate better

water status [61]. It has been reported that potassium has a crucial role in maintaining cell growth, cell stomatal regulation and turgor pressure, hydraulic conductance, leaf expansion, and root elongation, as well as in improving the photosynthesis and transport of photoassimilates between sources and sinks organs [58,62]. The enlargement of potato tubers could be attributed to the role of potassium in cell division and the photosynthesis process, its translocation through the phloem, and its involvement in starch production within storage organs [63].

In this experiment, biofertilizers, including bacteria and vermicompost, demonstrated significantly greater nitrogen uptake efficiency than the control. Recently, Hartmann and Six [64] highlighted that the role of biofertilizers could encompass atmospheric nitrogen fixation, a synthesis of various phytohormones and enzymes, and nutrient solubilization. Moreover, T4 and T5 led to the highest levels of Mg. As reported by Tränkner et al. [62], the adequate supply of nitrogen in conjunction with magnesium can enhance production and quality in potatoes, as magnesium enhances nitrogen uptake and stimulates photosynthesis activity.

The starch content in the produced tubers of 'Spunta' from plantlets exhibited more notable levels due to the use of biofertilizers T3 (0.301 mg kg $^{-1}$), T4 (0.290 mg kg $^{-1}$), and T5 (0.390 mg kg $^{-1}$), with the highest starch content observed in the vermicompost application (T5) (Table 7). This observation underscores the significance of potassium (K) in various aspects of potato plant growth, including the biochemical process of starch synthesis and the overall quantity and quality of tubers, as highlighted in a review by Ali et al. [59]. Khan et al. [60] reported that K enhances starch by activating the enzyme called starch synthase, which is responsible for starch synthesis.

5. Conclusions

In conclusion, biofertilization can effectively supplement mineral fertilizer applications, capitalizing on the synergistic potential of this combination. The highest seed tuber yield of 173.12 g was obtained from the 'Spunta + microtuber + vermicompost' combination. The second-highest tuber yield was achieved from the combination 'Spunta + microtuber + microalgae', being 126.55 g. In both varieties, *in vitro* microtubers led to a higher seed yield than *in vitro* plantlets. In terms of tuber diameter, tuber weight, and tuber number, the performance of the 'Spunta' cultivar was significantly higher than that of the 'Russet' cultivar. Seed tubers derived from *in vitro* microtubers had a larger diameter and were heavier than those derived from *in vitro* plantlets. However, seed tubers produced from *in vitro* plantlets were smaller in size but more in number. This strategic adjustment in fertilization practices holds promise in advancing potato cultivation methodologies, yielding improvements in multiple facets of production and promoting a more sustainable farming paradigm.

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