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Effects of the bio-fertilizers on potato mini tubers number and size produced from tissue culture plants

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Abstract

The present study aimed to increase mini tuber yield production of potato *in vitro* plants by decreasing mineral nutrients to 50% and applying biofertilizers micro-algae, bacteria, vermicompost, mycorrhizae and their combinations. The experiment was carried in controlled conditions in a growth chamber in pots with a capacity of 2L. The substrate was mixed soil with cocopeat (v/v). The evaluation of growth parameters and macro and micro elements was carried 30, 60, and 90 days after planting. Harvest was carried 120 days after planting and tuber numbers, size, and diameters were evaluated. The higher number of mini tubers obtained with 8.8, 8.2, and 7.6 per plant in control, algae, and the mixture of 4 biofertilizers, respectively. The higher tubers' diameter and weight values were 20.11 mm, 6.70 g, 18.65 mm, and 5.3 g in the plants treated with bacteria and vermicompost. For mini tuber seeds production, the number is important, yet the size and weight are the essential parameters to obtain high tuber yield. Thus, it is recommended that the seeds producers apply vermicompost and bacteria in their fertilizer's solution.

Keywords: In vitro plants, *Solanum tuberosum L*, tuber-size, tuber-yield

Introduction

Potato (*Solanum tuberosum L.*) is a versatile vegetable with almost 368 million tons overall worldwide production. More than 1 billion people worldwide consume potatoes; its prominence in agriculture follows cereals like rice, wheat, and maize (FAO, 2019). It belongs to the Solanaceae family comprising 26 genera and 2800 different species. Most potatoes' species have been native to the Andean highlands of South America, which produce underground stems in the form of a tuber (Fetena and Eshetu, 2016). It is considered one of the most economical crops due to its high yield and returns, but this yield is still insufficient to cover all the world's needs. The yield and quality of potato tubers are affected by many distinct factors such as genetics (cultivar properties), soil fertility, weather conditions, and chemical treatments (Torabian et al., 2021).

The potato is a highly heterozygous, tetraploid, and semi-perishable vegetable propagated by the tuber crop. It is also susceptible to many diseases and pests. The genetic nature, mode of spread, and vulnerability to disease/pests impose several inherent limitations in producing disease-free seeds. The production of pre-basic seeds is supported by tissue culture and generally requires in vitro culture techniques to rapidly multiply disease-free clonal plants and grow them under sanitary conditions (Tierno et al., 2013).

Plant tissue culture or micropropagation is a technique of maintaining in vitro parts of plants, cells, tissues, or organs on specified nutrient media under aseptic and controlled environmental conditions. It is based on the phenomenon of totipotence (Fazal Rehman et al., 2019). This technique has been used effectively to produce disease-free seed potatoes.

Current mini-tuber production only satisfies 1–2% of the production seed world; therefore, it is essential to improve the productivity and potential of this crop (Fazal Rehman et al., 2019).In Turkey, potato is one of the main crops with an average annual production of around 4.5 million tons (Çalışkan et al., 2010). Turkish potato production still depends on imported seeds, despite the efforts made in recent years to improve the output of mini tubers in terms of quantity and quality. Therefore, efficient

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mini-tuber production systems are needed to promote these efforts (Çalışkan et al., 2020).

Biofertilizers are preparations containing living or dormant cells, which benefit crop growth-promoting functions by producing phytohormones, macro, and micro-nutrient, improving soil's physical, chemical, and biological properties; thus, improve yield production (Kumar and Verma, 2018).

Generally, biofertilizers such as vermicompost, bacteria, algae, and mycorrhizae play a significant role in decomposing organic matter, which helps mineralize within the soil and increasing the availability of nutrients for crop yield (Rodríguez et al., 2006).

In recent research, biofertilizers have become essential practices for sustainable agricultural production and yield improvement through synthesizing phytohormones, metabolizing them, and acting on hormone biosynthesis in plants that affect plant growth, also by producing substances that work against soil-borne pathogens (Anelise Beneduzi, 2012).

Biofertilizers can be expected to promote the mini-tuber formation, increasing the number and size of mini tubers by some hormones, enzymes, and nutrients. Bio-fertilizers have biostimulant effects. Biofertilizers can increase the quantity and biodiversity of beneficial bacteria, such as plant growth-promoting rhizobacteria belonging to *Azotobacter*, *Bacillus*, *Burkholderia*, *Pantoea*, *Pseudomonas*, *Serratia*, and *Streptomyces* (Guo et al., 2019).

There is a beneficial interaction between biofertilizers and micro-organisms in the soil (Bulgarelli et al., 2015) that has advantageous effects on plants through direct or indirect pathways. Modern agriculture uses high amounts of chemical fertilizers to obtain actual product yields with improved cultivation efficiency. However, the excessive use of chemical fertilizers frequently causes severe environmental damage, such as water, soil, and atmosphere pollution (Savci, 2012). Furthermore, this excessive use leads to soil acidification and hardening, reducing the roots' respiration and vigour. This practice's population of beneficial micro-organisms is also decreased, resulting in a loss of soil fertility and a high incidence of root diseases (Chanda et al., 2019). Mahanty et al. (2017) recommended the application of biofertilizers in horticulture as an alternative to avoid the problems created by chemical fertilizers.

The objective of the present study, the effect of some biofertilizers; bacteria, micro-algae, vermicompost, and mycorrhiza on plant growth, plant nutrition and mini tuber yield production of in vitro plant Agria potato cultivar.

Materials and Methods

Plant material and experimental conditions

The present study was carried in the growth chamber at the University of Cukurova, Agriculture Faculty, Department of Horticulture in Adana/Turkey from May to August 2020. Potato cultivar Agria was used as plant material. The *in-vitro* plants were selected for the homogeneity of the plant height with approximately 12 cm height and transplanted in pots of 2 liters into a soil media (a cocopeat/soil mixture at 1:1 v/v). They were kept in controlled conditions for light at 16 h/8 h (day/night) and temperature at 24/16 °C (day/night).

The chemical and physical specifications of the experimental soil are given in Table 1.

Table 1. Chemical and physical characteristics of the experimental soil

Soil characteristics	Results
PH	7.70
EC (ds/m)	0.21
$CaCO_3$ (%)	20.33
Organic matter (%)	1.23
Phosphor (P_2O_5) (kg/da)	13.83
Potasium (K_2O) (kg/da)	72.52
Available Ca (mg/kg)	8132
Salinity (%)	0.01
Texture	Clay Loam
Available Zn (mg/kg)	0.603
Available Fe (mg/kg)	5.121
Available Mn (mg/kg)	2.124
Available Cu (mg/kg)	0.254
Water saturation (%)	59.40

Experimental design and management

The experiments were conducted under a Completely Randomized Design with 12 treatments, each with five replications. Each replication of treatment consisted of 5 pots and one plant in each pot. Bio-fertilizer treatments started after two weeks following plantation. Treatments details are given in Table 2.

Mineral nutrient solution composition and bio-fertilizers

The composition of the nutrient solution used for control treatment (mg L^{-1}) was as follows (Aydoner Coban et al., 2020) : 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. The nutrient solution concentrations used for control was decreased by 50% for all the nutrients and used for the bio-fertilizers. Mycorrhiza bio-fertilizer under the trade name "Endo Roots Soluble" (ERS) was used in the experiment. There were nine different mycorrhiza species as cocktail preparation: *Glomus intraradices, Glomus aggregatum, Glomus mosseae, Glomus clarum, Glomus monosporus, Glomus deserticola, Glomus brasilianum, Glomus etunicatum, Gigaspora margarita.* The commercial name of the Rhizofill was liquid bacteria bio-fertilizer used in the experiment. The bacteria

fertilizer contained three different bacteria species as *Basillus subtilis* ($1x10^9$), *Bacillus megaterium* ($1x10^9$) and *Pseudomonas fluorescens* ($1x10^9$). In vitro plants were inoculated with 1000 mycorrhizae spores during transplanting, and bacteria were applied every 7 days into the root by irrigation with 1ml Rhizofill in a 1 L nutrient solution. The commercial name of the Ekosolfarm was liquid vermicompost bio-fertilizer used in the experiment. The vermicompost composition were total organic matter 10%, total nitrogen 2%, organic nitrogen 2%, water-soluble phosphor pentaoxide (K_2O) 0.2%, free amino acids 10%. The vermicompost was applied every 7 days into the root by irrigation with 3 ml vermicompost in 1 L 50% nutrient solution. Eukaryotic green micro-algae *Chlorella vulgaris* produced in the Cukurova University Fishery Department has used $2x10^6$ microalgae in 1ml. This concentration was diluted 40 times with 50% nutrient solution (25 ml in 1L) during irrigation every 7 days.

Table 2. Treatments.

Treatment	Composition
C	Control :100% mineral nutrient solution
Myco	0.2 g Mycorrhizae for each plant
Bac	50% mineral nutrient + 50% Bacteria
Vermi	50% mineral nutrient + 50% Vermicompost
Alg	50% mineral nutrient + 50% Algae
Bac+Vermi	50% mineral nutrient + 25%Bacteria+25%Vermicompost
Bac+Alg	50% mineral nutrient + 25%Bacteria+25%Algae
Bac+Myco	50% mineral nutrient + 25%Bacteria+25% Mycorrhizae
Vermi+Myco	50% mineral nutrient + 25%Vermicompost+25% Mycorrhizae
Vermi+Alg	50% mineral nutrient + 25%Vermicompost+25% Algae
Alg+Myco	50% mineral nutrient + 25%Algae+25% Mycorrhizae
4 Bio	50% mineral nutrient + 12.5%Algae+12.5%Bacteria+12.5%Vermicompost +12.5% Mycorrhizae

Parameters determined

According to potatoes plants growth, three critical stages were fixed to evaluate growth parameters; plant length and diameter leaves number, leaves the area, chlorophyll content, and the dry matter and macro and micronutrients elements concentration in leaves.

Stage 1: Rooting and intensive growth of the above groundmass (30 days after planting)

Stage 2: Tuberization induction (60 days after planting)

Stage 3: Tuber growth (90 days after planting)

Chlorophyll measurements

A portable chlorophyll meter (SPAD-502, Minolta, Japan) was used to measure the leaf greenness of the fully matured leaves of all plants in each pot on the sampling day.

Leaves area measurement

In each development stage, and for each treatment, two leaves were taken from every plant. LI-3100C (Li-Cor) at a $1mm^2$ resolution area meter was used to determine the area of leaves cut from each plant in every treatment on the sampling day.

Leaf dry matter content

It was measured by weighing fresh material consisting of 2 mature and non-senescent leaves from each plant for every treatment. Leaves were weighed fresh, then dried for 24 h at 80 °C, and weighed again. According to the following formula, dry matter content was calculated: Leaf dry mass weight (g)/ Leaf fresh mass weight (g) x100

Macro and micro elements concentrations

Leaves were washed once with tap water and twice with deionized water. They were then dried in a forced-air oven at 65°C for 48 h and ground through a 40-mesh sieve for elemental analysis. The samples were dry-ashed in a muffle furnace at 550°C for 6 h. The ash was then dissolved in 0.1 M hydrochloric acid (HCl) solution. Concentrations of macro and micro elements (calcium, magnesium, potassium, copper, manganese, iron, zinc) were determined using an atomic absorption spectrophotometer (Jones, 2001). Nitrogen content in leaves was determined according to the Kjeldahl method.

Mini tuber potato harvest

The total number of potatoes per plant, weight, and diameter were determined 120 days after planting

Statistical Analysis

Data were subjected to ANOVA to determine the difference between the treatment means using JMP PRO14. The means were tested with the least significant difference (LSD) test, and the significance level was set at the 0.05 probability level.

Results and Discussion

Effect of the biofertilizers on growth parameters

Plant's length and diameter

The results obtained in Table 3, the effect of the different treatments growth parameters, showed a gradual increase in plants' height and diameter during the plant's development stages.

The highest heights were obtained in plants fertilized by bacteria and vermicompost. In fact, during the growth stage, plants' height rates increased about 40% and 30% for bacteria and vermicompost,

respectively. However, low's values were obtained for the treatment where it was mixing Bac+Myco in stage 3.

Regarding plants diameter, the highest value (6.0 mm) was observed in plants treated with bacteria, and the lowest (4.07 mm) was obtained in the mixture of Bac+Myco.

These results are in accordance with those of (Ali et al., 2020); in their work, the application of bacteria as a biofertilizer in potatoes plants increases the growth parameters and plants height with a rate of 15% compared to the control. Moreover, (Tuku, 2000) mentions that bacteria are significant micro-organisms that can improve vegetable yield growth and control pathogens through various mechanisms (Kang et al., 2021).

Leaves and branch number

The results obtained showed a significant effect of treatments and development stage of potato invitro plant. The maximum leaves number was obtained after 60 days of planting (stage2), in control and micro algae's treatments 15.0 and 15.4, respectively. In contrast, plants treated with Alg+Myco and Alg+Bac present the lowest leaves number at different development stages (Table 3). The vegetative development of tuber plants depends on the growth stage; it increases progressively to reach a high level in the tuber induction phase (Kolbe and Stephan-Beckmann, 1997).

Moreover, control and algae treatments developed more than one branch per plant. Pelealu et al. (2019) studies reported that the augmentation of branch number is related to the number of tubers produced.

Algae extracts contain phytohormones such as auxin (Romanenko, 2015; Stirk et al., 2013), an essential regulator of various plant developmental processes, such as cell division and elongation. Indole-3-acetic acid (IAA) and indole-3-butanoic acid (IBA), the two dominant types of auxins in microalgae, can both stimulate and inhibit the growth and metabolism of higher plants (Hashtroudi et al., 2013).

Chlorophyll SPAD readings and leaves area

The SPAD values and leaves area rates reflect the same evolution in function with the development stage; a significant increase during the second one followed by a decrease at the tuber stage growth (Table 3).

The control's both parameters' highest values were obtained, followed by micro-algae and mycorrhizae in the different development stages. In contrast, the mixture of the bio-fertilizers presents the lowest rates.

Algae are essential biofertilizers that promote plant growth and crop yield growth, the secretion of vitamins, and the enchanting of available nutrients such as nitrogen, phosphor, and potassium. Moreover, this nutrient improves cell growth, leaves expansion, transport between source and sink organs (Lee and Ryu, 2021). Additionally, NPK facilitates the diffusion of carbon dioxide (CO_2) through the leaf mesophyll, which plays a crucial role in photosynthesis and chlorophyll content (Torabian et al., 2021). Many research, reported the positive effect of mycorrhizal fungi in the agricultural system; it is a solution that improves the efficiency of phosphate used and contributes to the absorbance of potassium from soil (Sawers et al., 2010).

Potassium is very abundant in the soil, but its availability is deficient due to its strong mineral adsorption. Mycorrhizae play the role of mediator to accumulate and regulate this element and ensure its transport to the plant (Berruti et al., 2015). Thus, potassium increases the chlorophyll content in leaves and leaf areas (Torabian et al., 2021).

Dry matter

There is no statistical difference between treatments applied on dry matter content in leaves during the first and second stages (Table 3). On the contrary, there is a significant difference in the third stage with high values obtained in plants treated by biofertilizers and micro-algae, 10.7 and 10.6, respectively.

Dry matter content in leaves decreases parallel to the tuberization process in all the treatments with a dropping ratio varied from 3 to 18% in the tuber growth stage; explained by remobilization of minerals from leaves to mini tuber at the end of the vegetation period (Kolbe and Stephan-Beckmann, 1997).

According to Lee and Ryu (2021), using Eukaryotic green algae, *Chlorella vulgaris*, as biofertilizer, increased the fresh and dry weight, increasing the dry matter content in leaves.

Effect of the biofertilizers on mini tuber yield production

Applying the different treatments improves mini tubers' yield production. Tuku (2000) reveals that mini tubers produced per in vitro plant on soil media usually ranges from 2-5. The higher mini tuber number was obtained in the control, micro-algae, and Alg+Bac+Myco+Vermi, respectively 8.8,8.2 and 7.6. However, the lowest tubers numbers (3.4) were obtained in the mixture Alg+Myco and vermicompost (Table 4). Algae biofertilizers increase the number of mini tubers compared to the others; in fact, many researcher reported the beneficial fact of applying green algae "*Chlorella vulgaris*" in the yield production (Ergun et al., 2018; Farid et al., 2019; Tuku, 2000). Algae biofertilizers promote plant growth and crop yield and enhance plant robustness by a different process. Firstly, the production of phytohormones and regulators such as auxin and cytokinin increases plant growth and development and crop yield, secondly by producing macronutrients, vitamins, and insured nitrogen-fixing (Lee and Ryu, 2021). The control plants produce many tubers; however, their weight and size were lower than bacteria and vermicompost (Table 4). According to Altaf Hossain (2015), reported that mini tubers size classification: undersize ≤5 mm, pea-size (5-10 mm), small size (10-15 mm), medium size (15-20 mm), large size (20-25 mm) and extra-large ≥25

mm. Plants fertilized by bacteria and vermicompost produce a large-size mini tuber (20.11 and 18.65mm), respectively; Alg+Vermi, Mycorrhizae, and Myco+Vermi have a medium-size vary from 15.32 to 17.28 mm; the other application with control produces a small-size range from 12.49 to 14.96 mm. Many studies mention the effect of mini tuber size on seed production; seed tuber size is one of the significant factors affecting yield and quality in potatoes. Tuku (2000) revealed that larger mini tubers seeds give more vigorous plants than the small ones. Moreover, increasing the size of mini tubers seed is essential because size affects the duration of the dormancy, the plant's vigour, and the number of stems (Tuku, 2000), thus influencing the yield production and quality of the tuber obtained from the unit area (Ozkaynak, 2021). The weight of the protected mini tubers presents a highly significant difference in the treatments. The plants treated by bacteria and vermicompost produce the maximum tuber weight (6.7 g and 5.31 g), respectively.

Tuku (2000) and Özkaynak and Samanci (2006) mentioned that importance of mini tuber seeds weight in seed tuber production program; in fact, tuber number produced from mini tubers seeds with a high weight was significant (Table 3). Furthermore, the research of (Mahmoudpour, 2014), who studies the effect of different sizes of mini tubers on yield production of Agria potato variety, demonstrate the importance weight of mini tuber weight on yield potatoes production; and concluded that the range weight varies from 5 to 10 g is most suitable to obtain a high number and diameters of tubers production. In addition, Mahmoudpour (2014) mentions that the mini tubers lighter than 1g were unsuitable for planting.

The application of vermicompost increases the bioavailability of phosphorus in the soil, affecting plant growth in potato cropping and improving crop yield (Ansari, 2008). Bacteria's utilization in plant fertilization promotes the growth of plants with higher solubilization of tricalcium phosphate (TCP) by increasing nutrient uptake parameters and producing indole-3-acetic acid (IAA) and sierophores (Kang et al., 2021). The mixture Alg +Myco have the lowest tuber numbers, diameters, and weight values (Table 4). Generally, the mixture of biofertilizers can produce growth factors and exotoxins that promote or inhibit growth and development (Kang et al., 2021). In procedure research, some micro-organisms could not function with a high level of nutrients elements; the effect of mixtures of two biofertilizers or more simultaneously depends on the species, genotype, environmental conditions, and the concentration applied (Mujtaba and Lee, 2016). However, toxic relationships between the biofertilizers can inhibit the growth process by increasing the pH, dissolved oxygen concentration, soil temperature (Ribalet et al., 2008).

Table 4. Effect of different biofertilizers in mini tubers yield production

Treatments	Tuber/ Plant	Diameter/ Tuber (mm)	Weight/ Tuber (g)
C	8.80a	14.84c-e	2.51cd
Myco	5.60bc	15.80cd	3.23cd
Alg	8.20a	14.29de	2.31d
Bac	4.80c	20.11a	6.70a
Vermi	3.40c	18.65ab	5.31ab
4 Bio	7.60ab	12.49e	1.87d
Bac+Vermi	5.40bc	14.09de	2.31d
Myco+Vermi	4.40c	17.28b	4.01bc
Bac+Myco	4.60c	14.96c-e	2.76cd
Alg+Bac	4.60c	14.43de	2.44cd
Alg+Myco	3.40c	13.11de	1.83d
Alg+Vermi	4.40c	15.32cd	2.58cd

The differences between means shown with dissimilar characters in the same column is statistically important ($P < 0.05$). Differences between means shown with similar characters in the same column is not statistically important.

Effect of the biofertilizers on macro and micro nutrients in leaves

Nitrogen content

According to the results obtained in Table 5, a highly significant effect of the treatment and the development stage on nitrogen rate was noted. In fact, during the first stage, which corresponds to root and aboveground mass growth, the nitrogen level was higher, decreasing progressively during plant development. The most important values compared to the control were observed in micro algae treatment (4.28%) followed by bacteria (3.76%). During the second and the third stage, algae reduce the nitrogen content in leaves by about 36%, but Bacteria reduce it by 17%. The plants treated by the mixture Alg+Bac have the lowest nitrogen content in leaves. Microalgae *Chlorella vulgaris* can fix atmospheric nitrogen; also, this application to the plants improve nitrogen-fixing in the soil, thus increasing his availability and enhanced plant growth (Lee and Ryu, 2021). The application of bacteria in the soil increases the ratio of nitrogen uptake, thus improving the nutrient content in leaves and plant growth parameters (Adiloglu et al., 2021).

Macro elements

The calcium (Ca), magnesium (Mg), and potassium (K) contents show a significant difference for treatments and stages (Table 5). Their maximum values were recorded at the second development phase and decrease during the last stages. Araujo et al. (2019) mentioned that potato leaves assimilate a high level of nutrients during the vegetative growth phase; at the tuber growth stage, photoassimilates are translocation and remobilization stored in the aerial part of the plant to tubers. Compared to the control, the application of biofertilizers increases the Ca and K content rate in leaves. In addition, the regular content of Ca and K for potato leaves varies from 6 to 8% for K and 1.5 to 2.5% for Ca. Gondwe et al.

(2019) reported that algae application improves the rate of Ca and K content in leaves, a critical nutrient for the growth and development of potato tubers. Furthermore, Adiloglu et al. (2021) mentioned that the application of vermicompost, bacteria, and their combination increases Ca and K content in leaves. The effects of different biofertilizers on the Mg contents of potatoes leaves were not insignificant; we constate that the highest values it is obtained in the control. According to Adiloglu et al. (2021) and Chanda et al. (2019), the application of biofertilizers can stimulate plant growth and nutrient uptake; but this performance depends on the species of biofertilizers, soil parameters, and plant growth conditions (Çakmakçi et al., 2006).

Micro elements

Comparing to the control, the application of bio-fertilizers engenders a substantial effect on micro elements such as copper (Cu), manganese (Mn), iron (Fe), and zinc (Zn) by increasing their content in leaves (Table 6). Leaves acquire the most Cu, Mn, Fe, and Zn during vegetative growth; however, during tuber induction, these rates decrease significantly, which may be explained by the mobilization of nutritional elements from leaves to the tuber (Araujo et al., 2019; Kolbe and Stephan-Beckmann, 1997).

Table 3. Variation of the plant growth parameters affected by the different bio-fertilizers in leaves in the different growth stage of plant

Treatments	Plant height (cm)			Diam (mm)			Branch Number			Leaves Number		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
C	44.2a-c	56.8b-d	76.8bc	3.90cd	4.32de	4.75d	1.0a	1.8a	1.8a	8.0ab	15.0a	10.6b
Myco	48.8a	61.8a-c	83.2b	4.15bc	5.16b	5.66a-c	1.0a	1.0b	1.0c	7.4bc	12.6b	8.6de
Alg	48.0ab	53.6b-d	67.2ef	3.89cd	4.67cd	5.54a-c	1.0a	1.6a	1.6ab	9.0a	15.4a	13.6a
Bac	49.0a	66.0a	92.6a	4.35a	5.76a	6.00a	1.0a	1.0b	1.0c	8.4ab	11.4cd	9.4cd
Vermi	49.6a	65.6a	82.2bc	4.21ab	4.75c	5.18cd	1.0a	1.0b	1.0c	8.2ab	11.6c	9.6c
4 Bio	44.4a-c	52.6cd	69.0de	3.66de	4.39c-e	5.40bc	1.0a	1.0b	1.0c	7.4bc	10.2ef	8.6de
Bac+Vermi	39.4c	52.6cd	75.4d	3.48de	4.68cd	5.47a-c	1.0a	1.0b	1.0c	7.4bc	10.6de	8.6de
Myco+Vermi	42.2a-c	48.4d	61.4fg	3.55de	4.17ef	5.36bc	1.0a	1.0b	1.0c	8.6a	14.8a	13.4a
Bac+Myco	40.4bc	48.2d	55.0g	3.29de	3.21f	4.07e	1.0a	1.0b	1.0c	8.4ab	11.4cd	9.2c-e
Alg+Bac	40.2c	62.6ab	79.6bc	3.07e	3.91f	4.14e	1.0a	1.0b	1.0c	6.6cd	10.6de	8.6de
Alg+Myco	43.6a-c	58.4a-c	63.4ef	3.13e	4.34de	4.81d	1.0a	1.0b	1.0c	6.2d	9.4f	8.4e
Alg+Vermi	46.4a-c	60.4a-c	77.6bc	3.89cd	4.03ef	5.83ab	1.0a	1.2b	1.4b	8.4ab	11.4cd	9.4cd

Table 3. Variation of the plant growth parameters affected by the different bio-fertilizers in leaves in the different growth stage of plant (continuation).

Treatments	Chlorophyll (SPAD-values)			Leaves area (cm^2/two leaves)			Dry matter (%)		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
C	45.0a	50.8a	44.4a	101.8ab	232.1a	227.5a	10.3a	9.5a	9.2bc
Myco	43.3ab	46.1b	39.6bc	90.5c-e	188.8c	166.8c	10.0a	9.6a	9.2bc
Alg	42.7ab	48.9a	40.9b	99.8ab	221.9ab	201.4b	9.8a	9.9a	10.6ab
Bac	40.8bc	41.7cd	34.9d-f	87.2de	147.5ef	130.2ef	9.8a	9.3a	9.2a-c
Vermi	39.6cd	42.3cd	39.4bc	95.6b-d	157.8de	133.9d-f	9.7a	9.2a	9.4a-c
4 Bio	38.9c-e	42.8c	37.9cd	85.7e	138.1fg	105.6g	9.4a	9.6a	10.7a
Bac+Vermi	36.9ef	42.4cd	33.6ef	99.8ab	122.7gh	112.2fg	9.4a	8.9a	8.2c
Myco+Vermi	39.6cd	40.1df	37.3cd	104.1ab	166.6d	154.1cd	9.5a	10a	9.3a-c
Bac+Myco	37.2d-f	42.4cd	32.1f	107.3a	137.4fg	144.8c-e	9.8a	9.5a	8.6c
Alg+Bac	35.1fg	41.7cd	35.2de	97.8bc	208.4b	195.9b	10.4a	9.1a	8.6c
Alg+Myco	32.7g	37.3f	35.5de	100.7ab	117.9h	113.2fg	9.4a	9.0a	8.8c
Alg+Vermi	35.4f	39.2ef	37.9cd	100.8ab	160.3de	153.3cd	9.4a	10.1a	9.0c

The differences between means shown with dissimilar characters in the same column is statistically important ($P < 0.05$). Differences between means shown with similar characters in the same column is not statistically important.

Table 4.Variation of the macro-nutrients in leaves affected by the different biofertilizers in the different growth stage of plant (%)

Treatments	N (%)			Ca (%)			K (%)			Mg (%)		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
C	3.28c-e	2.60gh	2.43g	1.68f	1.98d	1.27f	5.22e	7.87bc	5.25b	2.57bc	4.85a	3.04a
Myco	2.92ef	2.46h	2.38g	1.52f	2.17cd	1.45ef	6.96a-c	7.92bc	8.70ab	2.42c	3.32ab	2.95a
Alg	4.28a	3.03de	2.76d	1.98e	2.75ab	1.54ef	5.63de	8.42bc	8.43ab	3.10bc	4.31ab	2.51ab
Bac	3.76b	3.39a	3.13a	2.05de	2.31b-d	1.75d-f	7.22ab	7.93bc	9.96a	2.98bc	3.33ab	2.31a-c
Vermi	3.15d-f	2.49h	2.76d	2.20d	2.25b-d	2.45bc	5.53e	7.89bc	10.25a	2.99bc	3.61ab	1.43bc
4 Bio	3.55bc	3.37ab	3.01b	1.97e	2.12d	1.72d-f	6.47b-d	6.70c	8.55ab	3.68ab	3.25ab	1.80bc
Bac+Vermi	3.41b-d	2.68g	2.52f	3.18a	2.34b-d	1.63ef	6.57a-c	7.59bc	7.25ab	3.37a-c	3.16b	2.05a-c
Myco+Vermi	2.63b	2.77a	2.39b-d	6.10c-e	8.54b	9.59a	3.30a-c	3.34ab	1.46bc	3.21c-e	3.05c-e	2.94bc
Bac+Myco	2.92ef	2.91ef	2.92c	2.42c	2.66a-c	2.09c-e	7.43a	10.76a	9.80a	3.23bc	3.20b	1.27c
Alg+Bac	2.80f	2.74fg	2.67e	2.70b	2.12d	2.58b	6.64a-c	9.22ab	8.85ab	4.45a	2.91b	1.38c
Alg+Myco	3.54bc	3.20bc	2.99bc	2.74b	1.90d	2.11c-e	6.51b-d	8.54b	8.76ab	4.47a	3.63ab	1.33c
Alg+Vermi	3.15d-f	3.09cd	2.77d	1.01g	2.73ab	3.44a	1.68f	8.32bc	9.67a	3.43a-c	3.21b	1.37c

The differences between means shown with dissimilar characters in the same column is statistically important (P <0.05). Differences between means shown with similar characters in the same column is not statistically important.

Table 5. Variation of the micro-nutrients in leaves affected by the different biofertilizers in the function of the growth stage of plant (ppm)

Treatments	cu (ppm)			mn (ppm)			fe (ppm)			zn (ppm)		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
C	88.5a-c	9.0c	14.0ab	84.0c-e	51.0e	31.0d	85.5ab	35.5a-c	40.0c	47.5b	26.5b	24.0c
Myco	107.0a	20.0ab	9.0b	85.5c-e	55.0de	35.5cd	82.5a-c	46.0ab	50.5bc	64.0a	26.5b	38.5a-c
Alg	29.5a-c	23.0a	12.0ab	86.0c-e	69.0bc	46.0a-c	67.5c-e	51.5a	50.5bc	25.5cd	27.5b	33.5bc
Bac	101.0ab	8.5c	15.5ab	80.5de	56.0de	60.0a	80.0b-d	39.5a-c	81.0a	41.5b	26.0bc	69.0ab
Vermi	26.5bc	12.5bc	13.5ab	88.5c-e	54.0de	46.0a-c	76.0b-d	44.5ab	68.5ab	28.0c	30.0b	74.5a
4 Bio	55.0a-c	5.0c	18.0a	88.5c-e	51.0e	42.0b-d	95.5a	26.5bc	68.5ab	44.0b	25.5bc	45.0a-c
Bac+Vermi	22.0c	22.0a	11.05ab	100a-c	69.0bc	33.0cd	81.5a-c	54.0a	45.0bc	39.0b	29.0b	29.0c
Myco+Vermi	12.0c	7.0c	9.0b	93.0b-d	79.5ab	37.0cd	72.5c-e	40.0a-c	31.0c	22.5cd	16.5c	26.0c
Bac+Myco	14.0c	4.0c	9.5b	75.0e	82.5a	42.5b-d	48.5f	40.0a-c	36.5c	19.5cd	26.0bc	30.5c
Alg+Bac	49.0a-c	5.0c	12.5ab	100.5ab	63.0c-e	33.5cd	65.5de	47.5a	35.0c	41.0b	34.0b	38.5a-c
Alg+Myco	17.5c	4.5c	10.0b	107.0a	66.05-d	46.0a-c	59.5ef	22.5c	69.5ab	22.0cd	44.5a	49.0a-c
Alg+Vermi	13.0c	7.0c	8.5b	39.0f	52.0e	55.5ab	22.5g	49.5a	58.0a-c	17.0d	34.0b	39.5a-c

The differences between means shown with dissimilar characters in the same column is statistically important (P <0.05). Differences between means shown with similar characters in the same column is not statistically important.

Conclusion

The results obtained showed a high effect of different biofertilizers and their mixture in mini tubers yield production.

The bacteria and vermicompost produce the highest tuber number per plant (4.8 and 3.4, respectively), tuber size (20.11 mm and 18.65 mm, respectively) and tuber weight (6.70 g and 5.31g, respectively). For mini tuber seeds production, the number is important, yet the size and weight are the essential parameters to obtain high tuber yield. Thus, it is recommended that the seeds producers apply vermicompost and bacteria in their fertilizer's solution.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this article, they have no actual, potential or perceived conflict of interest.

Author contribution

Hiba Boubaker performed the experiment in the growth chamber and laboratories and did data collection and manuscript writing. Hayriye Yildiz Dasgan contributes suggestions during the experiment and give ideas and reviewing the manuscript. Neji Tarchoun was read and revised the manuscript

Ethical approval

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Data availability

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