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Microbial fertilizer from PK rocks on lettuce nutrients and soil attributes in consecutive crops

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Abstract – The objective of this work was to evaluate the effect of a microbial fertilizer, produced from phosphate and potassic rocks and earthworm compost, as an alternative to conventional fertilizers, on lettuce (*Lactuca sativa*) nutrients and soil attributes. The experiment was conducted in consecutive cycles (30 and 60 days) in a soil from the Lorca region, in the municipality of Murcia, Spain. The fertilization treatments were: conventional fertilizer, 100% of the recommended rate (RR); microbial fertilizer, 50% of the RR (5 Mg ha^{-1}); microbial fertilizer, 100% of the RR (10 Mg ha^{-1}); microbial fertilizer, 150% of the RR (15 Mg ha^{-1}); and control, without N-P_2O_5-K_2O fertilization. The microbial fertilizers applied at 100 and 150% of the RR showed significant and positive effects, as well as the best results for plant characteristics. The microbial fertilizer increased total N and available P and K compared with the conventional fertilizer. A residual effect was observed in the successive cycle. The effectiveness of the microbial fertilizer shows it is a viable alternative to conventional fertilizers, with positive effects on plant productivity and soil attributes.

Index terms: *Lactuca sativa*, agromineral, organic fertilizer, residual effect.

Fertilizante microbiano com rochas de P e K sobre nutrientes da alface e atributos do solo em cultivos consecutivos

Resumo – O objetivo deste trabalho foi avaliar o efeito de fertilizante microbiano, produzido a partir de rochas fosfática e potássica e vermicomposto de minhoca, como alternativa a fertilizantes convencionais, sobre os nutrientes da alface (*Lactuca sativa*) e os atributos do solo. O experimento foi conduzido em cultivos consecutivos (30 e 60 dias) em solo da região de Lorca, no município de Múrcia, Espanha. Os tratamentos com fertilização foram: fertilizante convencional, 100% da dose recomendada (DR); fertilizante microbiano, 50% da DR (5 Mg ha^{-1}); fertilizante microbiano, 100% da DR (10 Mg ha^{-1}); fertilizante microbiano, 150% da DR (15 Mg ha^{-1}); e controle, sem fertilização com N-P_2O_5-K_2O. Os fertilizantes microbianos aplicados a 100 e 150% da DR apresentaram efeito significativo e positivo, além de os melhores resultados quanto às características das plantas. O fertilizante microbiano aumentou o N total e o P e K disponíveis comparado ao fertilizante convencional. Observou-se efeito residual no ciclo sucessivo. A efetividade do fertilizante microbiano mostra que é alternativa viável a fertilizantes convencionais, com efeitos positivos sobre a produtividade das plantas e os atributos do solo.

Termos para indexação: *Lactuca sativa*, agromineral, fertilizante orgânico, efeito residual.

Introduction

The effects of organic fertilizers on plant production are part of the guidelines for the development of a sustainable agriculture that meets economic, social, and environmental standards (Lal, 2010). Depending on the applied fertilizer, soil conditions and, consequently, agriculture may be negatively affected by organic matter loses, nutrient depletion, soil erosion, and groundwater contamination (Montemurro et al., 2015).

Therefore, recently, in order to enhance nutrient availability in the soil, microorganisms have been added to rocks (Meena et al., 2015; Stamford et al., 2016; Satyaprakash et al., 2017), especially the *Acidithiobacillus thiooxidans* (Waksman & Joffe 1922) Kelly & Wood 2000 oxidative bacteria, important in nutrient recycling in the soil and in releasing elements contained in rocks (Stamford et al., 2015).

Studies carried out with melon (*Cucumis melo* L.), sugarcane (*Saccharum officinarum* L.), and banana (*Musa* spp.) on different soils have shown the efficacy of phosphate and potassic rocks as an alternative to conventional fertilizers (Oliveira et al., 2014; Stamford et al., 2016, 2017). However, to produce an effective microbial fertilizer inoculated with *Acidithiobacillus*, for example, the inclusion of organic matter is necessary to neutralize the acidity caused by sulfuric acid production (Oliveira et al., 2015). Furthermore, to improve nitrogen content, the addition of free-living diazotrophic bacteria, such as *Beijerinckia indica* (Starkey & De 1939) Derx 1950, is also important due to their effectiveness in enriching N by the biological nitrogen fixation process, as reported by Lima et al. (2010).

Microbial fertilizers provide the necessary nutrients to increment plant growth and yield, particularly when inoculated with *Cunninghamella elegans* Lendn. (1907), a fungus that contains chitosan in its cellular walls (Franco et al., 2011). Inoculated organic matter increases soil nutrients, especially nitrogen, phosphorous, and potassium, as well as antimicrobial activity, improving plant protection against diseases (Berger et al., 2013).

Therefore, the hypotheses of the present study is that microbial fertilizers with phosphate and potassic rocks and organic matter (earthworm compost) can provide nutrients to plants by incrementing soil attributes in a gradual and constant form, which allows a residual effect on successive crops, indicating a sustainable cultivation strategy.

The objective of this work was to evaluate the effect of a microbial fertilizer, produced from phosphate and potassic rocks and earthworm compost, as an alternative to conventional fertilizers, on lettuce (*Lactuca sativa* L.) nutrients and soil attributes.

Materials and Methods

The soil used in the experiment was collected in the 0–30 cm layer at a horticultural farm, where lettuce was grown long term, in the Lorca region, in the municipality of Murcia, Spain. The soil is classified as a Haplic Calcisol (IUSS Working Group WRB, 2015), and its chemical analysis showed: pH_{H2O} 8.23, 0.61 g kg^{-1} total carbon, 2.0 mg kg^{-1} phosphorous (Mehlich-1), 49 mg kg^{-1} exchangeable potassium (Mehlich-1), 16 mg kg^{-1} carbon, 7.0 mg kg^{-1} magnesium, as well as heavy metals, including 10.1 mg kg^{-1} lead, 2.2 mg kg^{-1} cadmium, 22.9 mg kg^{-1} zinc, and 22.3 mg kg^{-1} chromium.

For the experiment, ‘Veneranda’ lettuce was grown in 4 kg plastic pots containing sieved soil (4 mm mesh), in a (5x2)+1 factorial arrangement, in a randomized complete block design. Five fertilization treatments were applied, being evaluated in two consecutive cycles with a growth time of 30 and 60 days, with four replicates.

The fertilization treatments were: conventional fertilizer, 100% of the recommended rate (RR); microbial fertilizer, 50% of the RR (5 Mg ha^{-1}); microbial fertilizer, 100% of the RR (10 Mg ha^{-1}); microbial fertilizer, 150% of the RR (15 Mg ha^{-1}); and control, without the application of the N-P_2O_5-K_2O fertilizer in the second cycle, since the aim was to evaluate the residual effects of the fertilization treatments. The conventional fertilizer contained ammonium sulphate (N=20%), simple superphosphate (P_2O_5=20%), and potassium sulphate (K_2O=50%).

The fertilization rates applied were based on the soil analysis and on the recommendation of Instituto Agronômico de Pernambucano (IPA) (Cavalcanti, 2008) for lettuce. The quantities of the fertilizers applied at the different rates were calculated according to the N content of the microbial fertilizer (2%) and to the N content of ammonium sulphate (20%).

The microbial fertilizer from phosphate and potassic rocks was produced at the horticultural campus of Universidade Federal Rural de Pernambuco, using ground natural apatite (24% total P_2O_5) and ground potassic rock (8% total K_2O), which were obtained, respectively, in the municipality of Irecê-Bahia, in the state of Bahia, and in the state of Minas Gerais, Brazil. Elemental sulfur was also added, after being inoculated with the *Acidithiobacillus* oxidizing bacteria, at a ratio of 1:10 sulfur:rock according to Stamford et al. (2015). Then, the microbial fertilizer was mixed with organic matter (earthworm compost) at a ratio of 0.5:0.5:3.0 phosphate rock+potassic rock+organic matter. Finally, strain UCP 542 of the *C. elegans* fungus, which contains 8–10% chitosan and 14% chitin in its cellular walls (Franco et al., 2011), was included. The microbial fertilizer was incubated for 30 days, maintaining moisture near maximum water capacity. The chemical characteristics of the microbial fertilizer are shown in Table 1.

The analysis of the used phosphate and potassic rocks, extracted by Mehlich 1 (Silva, 2009), showed the following results, respectively: pH 3.1 and 48 g kg^{-1} available P; and pH 3.3 and 10 g kg^{-1} available K. In addition, organic matter presented: pH 7.95, 100.7 g kg^{-1} organic C, 8.6 g kg^{-1} total N, 2.98 g kg^{-1} total K, and 1.12 g kg^{-1} total P, which was enriched in N through inoculation with strain NFB 10001 of the select free-living diazotrophic bacteria, *B. indica*, according to Lima et al. (2010).

The plants were harvested and height and shoot diameter were obtained at two growth times: 30 days, cycle 1; and, 60 days, cycle 2. The plant samples were oven dried at 65°C, during three days, to determine dry weight. For the analysis of nutrients (total N, P, and K) and heavy metals, the plants were passed through a 2 mm sieve.

Table 1. Chemical characteristics of the microbial fertilizer used in the experiment, with quantities limited according to the European Union Commission (European Comission, 2001) for important heavy metals (classes A and B).

Chemical characteristic	Microbial fertilizer(1)
pH	3.50 (0.02)
Electrical conductivity (dS m^{-1})	4.90 (0.01)
Total carbon (g kg^{-1})	60.3 (1.0)
Organic carbon (g kg^{-1})	54.2 (0.8)
Total nitrogen (g 100 g^{-1})	5.6 (0.1)
Total phosphorus (g kg^{-1})	14.7 (0.3)
Available phoshorus (g kg^{-1})	11.0 (0.2)
Total potassium (g kg^{-1})	81.0 (0.5)
Available potassium (g kg^{-1})	4.0 (0.2)

(1)The standard deviation in parenthesis represents (n=3).

Soil samples collected at 0, 30, and 60 days after seedling transplantation were sieved (2 mm) and stored at 4°C for the chemical analysis of: electrical conductivity, pH_{H2O}, total N, total C, and available P and K (Mehlich-1), following the methodology of Empresa Brasileira de Pesquisa Agropecuária (Embrapa) (Silva, 2009).

Electrical conductivity was measured in water extract 1:5 (m:v), using the CM 2200 analyzer (Crison Instruments, SA, Alella, Barcelona, Spain). A water solution of 1:5 (m:v) was used for pH determination with the 848 Titrino plus compact titrator (Metrohm AG, Herisau, Switzerland). In the soil, the total concentration of N, C, and organic C were obtained by the TruSpec C/N/S automatic analyzer (Leco Corporation, St. Joseph, MI, USA). Carbon and N (water) were determined in a soil extraction, subjected to agitation for 2 hours at a ratio of 1:5 soil:water. The samples were centrifuged, filtrated, and analyzed with the multi N/C 3100 automatic analyzer for liquid samples (Analytik Jena GmbH, Jena, Germany). The soil samples were analyzed for nutrients after digestion with microwaves (65% HNO_3) using the plasma optical emission iCAP 6500 duo spectrometer (Thermo Fisher Scientific, Waltham, MA, USA).

To determine significant differences between treatments, the data were subjected to the unidirectional analysis of variance (ANOVA) in each crop growth time. The ANOVA used Tukey's B-test (honestly significant difference) as a post hoc test in a 95% confidence interval (linear model). A factorial analysis was performed for the relative amount of phospholipid fatty acids, an indicator of microbial communities, in order to explore possible differences between treatments. The statistical analyses were carried out using the IBM-SPSS, version 19.0, statistics software (IBM, Armonk, NY, USA).

Results and Discussion

Total N showed similar results in cycles 1 and 2, and was significantly affected ($p<0.05$) by the application of the soluble fertilizer, when compared with the microbial fertilizer (Figure 1 A). With the microbial fertilizer treatments, the N content varied from 12.3 to 18.1 g kg^{-1} in cycle 1 and from 12.2 to 15.1 g kg^{-1} in cycle 2, whereas, with the soluble fertilizer, it ranged from 20.7 to 21.7 g kg^{-1} in cycles 1 and 2, respectively.

The total N content in lettuce was lower in the soil with the application of the microbial fertilizer, compared with the soluble fertilizer. This is explained by the slow mineralization of organic matter, leading to a more gradual absorption of N by the plants, which allows the action of metabolic processes and the production of organic structures, reducing nitrate accumulation in the plant shoot (Hernández et al., 2016). According to these authors, the use of microbial fertilizers that reduce the accumulation of N in leaves also minimizes soil pollution. These results agree with those of Ozgen et al. (2014), who observed a reduction of 38–50% in nitrate concentration in lettuce leaves that received the application of an organic fertilizer, compared with the soluble conventional fertilizer.

The C content in lettuce leaves was similar in all fertilization treatments in cycle 2. However, in cycle 1, it was significantly higher in the treatment using the soluble fertilizer and in the control, when compared with the microbial fertilizer (Figure 1 B).

The P content in cycle 1 was significantly affected when the microbial fertilizer was applied, in comparison with the conventional fertilizer and the control, varying from 0.6 to 1.6 g kg^{-1}. The content of the nutrient was lower when the conventional fertilizer was applied, and, therefore, the best results were obtained with the microbial fertilizer (Figure 1 C). In cycle 2, the microbial fertilizer applied at 50% of the RR differed significantly from the other treatments, with a low effect when compared with the conventional fertilizer; however, P uptake was higher in the treatment with the microbial fertilizer at the higher rate.

The K content in lettuce leaves showed a behavior similar to that of P content. In cycle 1, the content of the nutrient was higher with the microbial fertilizer, compared with the soluble fertilizer and the control, varying from 22.5 to 29.6 g kg^{-1}; the lowest and highest values were obtained when the conventional and microbial (at the highest RR) fertilizers were applied, respectively (Figure 1 D). However, after cycle 2, the conventional fertilizer and the control showed a K content higher than that of the other treatments.

The P and K contents in lettuce leaves were significantly affected in the soil with the microbial fertilizer, compared with the soluble conventional fertilizer and the control, especially in cycle 1. Oliveira et al. (2016) also observed a positive and significant effect on the content of these nutrients in melon plants, when comparing a microbial fertilizer produced from phosphate and potassic rocks inoculated with the *Acidithiobacillus* sulfur-oxidizing bacteria with the soluble conventional fertilizer. Berger et al. (2013) and Stamford et al. (2015) reported similar results when they applied rock biofertilizers mixed with an earthworm compound inoculated with rhizobia bacteria on cowpea [*Vigna unguiculata* (L.) Walp.] grown in a tableland soil of the Brazilian rain forest region. These authors described the positive and significant response of P and K released from rocks by action of the *Acidithiobacillus* bacteria, probably due to the production of sulfuric acid, which increases the release of these elements. In the present study, the acidic effect was neutralized by the organic matter (earthworm compost) used to produce the microbial fertilizer.

Other authors observed the effectiveness of organic biofertilizers, compared with the soluble fertilizer, in total K absorption. Pereira-Stamford et al. (2011) found positive results in grape (*Vitis vinifera* L.) grown in a soil of the semiarid region of the San Francisco Valley, and Lima et al. (2007), in lettuce, in a soil of the Cariri region in the state of Ceará, Brazil, with similar results in two consecutive crops.

The fresh weight of the lettuce plants in cycle 1 was affected significantly by the microbial fertilizer treatments (Figure 2 A), varying from 14 g with the control to more than 51 g with the microbial fertilizer applied at 100 and 150% of the RR.

Regarding plant height, in cycle 1, there was no significant difference (p=0.05) between treatments. However, in cycle 2, a significant difference was observed between the microbial fertilizer and the soluble fertilizer.

These results confirm the positive and significant effects of the microbial fertilizer, especially at higher rates, on the total content of nutrients in the leaves and in the fresh weight of lettuce in cycles 1 and 2, compared with the soluble fertilizer and the control. Hernández et al. (2016) also found a positive effect of organic matter on the fresh biomass of lettuce grown in a Mediterranean soil fertilized with an organic compost. Chatterjee (2015), testing different sources of nutrients on lettuce in the Hymalaian region, observed that plant yield was affected by the treatment with 10 Mg ha^{-1} manure mixed with 2.5 Mg ha^{-1} earthworm compost inoculated with a phosphate-solubilizing bacteria, which lead to the best results for leaf fresh weight.

Plant diameter did not differ significantly between fertilization treatments in cycle 2 (Figure 2 D), except when the microbial fertilizer was applied at the highest RR of 150%, which resulted in the greatest values of 16.25 and 17.75 cm, respectively, in cycles 1 and 2. This parameter showed a behavior similar to that of plant height, with greater values when the microbial fertilizer was applied at the highest rates, which also increased the number of leaves and, consequently, plant diameter. In both cycles, the microbial fertilizer significantly improved plant growth ($p \leq 0.05$), compared with the soluble fertilizer and the control, promoting a residual effect.

Plant diameter and fresh weight differed significantly in cycle 2, and the best results were obtained when the microbial fertilizer was applied at the highest RR. However, there was no significant effect due to the application of the microbial fertilizer at lower rates and the soluble fertilizer. Hosseny & Ahmed (2009) evaluated the N effectiveness of biofertilizers on N absorption by ‘Romana’ lettuce grown in a sandy soil, and observed that plant head diameter increased with a higher amount of N or with increased levels of organic fertilizers. The authors concluded that the organic fertilizers showed the best results for lettuce yield, with a reduction in N, confirming the hypothesis

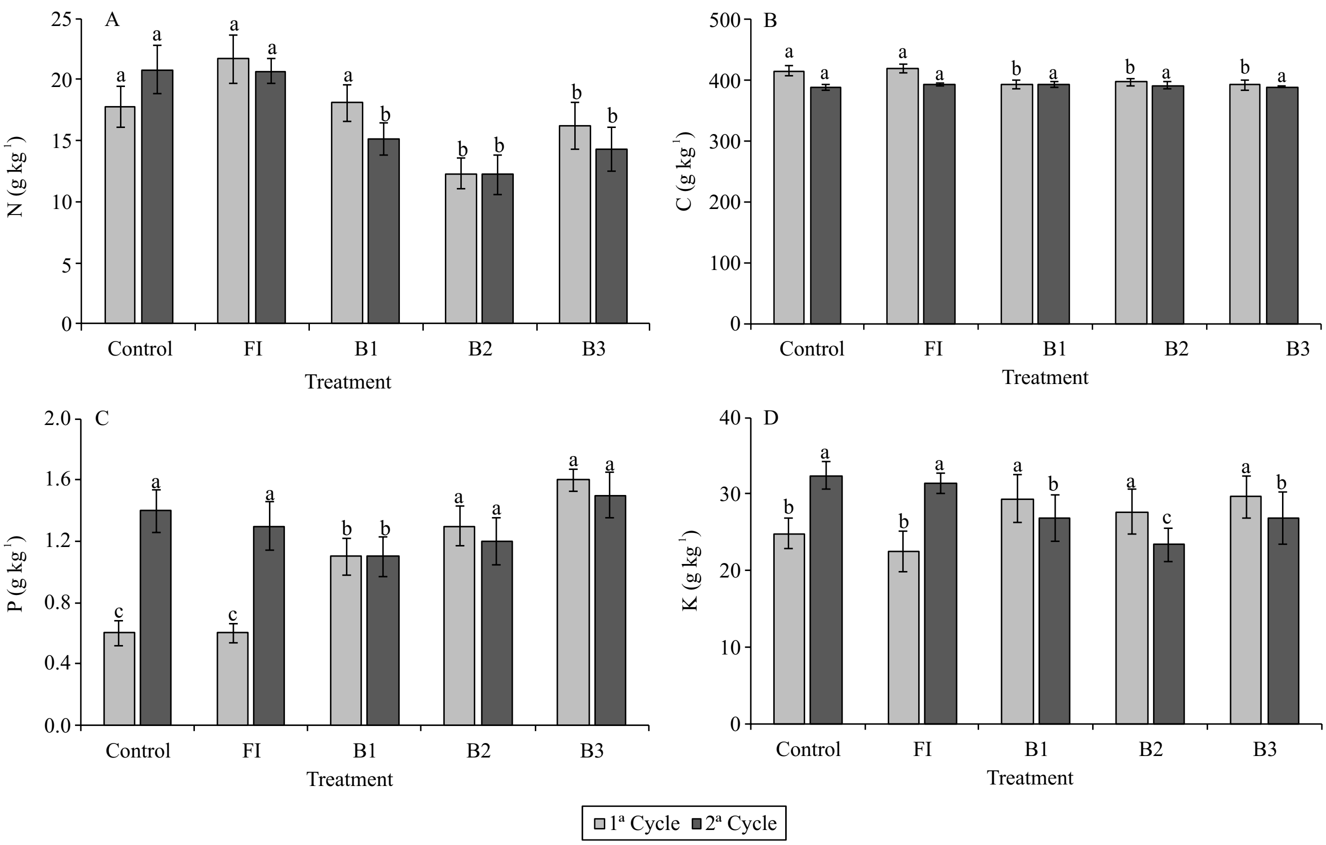

Figure 1. Effects of fertilization on the content of the following nutrients in lettuce (*Lactuca sativa*) leaves: nitrogen (A), carbon (B), phosphorous (C), and potassium (D). Treatments: control, no fertilizers applied; FI, conventional fertilizer, 100% of the recommended rate (RR); B1, microbial fertilizer, 50% of the RR (5 Mg ha^{-1}); B2, microbial fertilizer, 100% of the RR (10 Mg ha^{-1}); and B3, microbial fertilizer, 150% of the RR (15 Mg ha^{-1}). Bars represent standard deviation. Data followed by equal letters do not differ by Tukey’s test ($p<0.05$).

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that the microbial fertilizer may be an alternative to conventional soluble fertilization when applied at an adequate rate.

The effects of the treatments on soil chemical characteristics are shown in Table 2. Soluble N in the soil was significantly affected by the fertilization treatments ($p<0.05$) and varied from 32 to 118 mg kg^{-1}, being lower in the control and higher in the conventional fertilizer applied in the initial growth time (T0). At this time, the soluble N in the soil differed significantly ($p<0.05$) in all treatments; however, after 30 (T30) and 60 (T60) days, the soluble N in the soil was close to 0 mg kg^{-1} (Table 2).

Soil pH was affected by the fertilization treatments, decreasing significantly when the microbial fertilizer was applied at increasing rates, probably due to the presence of the *Acidithiobacillus* oxidizing bacteria, which metabolically produces sulfuric acid, whose acidity increases the release of P and K from rocks. Some authors agree that the addition of microorganisms for P and K solubilization reduce soil pH and, in some conditions, may release organic acids (Namli et al., 2017). Stamford et al. (2007, 2009, 2015), for example, observed a reduction in soil pH when sulfur inoculated with *Acidithiobacillus* was applied to a soil of the Brazilian semiarid region. In the present study, the total N in the soil showed an inverse behavior, with values increasing after T30 and T60, being the highest in the control and in the microbial fertilizer treatment applied at 150% of the RR (0.69 g kg^{-1}); at T60, the microbial fertilizer promoted a value (0.59 g kg^{-1}) higher than that of the soluble fertilizer (0.53 g kg^{-1}).

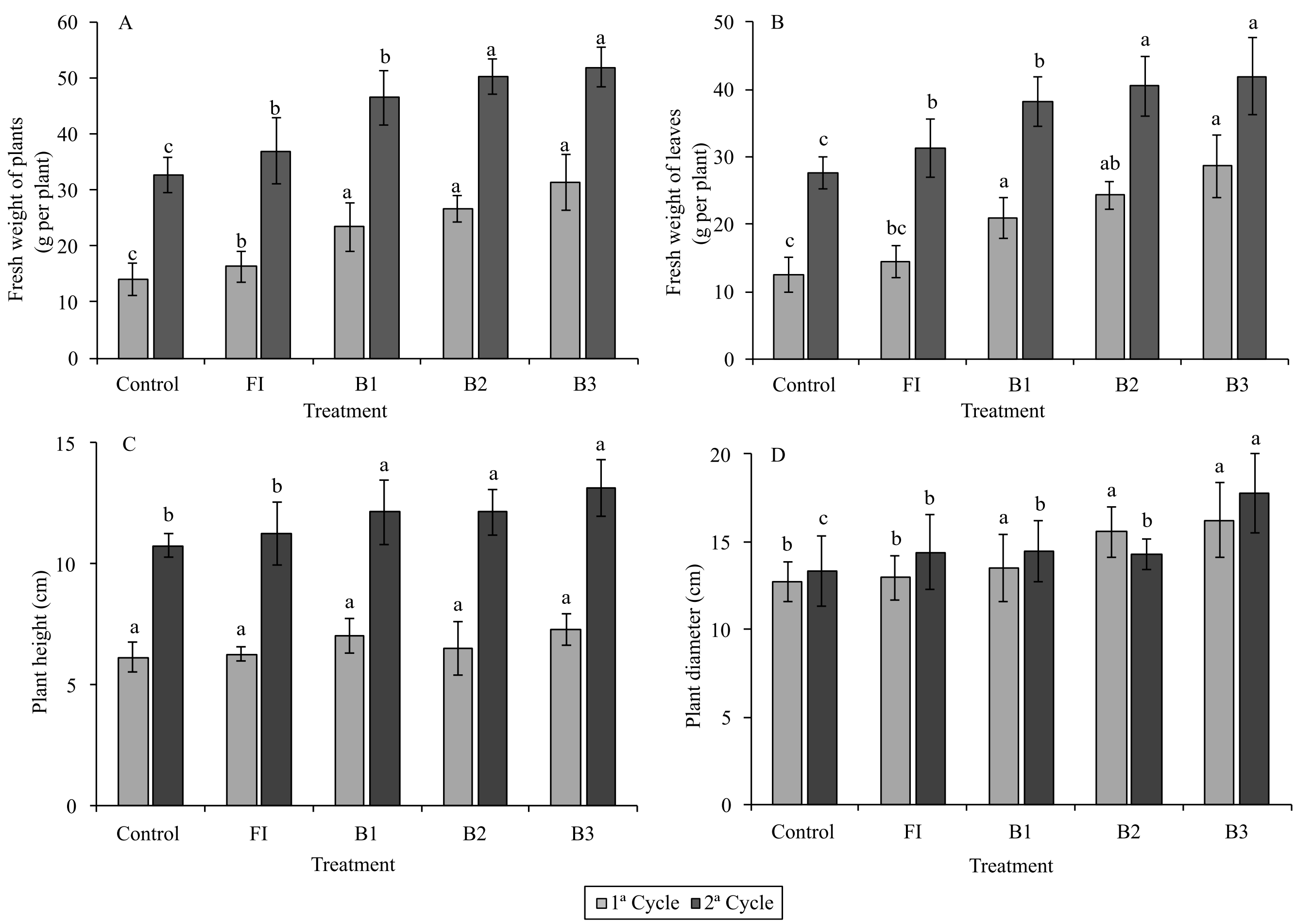

Figure 2. Effects of fertilization on plant fresh weight (A), leaf fresh weight (B), plant height (C), and plant diameter (D). Treatments: control, no fertilizers; FI, conventional fertilizer, 100% of the recommended rate (RR); B1, microbial fertilizer, 50% of the RR (5 Mg ha^{-1}); B2, microbial fertilizer, 100% of the RR (10 Mg ha^{-1}); and B3, microbial fertilizer, 150% of the RR (15 Mg ha^{-1}). Bars represent standard deviation. Data followed by equal letters do not differ by Tukey's test ($p<0.05$).

However, no significant difference was observed between fertilization treatments (Table 2).

In another study, total N in the soil increased when evaluated during a longer growth time under sewage and earthworm compost as an alternative inorganic fertilizer for lettuce, and the mineralization of organic N was shown to depend extensively on the source of organic matter used and on other biotic and abiotic parameters (Hernández et al., 2016).

Soil P was significantly affected by the fertilization treatments ($p<0.05$). The available P in the soil varied from 2.00 to 29.17 mg kg^{-1} in T0 and from 1.40 to 51.74 mg kg^{-1} in T60, with a lower increase when the control treatment was applied. In addition, the highest available P was obtained at both times when the microbial fertilizer was applied at 150% of the RR, with a significant difference for the others treatments, except when the microbial fertilizer was applied at 100% of the RR in T60 (Table 2).

The available K in the soil in T0 presented the best results; however, no significant effect was observed in the fertilization treatments ($p>0.05$), which showed a medium value of 227.05 mg kg^{-1}. In general, the highest contents of this nutrient were found in soils treated with the microbial fertilizer, when compared with the control (Table 2).

The effect of the microbial fertilizer on total N and available P, when applied at increased rates and in the longer growth times (T30 and T60), was significant and residual. These results are in alignment with those obtained by Stamford et al. (2014) for grape, Stamford et al. (2016) for sugarcane, and Oliveira et al. (2014) for melon. According to these authors, the observed effect is due to an increase in organic matter that, consequently, increases nutrient release and available P in the soil. Lima et al. (2007) also reported a residual effect on P availability after the application of a biofertilizer produced from P and K rocks mixed with sulfur inoculated with *Acidithiobacillus* and earthworm compost, whose effect on lettuce yield and nutrient absorption was compared with that of soluble conventional fertilizers in two consecutive cycles in an Oxisol. Franco et al. (2011) concluded that the increase in available P and in other elements contained

Table 2. Effects of fertilization on soil chemical attributes in consecutive cycles(1).

Treatment(2)	pH_{H2O} (1:5)	EC(3) (dS m^{-1})	Soluble N	Soluble C	Available P	Available K	Total N
			(mg kg^{-1})				
Initial growth time							
Control	8.33c (0.03)	0.15c (0.03)	32.89e (0.61)	38.66b (0.09)	2.00d (0.48)	27.10b (1.44)	0.021a (0.0)
FI	8.27b (0.02)	1.47b (0.32)	118.00a(1.96)	45.88a (0.05)	10.79c (0.25)	226.53a (1.23)	0.017ab(0.0)
B1	8.07a (0.04)	2.34a (0.51)	94.38b (0.71)	46.68a (0.58)	19.19b (0.37)	227.05a (1.93)	0.028a (0.0)
B2	8.05a (0.03)	0.77bc(0.28)	81.95c (0.39)	52.63a (0.32)	28.86a (0.44)	228.73a (1.61)	0.029a (0.0)
B3	8.04a (0.02)	1.07bc(0.11)	77.52d (0.61)	48.53a (0.37)	29.17a (0.51)	228.30a (1.62)	0.020a (0.0)
Growth time – 30 days							
Control	8.48c (0.03)	0.83ab(0.02)	12.16a (0.32)	57.82a (0.74)	1.58d (0.70)	6.02b (0.62)	0.069a (0.0)
FI	8.46c (0.03)	0.85ab(0.05)	10.40a (0.57)	54.42a (0.07)	6.70c (0.40)	35.10a (0.59)	0.050ab(0.0)
B1	8.40b (0.03)	1.26a (0.07)	0.55b (1.10)	57.46a (0.61)	21.31b (0.59)	37.70a (0.38)	0.041b (0.0)
B2	8.27a (0.02)	1.05a (0.03)	0.00b (0.00)	60.13a (0.12)	24.68b (0.36)	42.50a (0.46)	0.049ab(0.0)
B3	8.22a (0,01)	1.24a (0.06)	0.00b (0.00)	58.32a (0.45)	37.00a (0.34)	37.50a (0.37)	0.069a (0.0)
Growth time – 60 days							
Control	8.59c (0.06)	0.61a (0.05)	11.11a (0.51)	64.47b (0.51)	1.40d (0.69)	6.45b (0.20)	0.064a (0.0)
FI	8.54c (0.01)	0.87a (0.05)	0.00b (0.00)	66.38b (1.09)	7.19c (0.50)	60.00a (0.18)	0.053ab(0.0)
B1	8.36b (0.04)	1.04a (0.03)	1.04b (1,20)	89.02ab(0.44)	25.63b (0.16)	56.10a (0.18)	0.059ab(0.0)
B2	8.29ab(0.03)	1.15a (0.01)	0.00b (0.00)	122.79a (1.26)	46.67a (0.56)	59.30a (0.14)	0.056ab(0.0)
B3	8.21a (0.03)	0.97a (0.05)	0.00b (0.00)	84.83ab(0.95)	51.74a (0.64)	57.00a (0.23)	0.053ab(0.0)

(1)Data followed by equal letters do not differ by Tukey's test ($p<0.05$). (2)FI, conventional fertilizer, 100% of the recommended rate (RR); B1, microbial fertilizer, 50% of the RR (5 Mg ha^{-1}); B2, microbial fertilizer, 100% of the RR (10 Mg ha^{-1}); and B3, microbial fertilizer, 150% of the RR (15 Mg ha^{-1}). (3)Electrical conductivity.

in rocks may be attributed to the interactive effect of fungi that produce phosphatases and contain chitosan. Kowalski et al. (2006) and Goy et al. (2009) suggested that chitosan addition promotes an increase in N, P, and K availability.

In general, a greater content of soluble C was observed in the soil that received the application of the microbial fertilizer, compared with the soluble fertilizer and the control (Table 2). The soluble C in the soil showed the same tendency in T0 and T30, with no significant effect of fertilization treatments. However, its values ranged from 64.47 to 122.79 mg kg^{-1} in T60, showing a significant ($p<0.05$) effect of growth time, as well as a residual effect. The lowest value was found in the control (38.66 mg kg^{-1}), which did not differ in the different growth times. The best results were obtained when the microbial fertilizer was applied at 100% of the RR, which promoted a significant increase compared with the other treatments.

Conclusions

1. The microbial fertilizer applied at higher rates significantly affects soil nutrient availability, especially of available P and K, confirming that the fertilizer increments the chemical attributes of the soil.
2. The microbial fertilizer applied at higher rates may be a viable alternative to the conventional fertilizer to increase lettuce productivity.
3. Residual effects of the microbial fertilizer are observed in consecutive cycles, especially when it is applied at the highest rates.

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