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Effect of PGPR, Phosphate Sources and Vermicompost on Growth and Nutrients Uptake by Lettuce in a Calcareous Soil

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ABSTRACT

In order to study the effect of plant growth promoting rhizobacteria (PGPR), vermicompost and phosphate sources on the growth and nutrients uptake by lettuce, a greenhouse experiments was conducted. Treatments consisted of PGPR (Pseudomonas fluorescens) (with and without inoculation), vermicompost (0 and 1% w/w) and phosphate sources (control, rock phosphate (RP), tricalcium phosphate and triple super phosphate (TSP) at 25 mg P kg^{-1} level). Biological fertilizers, RP and TSP significantly increased shoot dry matter (SDM) and some measured nutrients uptake. Co-application of PGPR and RP, in non-vermicompost treatments significantly increased SDM, shoot nitrogen (N), phosphorus (P), potassium (K), zinc (Zn) and manganese (Mn) uptake rates. Shoot P uptake had no significant difference between TSP and RP treatments. Co-application of PGPR, vermicompost and TSP significantly decreased SDM which may be due to the P toxic levels in the plant aerial parts and/or the inhibition of the bacterial activities in the rhizosphere soil.

Keywords: Calcareous soil, insoluble phosphates, lettuce, Pseudomonas fluorescens.

INTRODUCTION

Lettuce is a significantly popular plant which is cultivated for its leaves most of the times. It is a cool season plant which needs high light values. Lettuce improves human health due to its anti-oxidant compounds e.g. (vitamin C and E, carotenoids and polyphenols), fiber and some minerals in its tissues (Baslam et al., 2011). The Ferdos lettuce is good quality and marketing in Iran. It is a strong and leafy variety of cos lettuce with compact head, perfect taste, and good fragility leaves.

Nowadays producing organic and chemical free products is a substantial aim of sustainable agriculture and production. Inoculation of beneficial microorganisms into soils can reduce requirements of chemical fertilizers which are environmentally deleterious (Barea et al., 1997). Plant growth promoting rhizobacteria (PGPR) are number of bacterial species which can get associated with plant rhizosphere and make beneficial effects on plant growth (Glick, 1995). Among the Gram-negative soil bacteria, Pseudomonas is the most abundant genus in the rhizosphere soil and the PGPR activities of some of these strains has been known in different regions (Kumar et al. 2015). These species can promote plant growth by production of phytohormones, antibiotics, siderophores and enzymes (Esitken et al., 2010; Kumar et al. 2015).

Phosphorus is one of the most important plant essential macro nutrients. Phosphorus availability is low in calcareous soils, due to the high amounts of calcium carbonate, high pH values and low organic matter and moisture content (Dey, 1988). Therefore using P fertilizers in these soils has been increased recently. However, phosphate immobilization in calcareous soils is a common feature (Dey, 1988). High concentrations of calcium will result in the phosphorus being fixed in these soils and unavailable to the crop (Goldstein, 1995).

Rock phosphate (RP) or phosphate rock is the primary raw material in the production of P fertilizers. Direct application of RP (DARP) is a low cost option for phosphorus fertilization in soils of some countries with pH 5.5 or less, such as tropical and subtropical soils (Haynes, 1984). However, DARP in calcareous soils of Iran is limited because of its low availability. In this regards, it should be used phosphate-solubilizing microorganisms as best eco-friendly means for P nutrition of crop (Nahas, 1996). Other than the mentioned PGPR traits, phosphate solubilization is also one of the most important mechanisms which can increases phosphorus uptake by inoculated plant. Using phosphate solubilizing (PS) PGPR with insoluble phosphate sources has become more attractive in recent years. These bacteria can solubilize the inorganic phosphate by releasing organic acids, H^+ and chelation compounds (Goldstein, 1995). Also PS-PGPR can mineralize the organic P by producing phosphatase enzymes (Rodriguez and Fraga, 1999).

On the other hand, vermicompost is known as a compound with high values of essential nutrients such as phosphorus and nitrogen (Edwards and Burrows, 1988). Application of vermicompost increased lettuce height and dry weight (Ali et al., 2007). Hernandez et al. (2010) reported that application of vermicompost increased potassium (K), calcium (Ca), magnesium (Mg) and manganese (Mn) to optimal concentration in lettuce. Today, however, no data is available in the literatures about the effects of co-applications of PGPR, phosphate sources and vermicompost in lettuce plants, therefore the present study was conducted to evaluate the effect of PGPR inoculation with soluble and insoluble sources of phosphate and vermicompost, on growth and nutrients uptake by lettuce in a calcareous soil.

MATERIALS AND METHODS

Bacterial Inoculum Preparation

Pseudomonas fluorescens bacterium, obtained from Soil Biology Lab, Department of Soil Science and Engineering, Tehran University. Pure bacterial culture was grown on nutrient broth (NB) medium in a shaker incubator, at 28°C for 24h. The PGPR strain was able to produce auxin and siderophore and also to solubilize the insoluble inorganic and organic phosphates (Data not shown).

Soil, Vermicompost and Rock Phosphate (RP) Characteristics

Calcareous soil was collected from surface horizon (0-30 cm) of Daneshkadeh series uncultivated lands (Fine, mixed (calcareous), mesic, Typic Calcixerepts) (Soil Survey Staff., 1998) in Bajgah, Fars, Iran (1810 m above sea level, 29º36´ N, 52º32´ E), in fall 2014. This region has an average annual rainfall of 307 mm and a mean annual temperature of 17.3ºC. Soil sample was air dried, passed through a 2-mm sieve and mixed thoroughly. Some physical and chemical characteristics of soil were measured based on standard methods. The soil was silty clay loam (Gee and Bauder, 1986) with pH of 7.5 (Thomas, 1996) and electrical conductivity

(EC) of 0.63 dS.m^{-1} (Rhoades, 1996), organic matter content of 0.85% (Nelson and Sommers, 1996), cation exchange content (CEC) content of 24 cmol$_{(+)}$ kg^{-1} (Summer and Miller, 1996), total nitrogen content of 0.03% (Bremner, 1996), Olsen P of 12.4 mg kg^{-1} (Olsen et al., 1954), available K of 620 mg kg^{-1} (Page et al., 1982) and diethylenetriaminepentaacetic acid (DTPA)-extractable iron (Fe), zinc (Zn), copper (Cu), and Mn of 5.5, 0.6, 1.6 and 12.2 mg kg^{-1}, respectively (Lindsay and Norvell, 1987). Vermicompost was produced from cow manure in the Windrow method using Eisenia fetida. Vermicompost was air dried, passed through a 2-mm sieve, mixed uniformly and its characteristics were measured by standard methods. The vermicompost was with pH of 7.75 (Thomas, 1996) and EC of 5.8 dS.m^{-1} (Rhoades, 1996) in the ratio of 5:1 of water: vermicompost suspensions, organic matter content of 44.2% (Nelson and Sommers, 1996), total nitrogen content of 2.15% (Bremner, 1996), total P of 14194 mg kg^{-1} (Chapman and Pratt, 1961), total K of 10000 mg kg^{-1} (Page et al., 1982) and total Fe, Zn, Cu, and Mn of 3274, 112.3, 28.7 and 248.8 mg kg^{-1}, respectively (Chapman and Pratt, 1961).The RP powder was provided from Yazd Esfordi mining and industrial complex, Iran.

Greenhouse Experiment

The experiment was carried out under greenhouse condition with day/night temperature of 24±3ºC/15±3ºC, 60-70% average relative humidity (RH) and a photoperiod of 14 (h) with photosynthetic photon flux (PPF) of 800 µmol m^{-2} s^{-1}. It was conducted with a factorial arrangement in a completely randomized design with three factors: two levels of bacteria (without inoculation (B_0) and with inoculation (B_1) (containing 10^7 CFU (colony forming unit) ml^{-1})), two levels of vermicompost (0 (V_0) and 1% w/w (V_1)) and four levels of phosphate sources (control, rock phosphate powder (RP), tricalcium phosphate (TCP) and triple super phosphate (TSP) at rate of 25 mg P kg^{-1}). Three replications per treatment were made to give a total of 48 pots. The soil was passed a 4 mm sieve and 4 kg of soil were filled into the plastic pots (21 cm diameter and 20 cm depth). Essential nutrient elements (except P) based on the results of the soil test analyses were added to all pots uniformly at the rate of 150 mg N kg^{-1} of soil (as Urea, 46%), Fe, Zn and Cu at the rate of 5 mg kg^{-1} of soil (as Iron(II) sulfate heptahydrate ($FeSO_4$. $7H_2O$), zinc sulfate heptahydrate ($ZnSO_4$. $7H_2O$), copper(II) sulfate pentahydrate ($CuSO_4$. $5H_2O$); respectively). Treatments of vermicompost and phosphate sources were added to soil of pots and mixed uniformly. Five seeds of lettuce (Lactuca sativa cv. Ferdos) were planted in each pot. In bacterial treatments each seed were inoculated with 2 ml of NB containing 10^7 CFU ml^{-1} of Pseudomonas fluorescens. Plants were thinned to two uniform stands after germination and watered to field capacity with distilled water.

Plant Analysis

Plant aerial parts were harvested after 10 weeks after planting, dried to a constant weight in oven at 65°C, shoot dry matter (SDM) was recorded and then ground into powder for chemical analysis. Shoot total nitrogen concentration was measured by Micro-Kjeldahl method (Bremner, 1996), shoot total phosphorus concentration by vanadate-molybdate yellow method (Chapman and Pratt, 1961). Shoot total Ca, Mg, Fe, Zn, Cu, and Mn concentrations were determined using the dry ash method, dissolved in 2N hydrochloric acid (HCl), filtered and then measured with atomic absorption Shimadzu-AA670 (Chapman and Pratt, 1961). Shoot total K and Na concentrations were determined by flame photometer CORNING 405, Gallenkamp, London, UK (Page et al., 1982). Nutrient uptake is calculated by multiplying plant dry weight by nutrient concentration.

Statistical Analysis

Data collected were subjected to statistical analyses by ANOVA. Means were compared by least significant difference (LSD) at 5% level of significance using SAS statistical software (SAS Inc., Carey, NC).

RESULTS AND DISCUSSION

Shoot Dry Matter (SDM)

Results showed that bacterial inoculation significantly increased SDM by 8%, compared to control ones (Table 1). Vermicompost application significantly increased SDM by 37%. Also, RP and SP application increased SDM by 14.8 and 15.5%, respectively compared to control (Table 1). Application of vermicompost significantly increased SDM by 37% compared to control (Table 1). Co-application of PGPR and RP, in non-vermicompost treatments (V_0) significantly increased SDM by 49% compared to single application of RP (Table 2). In bacterial treated samples (B_1), simultaneous application of vermicompost and TSP, significantly decreased

SDM by 18% compared to those of non-bacterial treatments (B_0). Application of vermicompost (V_1) along with phosphate sources (RP, TCP and TSP) significantly increased SDM in non-bacterial treatments by 69, 35 and 46%, respectively. The maximum SDM was obtained in combined application treatments of vermicompost and TSP in non-bacterial treatments (Table 2). Biofertilizers such as vermicompost and PGPR can increase plants growth and yield due to increasing biological activities and nutrients availability and also improving soil structure (Sansamma and Pillai, 2000).

Vermicompost is rich in nutrients and beneficial microorganisms which convert nutrients into plant-available forms (Edwards and Burrows, 1988). High values of nutrients specially P and N in compost can increase fresh and dry matters of lettuce (Bustamant et al., 2008). Plant growth-promoting traits of Pseudomonas fluorescens used here were consisted of the auxin and siderophore producing ability, and phosphate solubilizing activity. Inoculation of lettuce with Bacillus subtilis increased SDM (Kohler et al., 2007). The inoculation of PGPR strains significantly improved the germination characteristics of tomato and lettuce seeds. The PGPR effects could be attributed to their unique metabolic properties particularly the ability to produce growth regulators (Mangmang et al., 2014). The findings of present study showed that application of RP and TSP increased SDM; which was consistent with the results of Grant lipp and Goodall (1957) who reported that dry matter increased with increasing P in soil. Co-application of PGPR and RP or vermicompost and RP increased SDM. It may be related to PGP characteristics.

The results showed that co-application of PGPR, vermicompost and TSP, significantly decreased SDM which may be due to increasing available P. Excess P, can be toxic for plant growth.

Normally, excess P results in an imbalance which affects plants growth and the availability, uptake, and utilization of other essential elements (Jones, 1998). The negative effects were also observed on shoot N, K, Ca, Mg. Zn and Mn uptake rates (Tables 2 and 4). Barker and Bryson (2006) reported that the sufficient level of phosphorus for lettuce plant was 0.25 (percent of a dry mass) at the harvest time. Shoot phosphorus concentration range in present study was 0.39-0.51 (percent of a dry mass) at the harvest level. Also, Najafi and Towfighi (2009) reported that overuse of available phosphorus in soil may have negative effect on plant growth. Rather than a toxic effect of P, the results may be linked to the inhibition of the bacterial activities in the presence of high values of soluble P, which can negatively affect the plant growth.

Shoot N Uptake

Results showed that PGPR inoculation and application of RP and TSP significantly increased shoot N uptake by 8, 15 and 21%, respectively compared to control treatments (Table 1) and application of vermicompost significantly increased shoot N uptake by 17% (Table 1). In non-bacterial treatments, combined application of vermicompost with RP or TSP, significantly increased shoot N uptake compared to non-vermicompost treatments. The maximum shoot N uptake was obtained in co-application of vermicompost with TSP which had no significant difference with the simultaneous application of vermicompost with RP in non-bacterial treatments (Table 2). In non-vermicompost treatments (V_0), co-application of PGPR (B_1) and RP significantly increased shoot N uptake compared to non-bacterial (B_0) treatments, while in vermicompost treatments (V_1), simultaneous application of PGPR (B_1) with RP or TSP significantly decreased shoot N uptake compared to non-bacterial treatments (Table 2).

Organic fertilizers increased biological and enzymatic activities and also increased some nutrients availability and uptake such as N. Vermicompost is rich in N and may have nitrogen fixing and decomposers microorganisms. Patten and Glick (2002) reported that PGPR enhanced nutrients uptake by increase of root elongation and growth due to IAA production and other PGP activities. Nutrients uptake is also closely connected to plant growth (Leggett and Frere, 1971). The used bacterium had the ability to auxin production and other PGP characteristics. Phosphorus application increased shoot N uptake due to increasing root uptake area and root development (Zeidan, 2007). Results of present research showed that co-application of vermicompost and TSP, significantly decreased shoot N uptake by lettuce due to the reduction in dry matter yield.

Shoot P Uptake

Application of vermicompost, PGPR, RP or TSP significantly increased shoot P uptake by 44, 16, 18 and 11%, respectively compared to those of control (Table 1). In non-vermicompost (V_0) and vermicompost (V_1) treatments, combined application of RP and PGPR significantly increased shoot P uptake (Table 2). In non-bacterial (B_0) and bacterial (B_1) treatments, combined application of RP and vermicompost significantly increased shoot P uptake (Table 2). Shoot P uptake had no significant difference between TSP and RP treatments in bacterial or non-bacterial treatments.

Vermicompost contains large amounts of P organic and inorganic compounds that can convert to available forms easily by microbial activities (Edwards and Burrow, 1988). Phosphate solubilizing bacteria enhance the supply of P to plants as a result of their ability to solubilization organic and inorganic P (Richardson, 1994). Enhancing P uptake by phosphate solubilizing-PGPR is due to hormone releasing, increasing root development and therefore nutrients uptake by plant (Brown, 1974) and also releasing organic acids, H^+, chelation compounds and phosphatase enzymes, that caused solubilization of inorganic and organic insoluble phosphates and changing them to available forms (Azcon et al., 1976). Alexander (1977) showed that PGPR increased P and K availability in soil which resulted in higher uptake of these nutrients due to enhanced plant growth. Co-application of cow manure and calcium phosphate increased P uptake in spinach (Zahedifar et al., 2011). Shoot P uptake of lentil increased with combined application of RP and phosphate solubilizing rhizobia (Zarei et al., 2006).

Shoot K Uptake

PGPR inoculation or application of RP and TCP increased shoot K uptake insignificantly (Table 1). Application of TSP and vermicompost significantly increased shoot K uptake by 37 and 18%, respectively compared to control ones (Table 1). Also combined application of RP and PGPR significantly increased shoot K uptake in non-vermicompost treatments. Simultaneous application of vermicompost and TSP significantly decreased shoot K uptake by lettuce in bacterial treatments (Table 2). The highest shoot K uptake was obtained in co-application treatments of TSP with vermicompost in non-bacterial treatments which was significantly higher than the same treatment in bacterial treatments (Table 2).

Biological fertilizers increased P and K availability by releasing organic acids and other chemical compounds (Alexander, 1977). Composts from distillery wastes increased K uptake by plants (Bustamant et al., 2008). Our results is consistent with findings of Han et al., (2006) who showed that co-application of phosphate solubilizing bacteria and RP increased P and K uptake in pepper.

Shoot Ca and Mg Uptake

Results showed that PGPR inoculation and vermicompost application significantly increased shoot Ca uptake by 111% and 68%, respectively compared to control treatments (Table 1). Phosphate sources had no significant effect on shoot Ca uptake (Table 1). However, combined application of vermicompost and RP/TSP significantly increased shoot Ca uptake in bacterial treatments (B_1). The maximum Ca uptake was obtained in simultaneous application treatments of vermicompost with RP and PGPR (Table 2).

PGPR inoculation and vermicompost application increased shoot Mg uptake by 48 and 18% respectively compared to those of control (Table 1). Application of TCP significantly decreased shoot Mg uptake by lettuce (Table 1). Co-application of vermicompost with RP significantly increased shoot Mg uptake in non-bacterial (B_0) and bacterial (B_1) treatments (Table 2). In the presence of TSP/RP/TCP, co-application of vermicompost and PGPR significantly decreased shoot Mg uptake. The highest shoot Mg uptake (109.82 mg pot^{-1}) was observed in simultaneous application treatments of vermicompost with PGPR in the absence of phosphate sources.

Hernandez et al. (2010) reported that vermicompost application increased Ca and Mg contents in lettuce leaves. PGPR increased K, Mg and P availability in the soil by mineralization of organic compounds (Esitken et al., 2010). Pseudomonas flouresence with the ability to IAA production could increase root growth and N, P, K and Mg uptake (Farzana and Radziah, 2005). Singh and Kapoor (1988) reported that co-application of RP and phosphate solubilizing microorganisms could increase nutrients uptake such as Ca and Mg.

Shoot Fe and Mn Uptake

Results showed that PGPR inoculation increased shoot Fe uptake insignificantly (Table 3). Application of TSP, RP and TCP increased shoot Fe uptake by 50%, 32% and 12% respectively compared to control treatment (Table 3). Also, vermicompost application significantly increased shoot Fe uptake by 50% compared to those of control (Table 3). The highest shoot Fe uptake was observed in co-application treatment of RP and vermicompost in bacterial treatment (B_1) which increased shoot Fe uptake by 46% compared to simultaneous application of RP with vermicompost in non-bacterial treatments (B_0) (Table 4). In bacterial treatments (B_1), combined application of phosphate sources (RP, TCP and TSP) and vermicompost significantly increased shoot Fe uptake compared to non-vermicompost treatments (Table 4). Singly application of PGPR and vermicompost, significantly increased shoot Mn uptake by 33% and 11%, respectively compared to control ones (Table 3). TCP decreased shoot Mn uptake insignificantly compared to that of control (Table 3). In non-vermicompost treatments (V_0), co-application of RP and PGPR, significantly increased shoot Mn uptake compared to non-bacterial treatments. Simultaneous application of vermicompost and phosphate sources significantly increased shoot Mn uptake in non-bacterial treatments compared to non-vermicompost treatments (Table 4). The maximum shoot Mn uptake was obtained in combined application of TSP and vermicompost in non-bacterial (B_0) treatments.

Improved Fe and Mn uptake may be related to PGP characteristics of bacterium (Patten and Glick, 2002) and chelating agents or presence of ingredients bearing nutrients in vermicompost. Nutrients uptake might be also enhanced due to increased plant growth (Leggett and Frere, 1971).

Shoot Cu and Zn Uptake

Bacterial inoculation significantly decreased shoot Cu uptake by 22% (Table 3). Application of RP significantly increased shoot Cu uptake by 99% compared to that control but TSP application significantly decreased shoot Cu uptake by 31% (Table 3). In bacterial and non-bacterial treatments, co-application of vermicompost with TSP decreased shoot Cu uptake compared to non-vermicompost treatments (Table 4). Combined application of vermicompost with RP in bacterial and non-bacterial treatments significantly increased shoot Cu uptake compared to non-vermicompost treatments. Also in vermicompost treatments (V_1), simultaneous application of PGPR and phosphate sources (RP, TCP and TSP) decreased shoot Cu uptake by lettuce compared to non-bacterial treatments. The lowest shoot Cu uptake was obtained in the combined application treatments of vermicompost with TCP or PGPR (Table 4).

RP application significantly increased shoot Zn uptake by 17% compared to control (Table 3). Also vermicompost application significantly increased shoot Zn uptake by 15% compared to control one (Table 3). Some chelating agents in vermicompost like humic acids may be caused increased Zn uptake in plants. Co-application of RP and vermicompost significantly increased shoot Zn uptake in non-bacterial treatments (B_0), and significantly decreased in bacterial treatments (B_1) compared to non-vermicompost treatments (Table 4). The maximum Zn uptake was observed in combined application of RP and vermicompost in non-bacterial treatments (B_0). Lambert et al. (1979) reported that increasing phosphorus fertilizers in soil reduced Cu and Zn uptake by plants; due to the interaction between these nutrients and phosphorus. Increasing P availability and uptake in plants by PGPR may decrease shoot Cu and Zn uptake. This result is consistent with findings of Bagyako et al. (2000). As can be seen in the results, PGPR inoculation significantly decreased shoot Zn uptake in co-application of RP with vermicompost. This result may be due to phosphorus solubilization activities of the microorganisms and presence of soluble phosphorus in vermicompost which increased the available phosphorus form and consequence its uptake by plant. This is naturally followed by a sharp decrease in Zn uptake by lettuce. Jones (1998) reported that increasing P significantly reduced Zn uptake by plants. Also Barker and Bryson (2006) showed that P can lead to Zn or Cu deficiencies in plants.

Shoot Na Uptake

PGPR inoculation and vermicompost application significantly increased shoot Na uptake by 12 and 29%, respectively compared to control treatments. Also RP application significantly increased shoot Na uptake by 22% compared to that of control (Table 3). Simultaneous application of vermicompost with RP/TSP significantly increased shoot Na uptake in bacterial treatments (B_1) compared to non-vermicompost treatments (Table 4). The highest shoot Na uptake was obtained in RP and vermicompost combined treatments. Also in vermicompost treatments (V_1), combined application treatments of TSP and PGPR, increased shoot Na uptake compared to non-bacterial treatments (Table 4). Bustamant et al. (2008) showed that compost application increased shoot Na uptake in lettuce that is in agreement with our findings. Also, Esitken et al. (2010) reported that PGPR inoculation increased Na in strawberry's leaves.

CONCLUSION

Applications of vermicompost significantly increased SDM and shoot nutrients uptake except for shoot Cu uptake. PGPR inoculation significantly increased SDM and shoot N, P, Ca, Mg, Mn and Na uptake rates, but it significantly decreased Cu uptake. Shoot N, P, Cu, Fe, Zn and Na uptake rates increased in RP treatments, while shoot K and Mn uptake rates had no significant differences. TSP application decreased shoot Zn and Cu uptake rates but it increased SDM and other shoot nutrients uptake.

Co-application of PGPR and RP, in non-vermicompost treatments significantly increased SDM, shoot N, P, K, Zn and Mn uptake rates, while in vermicompost treatments it significantly increased shoot P, Ca and Fe uptake rates and decreased shoot N and Zn uptake rates.

Application of vermicompost with RP significantly increased SDM, and shoot N, P, K, Mg, Mn, Cu, Zn and Na uptake in non-bacterial treatments.

Our results also showed that SDM, shoot P, N, Mg, Fe and Mn uptake rates in the co-application treatments of RP or TSP with biofertilizers had no significant differences. Therefore using these biological fertilizers instead of chemical phosphorus fertilizers can reduce costs and environmental hazards.

Simultaneous application of PGPR, vermicompost and TSP significantly decreased SDW that may be due to the P toxic levels in the plant aerial parts. This in turn had also some negative effects on shoot N, K, Ca, Mg. Zn and Mn uptake rates. It may be also because of the inhibition of the bacterial activities in the presence of high values of soluble P. Regarding the results, it should be noted that co-application treatment of PGPR, TSP and vermicompost may be not suitable or recommendable in calcareous soils during greenhouse production of lettuce.

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Table 1. Main effect of PGPR, vermicompost and sources of P on shoot dry weight (SDM) (g pot^{-1}), shoot (Sh) N, P, K, Ca and Mg uptake (U) (mg pot^{-1}) by lettuce.

Factors		Parameters					
		SDM	Sh(N)U	Sh(P)U	Sh(K)U	Sh(Ca)U	Sh(Mg)U
PGPR	B_0	20.20 B	324.00 B	87.01 B	1198.00 A	92.05 B	55.14 B
	B_1	21.88 A	352.89 A	100.97 A	1266.00 A	154.91 A	65.58 A
Vermicompost	V_0	17.69 B	297.65 B	76.94 B	1036.00 B	79.31 B	48.66 B
	V_1	24.39 A	379.24 A	111.04 A	1428.00 A	167.66 A	72.07 A
Sources of P	P_0	19.62 B	312.02 B	86.71 C	1164.00 B	122.56 A	67.18 A
	RP	22.525 A	359.51 A	96.81 AB	1165.00 B	100.82 A	58.36 AB
	TCP	19.36 B	301.78 B	89.85 BC	1215.00 B	96.50 A	50.67 B
	TSP	22.67 A	380.46 A	102.60 A	1384.00 A	174.06 A	65.25 A

*****Values followed by the same capital letter are not significantly different using LSD test ($P < 0.05$).

B_0: non-bacterial inoculation, B_1: inoculation with Pseudomonas fluorescens. V_0: without vermicompost, V_1: 1% vermicompost. RP: Rock phosphate, TCP: Tricalcium phosphate, TSP: Triple super phosphate. Sh: Shoot, U: uptake

Table 2. Effect of PGPR, phosphate sources and vermicompost on shoot dry mater (SDM), shoot N, P, K, Ca and Mg uptake by lettuce.

Treatments								
	B_0				B_1			
	Control	RP	TCP	TSP	Control	RP	TCP	TSP
	SDM (g pot^{-1})							
V_0	9.37 i	16.23 gh	16.03 h	19.07 e-h	20.4 d-g	22.07 c-f	18.07 fgh	20.33 d-g
V_1	23.20 b-e	27.50 ab	22.03 c-f	28.20 a	25.50 abc	24.30 a-d	21.30 c-f	23.10 cde
	Shoot N uptake (mg pot^{-1})							
V_0	177.8 h	243.8 gh	252.4 fgh	307.9 d-g	373.4 bcd	398.4 bc	281.0 efg	346.9 cde
V_1	355.4 cde	441.8 ab	327.6 c-f	485.7 a	341.9 cde	354.1 cde	346.0 cde	381.3 bcd
	Shoot P uptake (mg pot^{-1})							
V_0	40.89 h	52.40 g	96.23 def	86.29 efg	95.30 def	100.54 cde	74.59 fg	79.27 efg
V_1	104.02 cde	109.16 bcd	90.42 d-g	122.67 b	106.63 bcd	125.13 a	108.15 bcd	118.17 abc

	Shoot K uptake (mg pot^{-1})							
V_0	618.00 f	843.00 ef	974.90 de	1120.20 cde	1223.10 bcd	1148.10 cd	1145.60 cd	1217.50 bcd
V_1	1368.80 bc	1439.20 b	1381.80 bc	1840.70 a	1447.70 b	1233.10 bcd	1360.40 bc	1359.20 bc
	Shoot Ca uptake (mg pot^{-1})							
V_0	16.80 e	29.32 de	108.85 b-e	172.54a-e	176.82 a-d	45.98 cde	39.49 cde	44.63 cde
V_1	52.75 cde	58.29 cde	40.73 cde	257.13ab	243.85 ab	269.67 a	196.91 abc	221.93 ab
	Shoot Mg uptake (mg pot^{-1})							
V_0	26.07 f	39.39 ef	42.33 def	62.14bcd	52.20 cde	53.25 cde	42.62 def	71.28 bc
V_1	80.62 b	62.18bcd	62.17 bcd	66.23 bc	109.82 a	78.62 b	55.54 cde	61.33 b-e

*Values followed by the same letter are not significantly different, using LSD test ($P < 0.05$).

B_0: non-bacterial inoculation, B_1: inoculation with Pseudomonas fluorescens, Control: without phosphate sources, RP: rock phosphate, TCP: Tricalcium phosphate, TSP: triple superphosphate.

V_0: without vermicompost, V_1: 1% vermicompost.

Table 3. Main effect of PGPR, vermicompost and Sources of P on shoot (Sh) Fe, Mn, Cu, Zn and Na uptake (U) (mg pot^{-1}) by lettuce.

Factors		Parameters				
		Sh(Fe)U	Sh(Mn)U	Sh(Cu)U	Sh(Zn)U	Sh(Na)U
PGPR	B0	3364.70 A	600.92 B	163.49 A	623.71 A	38.22 B
	B1	3421.20 A	671.81 A	126.47 B	597.57 A	33.94 A
Vermicompost	V0	2709.60 B	545.21 B	144.19 A	567.87 B	31.45 B
	V1	4076.30 A	727.53 A	145.77 A	653.40 A	40.72 A
Sources of P	P0	2750.50 C	628.63 AB	118.49 B	586.57 BC	32.47 B
	RP	3646.50 AB	614.71 AB	236.32 A	689.87 A	39.73 A
	TCP	3090.70 BC	607.59 B	143.81 B	652.45 AB	36.32 AB
	TSP	4084.10 A	694.53 A	81.29 C	513.65 C	35.80 AB

*****Values followed by the same capital letter are not significantly different using LSD test ($P < 0.05$).

B_0: non-bacterial inoculation, B_1: inoculation with Pseudomonas fluorescens. V_0: without vermicompost, V_1: 1% vermicompost. RP: Rock phosphate, TCP: Tricalcium phosphate, TSP: Triple super phosphate. Sh: Shoot, U: uptake.

Table 4. Effect of PGPR, phosphate sources and vermicompost on shoot Fe, Mn, Cu, Zn and Na uptake by lettuce.

Treatments								
	B_0				B_1			
	Control	RP	TCP	TSP	Control	RP	TCP	TSP
	Fe uptake (µg pot^{-1})							
V_0	1765.7 h	2908.1 d-h	2269.3gh	4012.7 bcd	2399.9 gh	2525.1 fgh	2567.5 fgh	3228.5 c-g
V_1	4128.3 bc	3706.3 b-f	3693.3 b-f	4434.2 abc	2708 e-h	5446.6 a	3832.8 b-e	4661.2 ab
	Shoot Mn uptake (µg pot^{-1})							
V_0	293.68 f	428.25 ef	462.79de	606.89 cd	708.32 abc	611.85 cd	617.09 cd	632.78 bc
V_1	732.54 abc	736.91 abc	697.77 abc	848.55 a	780.00 ab	681.83 bc	652.71 bc	689.91 abc
	Shoot Cu uptake (µg pot^{-1})							
V_0	109.14 ef	189.44 cd	139.23 de	80.06 fg	112.03 ef	173.36 cd	190.58 cd	159.68 de
V_1	217.30 c	302.23 a	225.68	44.84 gh	35.49 gh	280.27	19.74 h	40.58 gh

			bc			ab		
	Shoot Zn uptake (µg pot^{-1})							
V_0	386.53 e	523.92 cde	674.15 bc	520.26 cde	622.94 bcd	759.88 b	584.22 bcd	548.63 cde
V_1	649.90 bc	1026.90 a	765.25 b	542.98 cde	686.93 bc	448.78 de	586.21 bcd	442.75 de
	Shoot Na uptake (µg pot^{-1})							
V_0	14.27 f	32.76 de	37.71 b-e	29.09 e	35.48 de	30.69 e	36.30 cde	35.27 de
V_1	42.33 a-d	48.06 a	33.93 de	33.36 de	37.79 b-e	47.43 ab	37.36 cde	45.47 abc

*Values followed by the same letter are not significantly different, using LSD test ($P < 0.05$).

B_0: non-bacterial inoculation, B_1: inoculation with Pseudomonas fluorescens,

Control: without phosphate sources, RP: rock phosphate, TCP: Tricalcium phosphate, TSP: triple superphosphate.

V_0: without vermicompost, V_1: 1% vermicompost.