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Effect of applying bio-enriched rock phosphate on soil properties and wheat plant growth

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THIS STUDY was conducted at the Soil and Water Department, Faculty of Agriculture, South Valley University, Qena, Egypt. The study included two incubation experiments to test the efficiency of *Bacillus megathirum, Bacillus polymyxa, and Trichoderma harzianum* in their ability to release phosphorus from rock phosphate. In addition, a pot experiment was conducted to study the effectiveness of bio-enriched rock phosphate as phosphate fertilizer and its effect on some soil chemical properties and phosphorus availability as well as growth and nutrient uptake of the wheat plant during growth season (winter 2021/22022). Results showed that, liquid medium inoculation by the three different microbials strains under study improved P release from rock phosphate compared to the uninoculated treatment (control). Also, the highest values of available phosphorus were recorded with isolating *Bacillus polymyxa*+*Trichoderma harzianum* and *Bacillus megathirum*+ *Trichoderma harzianum* in most of the incubation periods. Moreover, applying rock phosphate bio-enriched by *Bacillus polymyxa*+*Trichoderma harzianum* and *Bacillus megathirum*+ *Trichoderma harzianum* resulted in enhancing available soil phosphorus, plant height, root lengths, dry matter as well as N, P and K uptake of wheat plants compared to the other treatments. Also, in most treatments, all previous measurements were enhanced by applying bio-enriched rock phosphate at the level of 1.2 ton ha^{-1} compared to the same biological treatments at the level 2.4 ton ha^{-1} and Superphosphate treatment. Hence, it was concluded that bio-enriched rock phosphate by *Bacillus polymyxa* + *Trichoderma harzianum* or *Bacillus megathirum*+ *Trichoderma harzianum* before direct application to the soil enhanced the release of P from rock phosphate and proved to be a suitable approach to use rock phosphate**.**

Keywords: Available Phosphorus, *Bacillus*, Rock Phosphate**,** Soil Properties**,** Trichoderma**.**

1. Introduction

Phosphorus is one of the major nutrients after nitrogen required for plant growth and is one of the essential nutrients to sustain all forms of life, but it is an insufficient nutrient, this is due to its complex chemical reactions in soil, and the speed of its transformation to insoluble form, makes it highly deficient nutrient in most soils especially calcareous soils (Roy et al. 2016; Hellal et al 2019). The cost of producing fertilizers, especially phosphate fertilizers, has increased, markedly worldwide (Straaten, 2002, 2006; Chien et al., 2011). Finding alternative sources of fertilizer that can offer P or other elements for the sustainable production of crops is doable, especially if it's a long-term, low-cost plan that takes into account the needs of impoverished farmers. (Appleton, 2002; Straaten, 2006; Zapata and Roy, 2004).

Rock phosphate is an important natural source of P and is used as raw material for the production of chemical phosphatic fertilizers (Reddy et al. 2002). Apatitic rocks It contains a high concentration of P and may contain a wide range of minor chemical elements, some of which are useful for plant nutrition (IAEA, 2002; Straaten, 2006; Szilas et al., 2007). Rock phosphate is extremely mineralogically relevant for replenishing P, Ca, and Mg. Given that African nations cannot afford to buy pricey water-soluble phosphatic fertilizers, their value is even more crucial. (Khasawneh and Doll, 1979; Appleton, 2002; Straaten, 2007). The direct application of rock Phosphate is considered inefficient due to limited solubility of the most of the phosphate compounds in rock phosphate, despite the above mentioned the direct use of rock Phosphate is still a possible option if it is applied

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after certain treatments for activation such as partial acidulation (Ahmad el al. 2019).

Biological solubilization of rock phosphate is environmentally friendly more than acidulation, Biological solubilization will make phosphorus available for plant use and reduce environment pollution, Rock phosphate inoculated with bacteria and Mycorrhizae proved to be a suitable approach to use rock phosphate for crop production (Hellal et al 2019). Inoculation of phosphate-solubilizing fungi and mycorrhizal fungi helps in releasing phosphorus from rock phosphate and transforming to available forms during composting. Moreover, it improves the physicochemical, biochemical, and biological soil properties (Santi et al. 2000). The higher amount of soluble phosphorus was attained from composts inoculated with Aspergillus Niger plus *Trichoderma* with or without farmyard manure and was much better than the superphosphate in maintaining the grown maize with available phosphorus (Hellal et al.2013). *Bacillus megathirum* is bacteria which have the ability to dissolve phosphorus from rock phosphate (Zhong and Huang 2005). By phosphate solubilizing bacteria, the plant can access the inaccessible P compounds. The active strains of phosphate solubilizing bacteria which have the ability to dissolve phosphorus from rock phosphate are Pseudomonas, Mycobacterium, Micrococcus, *Bacillus*, *Flavobacterium*, *Rhizobium*, *Mesorhizobium* and *Sinorhizobium13*. A significant improvement in P availability and promote plant growth occurred through many processes such as, decreasing the pH of the soil by producing organic acid and phytohormones (Sugihara et al. 2010; Sharma et al. 2013; Ganzour eat al. 2020).

The current work aimed to study the activity and efficiency of three different microbials strains (*Bacillus megathirum*, *Bacillus polymyxa*, and *Trichoderma harzianum*) on phosphorous solubility degree from rock phosphate and to investigate the feasibility of using rock phosphate bio-enriched by microbial strains as an alternative to phosphate chemical fertilizers and its effected-on soil properties and plant growth.

2.Materials and methods

2.1. Laboratory experiments

Two incubation experiments were performed-in the Soil Microbiology Laboratory, at the Soil and Water department, Faculty of Agriculture, South Valley University, Qena, Egypt. Our aim was to test the efficiency of the microbes under study (Bacillus megathirum, Bacillus polymyxa, and Trichoderma harzianum) on phosphorous (P) availability from rock phosphate. The rock phosphate was obtained from ELSebaiya site mines (25°10′30″N 32°40′43″E) Aswan Governorate, Egypt. The ground samples were (RP) crushed and sieved to pass through a 270-mesh screen and analysed for chemical composition Table 1. Also, the studied microorganisms (*Bacillus megathirum, Bacillus polymyxa, and Trichoderma harzianum*) were obtained from Microbiological Resources Center, Faculty of Agriculture. Ain Shams University, Cairo, Egypt.

2.1.1. The first incubation experiment

Bacillus megathirum, *Bacillus polymyxa*, and *Trichoderma harzianum were* isolates for testing efficiency in dissolving rock phosphate. Their efficiency was measured by preparing 250 ml conical flasks containing (100 ml) of Pikovskaya liquid medium (Pikovskaya, 1948) for *Bacillus megathirum* and *Bacillus polymyxa*, and potato dextrose Agar (PDA) liquid medium without agar for *Trichoderma harzianum*, according to (Girard, 1964 and Vásconez and Imbaquingo, 2017). Each flask received 0.25 g of rock phosphate. Then, all flasks were sterilized and inoculated with microbial strains under study. Incubation was carried out on a rotary shaker (150 rpm) at 30 °C, control treatments were utilized in the same manner without inoculation. After incubation periods (2, 5, 10 and 15 days), the flasks were tested for pH change and the amount of available phosphate. The available phosphorus was extracted by 0.5 M $NaHCO_3$ at pH 8.5 (Olsen et al. 1954) and spectrophotometrically determined according to Jackson (1973).

2.1.2. Second incubation experiment

This experiment was a continuation of the previous experiment. The experiment was conducted to measure the effectiveness of the microbial strains, as well as the effect of mixing microbial strains with each other on the degree of phosphorus dissolution from rock phosphate. 500 grams of rock phosphate placed in a bottle and inoculated with microbial strains at a rate of 20 liters from the inoculated liquid medium by the microbial strains under this study per ton of rock phosphate. Eight different treatments were used with three replicates (Table 2). After inoculating the rock phosphate with microbial strains, the mixture was moistened with a mixture of 5% (w/v) molasses and water (molasses used here as a carbon source to increase microbial activity). The bottles were incubated at 30 °C and maintaining an appropriate moisture level and stirring, after incubation periods (0, 5, 10, 15, 20, 25, 30 and 35 days) rock phosphate samples were collected from each bottle. The removal of adsorbed moisture by heating samples for 48 h at 105 °C according to (Aydin et al 2009 and Aydin et al. 2010), crushed with a wooden roller, then the samples were directly analyzed analyzed. The samples were tested for pH and amount of soluble phosphate (ppm). The available phosphorus was extracted by 0.5 M $NaHCO_3$ at pH 8.5 (Olsen et al.

1954) and determined spectrophotometrically determined according to Jackson (1973).

2.2. Pot Experiments

Pot experiments were conducted in the screen house of South Valley University's agricultural experimental farm's department of soils and water, Qena, Egypt, during the growth season (winter 2021/22022). The aim of this study the effectiveness of bio-enriched rocks phosphate as a fertilizer for direct application to the soil and its effects-on some soil chemical properties, nutrient availability, growth and nutrient uptake of wheat grown in sandy soil. The soil used in this experiment was collected from a surface layer (0 - 20 cm) of an agricultural experimental farm of the department of soils and water, faculty of agriculture, south valley university. Some physicochemical properties of soil are in Table 3. The pot experiment was prepared in a completely randomized design (CRD) using plastic pots of 40 cm in height and 30 cm diameter with a drainage aperture in the bottom. Each pot was filled with 8 kg of soil. Also, an air-dried farmyard manure was used at a level of 23.8 ton ha^{-1} (120 g pot^{-1}) and rock phosphate bio-enriched by different microbial strains were used at two levels of 1.2 ton ha^{-1} (6 g pot^{-1}) and 2.4 ton ha^{-1} (12 g pot^{-1}), The treatments that were applied in this study are shown in Table (2). The soil sample in each pot was thoroughly mixed with the investigated amendment and then 12 seeds of wheat were planted in each pot. All pots were directly irrigated after applying all treatments. The control treatment was carried out by applying superphosphate (15.5% P2O5) at a level of 476.19 kgha^{-1} (2.4 g pot^{-1}) and without applying the rock phosphate rock. In addition, ammonium nitrate (33.5 % N) at a level of 866.32 kg ha^{-1} (4.3 g pot^{-1}) and potassium sulphate (48% K2O) at a level of 119 kg ha^{-1} (0.6 g pot^{-1}) were applied in two doses. The first dose was after two weeks from planting and the other after five weeks from planting. After 70 days from planting, the plants were harvested completely from each pot, the soil attached to the plant was removed, and then the wet weight, plant height and root length were recorded, then plants washed using deionized water, air dried for three days and oven-dried at 70 °C for 48 h , then the plant dry weight was recorded. Plant samples for all pots were kept for chemical analysis. Moreover, after the harvest, soil samples were collected from each pot, air-dried, crushed. with a wooden roller, sieved to pass through a 2 mm sieve and kept for analysis.

2.2.1. Soil analysis

The pipette technique was utilized to determine the particle-size distribution. (Richards, 1954; Jackson, 1969) and the corresponding textural class was determined from the USDA textural class triangle. The soil organic carbon content was determined according to the modified (Walkley and Black method USDA, 1996). Inorganic carbonate content (% $CaCO_3$) was estimated by a Collins calcimeter (Jackson 1973; USDA, 1996). The soil pH in a 1: 2.5 ratio soil to water suspension was measured using a digital pH meter. The electrical conductivity (EC) of the 1:5 ratio soil to water extract was estimated using an electrical conductivity meter (Jackson, 1973). The total soil nitrogen was determined using the microkjeldahl method as described by Jackson (1973). The available phosphorus was extracted by 0.5 M NaHCO3 at pH 8.5 (Olsen et al. 1954) and spectrophotometrically determined according to Jackson (1973). The available potassium was extracted with 1 N ammonium acetate at pH 7.0 and determined using the flame photometer (Jackson,1973).

Table 1. The chemical composition analysis of rock phosphate (RP).

Chemical composition	Rock phosphate (RP)
P_2O_5	30%
SiO_2	9.1 %
MgO	0.6 %
AL_2O_3	0.8 %
Fe_2So_3	1.2 %
Na_2O	0.4 %
K_2O	0.05%
MnO	0.05 %
F	2.7 %
CaO	46.2 %
$CaCO_3$	8.82%
SO_3	0.6 %
pH	8.6
EC	3.55 dS m^{-1}

Table 2. The different treatments used in the experiments.

Treatment No.	Treatment content
Control	without inoculated
B.M.	*Bacillus megathirum*
B.P.	*Bacillus polymyxa*
Tr.	*Trichoderma harzianum*
B.M.+B.P.	*Bacillus megathirum and Bacillus polymyxa*
B.M. + Tr.	(*Bacillus megathirum and Trichoderma harzianum*
B.P. + Tr	*Bacillus polymyxa, and Trichoderma harzianum*
B.M. + B.P. + Tr.	(*Bacillus megathirum, Bacillus polymyxa, and Trichoderma harzianum*)

Table 3. Physical and chemical properties of the soil used in this experiment.

Sand	Silt	Clay	Texture	pH (1:2.5)	EC (1:5) (dS m^{-1})	$CaCO_3$ (%)	Organic Matter (%)	Total N (%)	Available P (mg kg^{-1})	Available K (mg kg^{-1})
82	10	8	Sandy	8.12	0.55	10.02	0.95	0.033	7.14	208

2.2.2. Plant analyses

A sample of 0.5 g of the dried plant material was digested using a 20 : 5 mixture of sulfuric acid to hydrogen peroxide (Agiza et al. 1985 and Lowther, 1980) and then potassium, phosphorus and nitrogen were determined in digests using the methods as described for the soil analysis. Total NPK uptake by plant was calculated according to (Dobermann 2005) as follow:

N, P or K Uptake (mg pot^{-1}) = N, P or K concentration (mg kg^{-1}) in plant part (dry matter) × dry biomass (g pot^{-1}) /1000

2.3. Statistical analyses

All data were analyzed using MSTAT-C (Russell, 1994) and the treatment means were compared by Duncan's multiple range test.

3.Results and Discussion

3.1. Laboratory experiments

3.1.1. The first incubation experiment

Three different microbials strains (Bacillus megathirum, Bacillus polymyxa, and Trichoderma harzianum) were tested for their ability in solubilizing rock phosphate in a liquid medium. The results in Figure (1) showed that, the microbial strains under study varied in their potential to release P from rock phosphate during the incubation experiment. Generally, in most period of the incubation experiment, inoculation by the three different microbials strains under study improved P release from rock phosphate compared to the uninoculated treatment. Moreover, in inoculated treatment by *Trichoderma harzianum* the lowest values of soluble phosphate 0 mg kg^{-1} were observed in the early period of the experiment (5 days incubation at 30 °C), while the highest values of soluble phosphate 23575 mg kg^{-1} were recorded at the end of the experiment period (15 days incubation at 30 °C). Also, Olsen extractable P content progressively increased with increasing incubation duration in inoculated treatment by Trichoderma harzianum period as shown in Figure 1. Several studies found that, some species of *Trichoderma* such as *Trichoderma harzianum* has the ability to solubilize phosphate that in tur became available to plant (Li et al. 2015). In another study, Bononi et al. (2020) found that, the isolated Trichoderma strains were able to solubilize phosphate, also produced different organic acids during the solubilization.

On the other hand, Figure (1). showed that, in treatment inoculated by *Bacillus megathirum* the highest values of soluble phosphate 19375 mg kg^{-1} were recorded in the early period of the experiment (5 days incubation at 30 °C). Olsen extractable P content progressively decreased after 5 days of incubation in this treatment, where the lowest value for extractable phosphorus 963.88 mg kg^{-1} was recorded at (15 days of incubation at 30 °C). On the other hand, the treatment inoculated by *Bacillus polymyxa* showed the highest values of soluble phosphate 14083.33 mg kg^{-1} was observed at (2 days of incubation at 30 °C). Also, Olsen extractable P content progressively decreased after 2 days of incubation in this treatment, where the lowest value for extractable phosphorus 5091.66 mg kg^{-1} was recorded at (15 days incubation at 30 °C). Acid-producing bacteria (APB) such as *Bacillus megathirum* and *Bacillus polymyxa*, have the ability to convert the insoluble forms of P into soluble ones through the production of organic and inorganic acids, as well as CO_2 that react and dissolve the rock phosphate Therefore, APB play an important role in increasing the availability of P, these results agree with those reported by Abo-baker (2017), Sanchez et al. (2018) and Farrag and Bakr (2021).

In addition, the data in Figure (1) reveal that, the pH values of the medium were increased in the early incubation period in all treatments, but they decreased at (15 days of the incubation) except for the incubation treatment with *Trichoderma harzianum* the pH values continued to increase. Some microorganisms such as acid-producing bacteria and some species of *Trichoderma*, have the ability to decrease the pH of the liquid medium by producing organic (gluconic acid) and mineral acids (Abo-baker (2017), Bononi et al. (2020) and Farrag and Bakr (2021). This explains the decreased pH of the liquid medium at the beginning of the incubation period, but the decreased pH values helped dissolve the calcium carbonate existing within the composition of the rock phosphate used. This helps to increase the pH value in the following periods of the incubation experiment and mineral P in the liquid medium may become unavailable because of precipitation reactions with cations such as Ca-P and Mg-P under increased pH, this explains the decrease of soluble phosphate in some periods of incubation experiment.

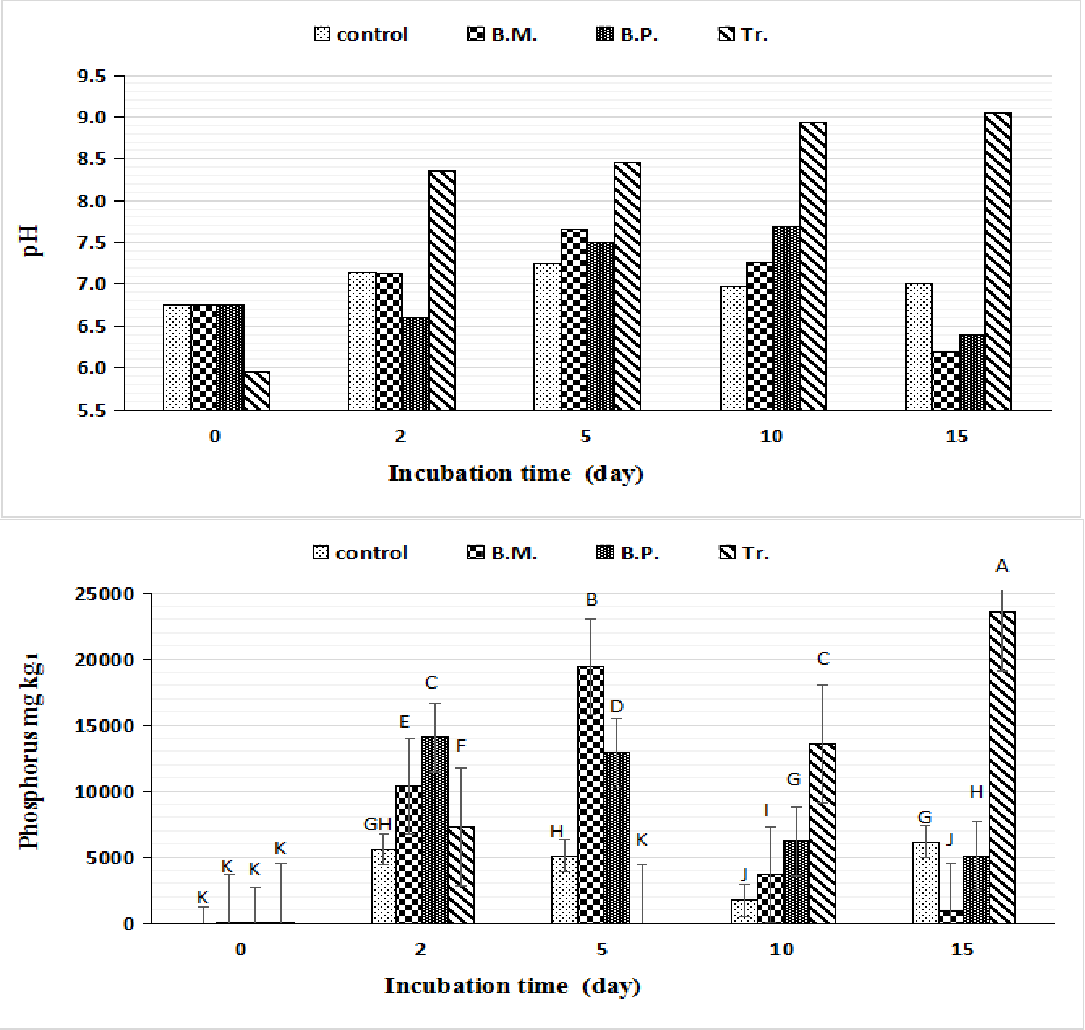

Fig. 1. Effect of microbial strains (Bacillus megathirum (B.M.), Bacillus polymyxa (B.P), and Trichoderma harzianum (Tr.) on phosphorus release from rock phosphate and changes of pH values in liquid medium.

3.1.2. The second incubation experiment

The experiment was conducted to measure the effectiveness of microbial strains, as well as the effect of mixing microbial strains with each other on the degree of phosphorus dissolution from rock phosphate. The data in Figure (2) show that, in most treatments, the highest values of available phosphorus were obtained in the first period of incubation (5 days incubation). The highest values of available phosphorus were recorded at incubation with isolate *Bacillus polymyxa+Trichoderma harzianum* and *Bacillus megathirum+ Trichoderma harzianum* which gave available phosphorus values of 471.72 and 461.53 mg kg^{-1}, respectively. Generally, both previous treatments gave the highest values of available phosphorus in most of the incubation periods. Also, the data in Figure (2) showed that, in most treatments, the values of available phosphorus were decreased with increasing incubation period compared to the first period of incubation (5 days incubation). In addition, in all treatments under this study, the lowest values of available phosphorus were recorded at the end of the incubation period, (35 days incubation at 30 °C). Also Figure (2) shows that, the pH values decreased in the first period of incubation (5 days incubation at 30 °C) in all treatments compared to zero time period of incubation, Lowest pH values of 7.3 were found with treatment *Bacillus megathirum+ Bacillus polymyxa* and after this incubation period, the pH values increased with increased the incubation period in all treatment.

In general, the results indicate that, There is an increase in the concentration of dissolved phosphorus in the first periods of the incubation experiment accompanied by a decrease in the value of pH, The reduction in the pH, depends upon the progress of decomposition and oxidation of organic compounds found in microorganism liquid medium and production of Inorganic and organic acids and CO_2 produced from the activity of microorganisms; these products help to convert the insoluble forms of P into soluble ones. In different studies (Rodriguez and Fraga 1999, Chen et al 2006, Sugihara et al. 2010and Xiao et al. 2017) they found that, through many processes such as, producing organic (gluconic acid) and mineral acids, alkaline phosphatases, phytohormones, H^+ protonation, anion exchange and chelation, acid-producing microorganism have the ability to modify P nutrition and increase its solubilization in soil. Also, in other studies (Yan et al. 2016 and Ahmad et al. 2019) concluded that raw phosphate rocks could release more P when activated with EDTA or low molecular organic acids like oxalic acid. After which there, the available phosphorus values decrease with an increased in the incubation period accompanied by an increase in the value of pH. This could be due to, the rock phosphate used contains a high percentage of calcium oxide and calcium carbonate Table 1.

3. 2.1. Soil properties

The changes in the soil pH, electrical conductivity (EC) and calcium carbonate ($CaCO_3$%) content induced by the investigated treatments are presented in Table 4.

3.2.1.1. Soil pH

Data in Table (4) showed that, all treatments decreased the soil pH compared to the rock phosphate application without inoculated treatment and the original soil pH. The lowest soil pH values of 7.67 and 7.70 were found with the application of rocks phosphate bio-enriched by *Trichoderma harzianum* (Tr.) and *Bacillus polymyxa, respectively* at the level of 1.2 ton ha^{-1}. Generally, from the data in Table (4) we observed that, all treatment when applied rocks phosphate bio-enriched at the level 1.2 ton ha^{-1} decreased the soil pH compared to the level 2.4 ton ha^{-1}. Reductions in the soil pH that occurred may be attributed to the activity of microorganisms producing organic acids and CO2 during the decomposition of organic matter and also root exudates may be have played a role in the decrease in the pH value, this is clear in the treatments when applied rock phosphate without inoculated and superphosphate treatment compared with original soil pH (Table 3). These results are in an agreement with those of El-Shinnawi et al. (2015), Abo-baker (2017), Farhat Barka et al (2018) and Farrag and Bakr (2021). Also, Aqarab et al. (2021) found that, decay of organic matter, root exudates and microorganisms including bacteria and fungi may be the cause decreased soil pH by producing large quantities of soil organic acids. In addition, an increase in the value of pH with an increase in the amount of rock phosphate added to the soil. This could be due to, the rock phosphate used contains a high percentage of calcium carbonate. With root exudates and production of inorganic and organic acids and CO_2 produced from the activity of microorganisms, this encourages the dissolution of calcium carbonate, with the released carbonate anion leading to increases in the pH value. Farrag and Bakr (2021) found that, an increase in calcium carbonate content ($CaCO_3$) in soil has important effects soil properties related to plant growth, it includes the high pH. Also, that found, Acid-producing bacteria through producing organic acids can help dissolve the calcium carbonate.

3.2.1.2. Electrical conductivity (EC)

From the data in **Table 4**. We observed that, in all treatments the soil electrical conductivity (EC) increased compared to the original soil (**Table 3**). This may be attributed that, the added amount of the farmyard manure to the soil before planting resulting in increases in the soil EC because of its high content of soluble salts. Farhat Barka et al (2018) and Farrag and Bakr (2021) found that, the organic manure application to the soil increased its, EC due to its high contents of soluble salt ions like Mg, Ca and Cl. Moreover, the decomposition of organic amendments its effects on the increases the dissolution of the soil salts. Also, the data in **Table (4)** found that, an increase in EC in most treatments when applied bio-enriched rock phosphate at the level of 2.4 ton ha^{-1} compared to the level of 1.2 ton ha^{-1} could be attributed to the activity of microorganisms producing organic acids and CO_2 during the decomposition of organic matter, it helps accelerate the rate of dissolution of rock phosphate and release of the excess salts from rock into the soil solution, this effect increases with increasing rate of rock phosphate used**.**

3.2.1.3. Soil calcium carbonate ($CaCO_3$) content

The data in Table (4) clear that, all investigated treatments decreased the soil calcium carbonate ($CaCO_3$) content compared to the rock phosphate application without inoculated treatment and the original soil (Table 3). The lowest calcium carbonate content 8.06, 8.10 and 8.50 % were found with the application of rock phosphate bio-enriched by *Bacillus megathirum +and Trichoderma harzianum* at the level 1.2 ton ha^{-1} rock phosphate bio-enriched by *Bacillus polymyxa + Trichoderma viride* at the level 2.4 ton ha^{-1} and rock phosphate bio-enriched by *Trichoderma* harzianum at the level 1.2 ton ha^{-1}, respectively. The negative effect of microorganisms under this study on the soil $CaCO_3$ content is attributed mainly to microbial activity, producing CO_2 and organic acids that react and dissolve the calcium carbonate of the soil. These results agree with those reported by Abo-baker (2017), Adnan et al (2017), Sanchez et al. (2018) and Farrag and Bakr (2021).

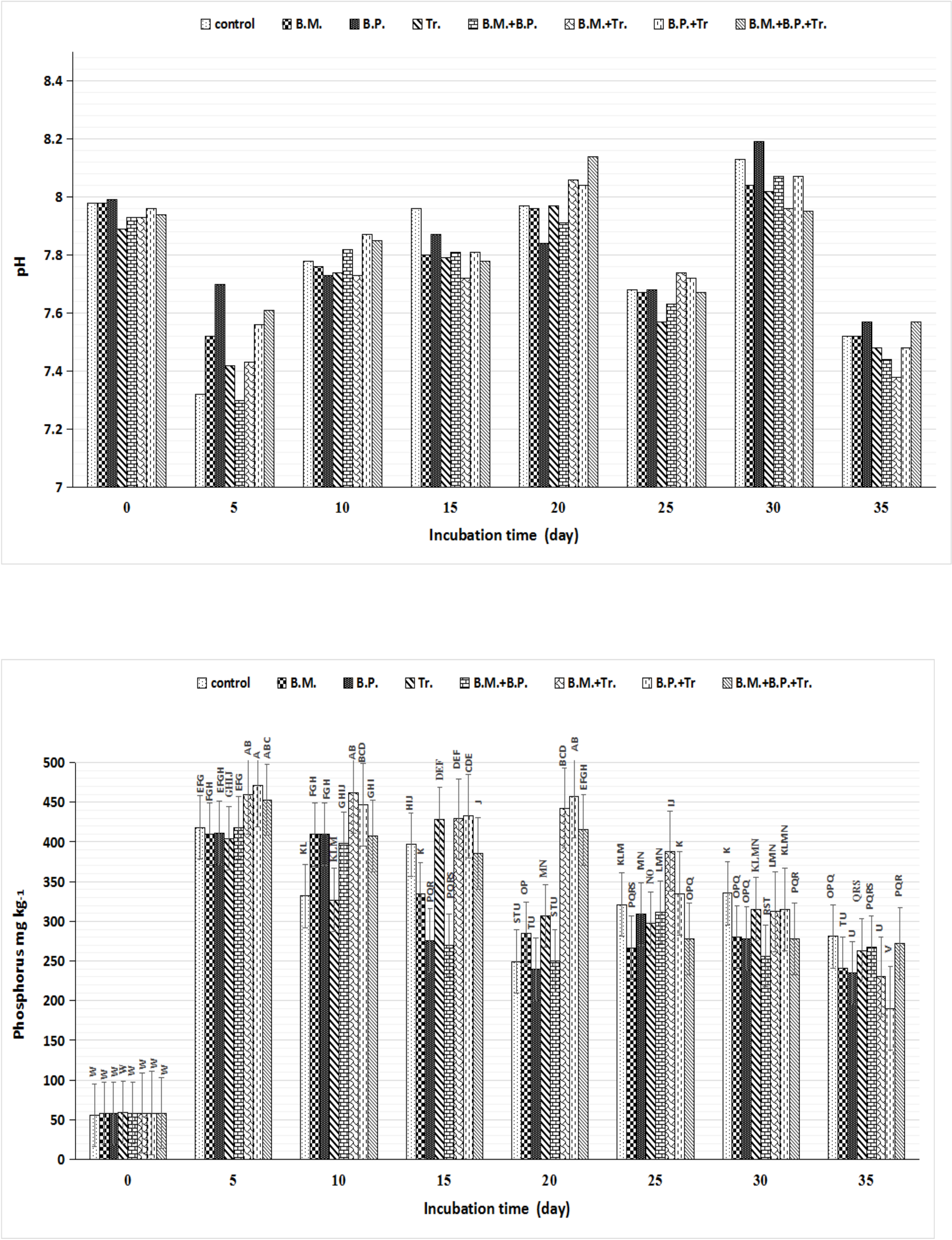

Fig. 2. Effect of microbial strains (*Bacillus megathirum* (B.M.), *Bacillus polymyxa* (B.P.), and *Trichoderma harzianum* (Tr.) as well as mixing microbial strains with each other on phosphorus release from rock phosphate and changes of pH values after the different incubation periods.

Table 4. Effect of rock phosphate bio-enriched application on some soil properties, plant growth and plant nutrients uptake.

Treatments		Soil analysis				Plant analysis								
		pH (1:2.5)	EC dS m^{-1}	$CaCO_3$ (%)	Available-P(mg kg^{-1})	Plant height (cm)	Root length (cm)	Dry matter g pot^{-1}	plant nutrient concentration (%)			plant nutrient uptake (mg pot^{-1})		
Phosphate rock level	Microbial inoculation								N	P	K	N	P	K
1.2 ton ha^{-1}	without inoculated	7.83	1.15bcde	9.8a	52.26c	31.75g	7.5bc	6.63f	1.18abc	0.20a	0.47de	78.23de	13.26e	31.16d
	B.M.	7.77	1.09cde	9.34ab	58.67bc	32.50fg	8.25abc	8.73cdef	1.58a	0.24a	0.62abcd	137.93bc	20.95cde	54.13cd
	B.P.	7.70	1.40abcd	9.43ab	53.81c	36.50cd	8.5abc	10.48bcd	1.00bcd	0.22a	0.52cde	104.8cde	23.06bcd	54.5bcd
.	Tr.	7.67	1.09cde	8.5bcd	63.19abc	37.50bc	10.50a	10.97abc	1.17abc	0.24a	0.66abc	128.35bc	26.33bc	72.4abc
	B.M.+B.P.	7.74	1.10bcde	8.7bcd	58.57bc	37.00bcd	9.50ab	11.09abc	1.31abc	0.26a	0.66abc	155.76b	30.91ab	78.47abc
	B.M.+Tr.	7.77	1.03de	8.06d	65.23abc	36.52cd	10.75a	11.89ab	1.52ab	0.28a	0.71ab	168.57ab	31.05ab	78.74ab
	B.P.+Tr	7.83	0.95e	8.96abc	68.32ab	40.50b	11.00a	12.86a	1.59a	0.28a	0.72a	204.47a	36.01a	92.59a
	B.M.+B.P.+Tr.	7.74	1.27abcde	9.08ab	60.34bc	54.00a	10.50a	9.33cde	0.99bcd	0.22a	0.46de	92.37cde	20.53cde	42.92d
2.4 ton ha^{-1}	without inoculated	7.92	1.27abcde	9.74a	52.62c	31.70g	7.17bc	6.69f	0.50 de	0.20a	0.45de	33.45e	13.38e	92.59a
	B.M.	7.78	1.41abc	8.68bcd	64.64abc	31.67g	8.67abc	9.15cde	0.36e	0.24a	0.40e	32.94e	21.96cde	36.6d
	B.P.	7.79	1.28abcde	9.27ab	63.04abc	36.17cdef	9.17abc	9.49cde	0.33e	0.23a	0.46de	31.32e	21.83cde	43.65d
	Tr.	7.78	1.09cde	8.68bcd	62.05abc	36.33cde	7.00bc	9.14cde	0.34e	0.24a	0.49cde	31.08e	21.94cde	44.79d
	B.M.+B.P.	7.74c	1.37abcd	9.02ab	56.38bc	33.50defg	8.67abc	9.35cde	0.99bcd	0.24a	0.48de	92.57cde	22.44cd	44.88d
	B.M.+Tr.	7.75	1.22abcde	9.61a	58.04bc	32.67efg	7.83bc	8.31def	0.80cde	0.23a	0.44de	66.48de	19.11cde	36.56d
	B.P.+Tr	7.73	1.18bcde	8.10cd	59.92bc	35.00cdefg	9.00abc	9.59cde	0.95cd	0.20a	0.47de	91.11cde	19.18cde	45.07d
	B.M.+B.P.+Tr.	7.72	1.09cde	8.98abc	61.1bc	31.67g	9.17abc	7.41ef	0.99bcd	0.20a	0.41e	73.36de	14.82de	30.38d
Superphosphate (15.5% P2O5)		7.69	1.48ab	8.53bcd	74.92a	31.67g	6.33c	6.62f	0.49 de	0.25a	0.54bcde	32.44e	16.55cde	35.75d

3.2.1.4. Available soil phosphorus

The data in Table 4 show that, most biological treatments varied significantly in their potential to release P from rock phosphate. The highest values of available soil phosphorus of 68.32, 65.23, and 63.19 mg kg^{-1} were found with applying phosphate rocks bio-enriched by *Bacillus polymyxa + Trichoderma harzianum*, *Bacillus megathirum +Trichoderma harzianum* and *Trichoderma harzianum* , respectively at the level of 1.20 ton ha^{-1}. On the other hand, the lowest values of available soil phosphorus of 52.26 and 52.62 mg kg^{-1} were found with applying rock phosphate without inoculation at level of 1.2 and 2.4 ton ha^{-1} respectively. Also, from the data in Table (4) we found that, the available soil phosphorus significantly increased with applying superphosphate (15.5% P_2O_5) compared to all treatments with applying rock phosphate bio-enriched, except the treatments with applying rock phosphate bio- enriched by *Bacillus polymyxa + Trichoderma harzianum, Trichoderma harzianum, Bacillus megathirum +Trichoderma harzianum* and *Trichoderma harzianum* at the level 1.2 ton ha^{-1} and the treatments with applying rock phosphate bio-enriched by *Bacillus megathirum* and *Bacillus polymyxa* at the level 2.4 ton ha^{-1} increased of available soil phosphorus with applying superphosphate (15.5% P_2O_5) compared to these treatments is no significantly Inorganic and organic acids and CO2 produced resulting from the activity of the microbial strains used and decomposition of organic matter additive before planting , this play a role to lower the soil pH and these products help to convert the insoluble forms of P into soluble ones. All of these have significant effects on increasing the phosphorus availability in the soil. Similar results were obtained by (Minja et al . 2014; Abo-baker 2017; Farrag and Bakr 2021; Habib 2021). Adnan et al. (2017) found that, that acid-generating bacteria like Bacillus can modify the nutrition of P and increase its solubility in soil by a variety of processes. For example, they can lower the pH of the soil by producing organic acid and mineral acids, which encourage P solubility in soil. Moreover, the ability of *Trichoderma* strains to solubilize phosphate through the production of various organic acids during the solubilization process is crucial for the availability of phosphorus for plants because these organic acids are able to transform the phosphate in the soil into di- or monobasic phosphates, which are easily absorbed by plants. (Li et al. 2015; Bononi et al. 2020; Lajim et al. 2021). In addition, in most treatments, the available P of the soil increased but not significantly with applying rock phosphate bio-enriched at the level 1.2 ton ha^{-1} compared to the same biological treatments at the level 2.4 ton ha^{-1} may be due to, the rock phosphate used contains a high percentage of calcium oxide and calcium carbonate Table (1). Under high pH conditions and the soluble calcium concentration increases, this leads to limited availability of P by a series of fixation reactions occur that gradually decrease phosphorus solubility, it is converted to less soluble. compounds such as dicalcium phosphate dihydrate or octa calcium phosphate. This could be the reason for the decrease in the soluble phosphorus concentration and this effect increases with the increasing rate of rock phosphate used. This result agrees with Reddy et al (2002) and moreover, the data in the Table (4) show that, increased available soil phosphorus in most treatments when applying rock phosphate bio-enriched by *Trichoderma harzianum* alone or its mixture combined with bacteria (*Bacillus megathirum* or *Bacillus polymyxa*) compared to other treatments.

3.2.2. Effect of phosphate rocks bio-enriched on the growth of the wheat plant

3.2.2.1. Plant height, Root length and Dry matter yield of wheat plants

The data in the Table (4) indicated that, most investigated treatments with applying rock phosphate bio-enriched significantly increased the height of wheat plant and root length compared to the treatments with applying superphosphate (15.5% P2O5) and rock phosphate without inoculated. The highest plants height were for applying rock phosphate bio-enriched by *Bacillus megathirum+Bacillus polymyxa +Trichoderma harzianum*, *Bacillus polymyxa+Trichoderma harzianum*, *Trichoderma harzianum, Bacillus megathirum + Bacillus polymyxa* and *Bacillus megathirum+Trichoderma harzianum* under level 1.2 ton ha^{-1} which recorded 54, 40.5, 37.5, 37 and 36.52 cm respectively compared to the superphosphate treatment (31.97 cm). In addition, the same treatments give the highest plants root length, which was recorded at 10.5, 11, 10.5, 9.5 and 10.75 cm respectively compared to the superphosphate treatment (6.33 cm). Moreover, the data presented in Table (4) show that, most investigated treatments with applying rock phosphate bio-enriched significantly increased the dry matter compared to the treatments with applying superphosphate (15.5% P_2O_5) and rock phosphate without inoculated, with highest dry matter were for applying rock phosphate bio-enriched by *Bacillus polymyxa+Trichoderma harzianum*, *Bacillus megathirum+Trichoderma harzianum*, *Bacillus megathirum + Bacillus polymyxa*, and *Trichoderma harzianum* under level 1.2 ton ha^{-1} which recorded 12.86, 11.89, 11.09 and 10.97 g pot^{-1}, respectively compared to the superphosphate treatment (6.62 g pot^{-1}). In most treatments, the plant height, root length and dry matter yield of wheat plants increased with applying rock phosphate bio-enriched at the level of 1.2 ton ha^{-1} compared to the same biological treatments at the level 2.4 of ton ha^{-1}. This may be due to, some microbial strains such as acid-producing bacteria and fungi, they directly enhance plant growth through produce substances that stimulate plant development and enhance the mobilizing of nutrients, also help to solubilize insoluble unavailable forms of nutrients compounds resulting in increased nutrients availability in the soil. They also improve the chemical and biological characteristics of soil by dissolving the calcium carbonate, reduction of soil pH and help accelerate leaching the soluble salts with irrigation water. This helps increase plant growth and reflected in the plant growth characteristics studied. Similar results found by (Abo-baker 2017; Farrag and Bakr 2021). Also from the data in Table (4) we found that, increased the plant height, root length and dry matter yield of wheat plants in most treatments when applying rock phosphate bio-enriched by *Trichoderma harzianum* combined with *bacteria (Bacillus megathirum or Bacillus polymyxa) compared to other treatments* . Our results agree with the results of Fiorentino et al. (2018) who have demonstrated that *Trichoderma* enhanced significantly the growth parameters of plants such as the plant height, biological yield, biomass yield and root length, over control. The positive effect of *Trichoderma* on the improvement of plant growth has been linked to several direct and indirect effects on plants, including the release of substances such as volatile organic compounds as well as small peptides , which enhance root system architecture (root length, branching, and density) with auxin activity. (, indole-3-carboxaldehyde, i.e., indole-3-acetaldehyde, and indole-3-ethanol) thus boosting plant growth (Rouphael et al., 2017a and Fiorentino et al. 2018). In another study Cai et al. (2013) found that Trichoderma may affect plant development through a number of methods, including the solubilization of minerals with limited solubility and the induction of systemic resistance in the host plant, This study also demonstrated Trichoderma's capacity to release harzianolide, an auxin-like phytohormone that markedly enhanced tomato plants' total root length and the number of root tips by 1.5 to 2.6-fold., Bononi et al. (2020) demonstrated that *Trichoderma* strains improved the efficiency of P uptake by up to 141% and increased soybean growth from 2.1% to 41.1%. Also, Lajim et al. (2021) found that, soil application with Trichoderma spp. showed a positive effect on plant height, germination, root length and root mass compared to control. In addition, one of the ways that Trichoderma and plants interact to promote growth, during the process of P solubilization, the pH of the medium becomes acidified, likely as a result of the production of organic acids by Trichoderma, which results in an inhibition of the primary root and, as a result, the development of secondary roots. As a mechanism to escape the acidification of the medium, this influences the biomass increase of the lateral roots, so *Trichoderma* enhances the lateral roots instead of the formation of new roots resulting in the early development of the roots process. (Contreras-Cornejo, et al. 2009; Kapri et al. 2010; Borges Chagas et al. 2015; Pelagio-Flores et al. 2017). All these findings support that the Trichoderma *harzianum* and *Bacillus megathirum or polymyxa* have the capability of plant growth promotion and these results reveal the potential of *Trichoderma harzianum* combined with bacteria *(Bacillus megathirum or Bacillus polymyxa)* as promising biofertilizer agents.

3.2.2.2. N, P and K content in wheat plant

Data presented in Table (4) indicated that, N, P and K percent in wheat plants showed response to inoculation with the selected microorganisms under the different doses of rock phosphate application. Significant increase in the amounts of N, P and K -percent in wheat plant in as a result of applying rock phosphate bio-enriched at the level of 1.2 ton ha^{-1} compared to the superphosphate treatment and rock phosphate without inoculated, also in most treatments, significant increase in the amounts of N, P and K -percent in wheat plant in as a result of applying rock phosphate bio-enriched at the level of 1.2 ton ha^{-1} compared to the same biological treatments at the level of 2.4 ton ha^{-1}. The highest values of N, P and K in wheat plants were recorded for rock phosphate at the level of 1.2 ton ha^{-1} when bio-enriched by *Bacillus polymyxa+Trichoderma harzianum* which displayed 1.59, 0.28 and 0.72 % N, P and K pot^{-1}, respectively, phosphate bio-enriched by *Bacillus megathirum+Trichoderma harzianum* that exhibited 1.52, 0.28 and 0.71 % N, P and K respectively and phosphate bio-enriched by *Bacillus megathirum* + *Bacillus polymyxa* which recorded 1.31, 0.26 and 0.66 % N, P and K respectively.

2.2.3. N, P and K uptake of wheat plants

Uptakes of N, P and K by wheat plants cultivated in the soil amended with the different treatments under study during growth season is considered an indication of the treatment effects on the ability of the plant to take advantage of the available amount of nutrients in the soil. The data in Table (4) demonstrated that, the highest uptake values of N, P and K by wheat plants were recorded for treatments when applied rock phosphate at the level of 1.2 ton ha^{-1} bio-enriched by *Bacillus polymyxa+Trichoderma harzianum* which displayed 204.47, 36.01 and 92.59 mg pot^{-1} N, P and K respectively, rock phosphate bio-enriched by *Bacillus megathirum+Trichoderma harzianum* that exhibited 168.57, 31.05 and 78.74 mg pot^{-1} N, P and K respectively and rock phosphate bio-enriched by *Bacillus megathirum + Bacillus polymyxa* which recorded 155.76, 30.91 and 78.47 mg pot^{-1} N, P and K respectively. On the other hand, from the data in the Table (4) we observed that, in most treatments, significant increase in the amounts of uptakes of N, P and K by wheat plants as a result of applying rock phosphate bio-enriched at the level of 1.2 ton ha^{-1} compared to the same biological treatments at the level 2.4 ton ha^{-1}. Also, a significant increase in the amounts of uptakes of N, P and K by wheat plants as a result of applying rock phosphate bio-enriched at the level of 1.2 ton ha^{-1} compared to the superphosphate treatment and rock phosphate without inoculated.

Application of rock phosphate combined with phosphate dissolving bacteria such as Bacillus led to an increase in the uptake of N and K by plants, which may be due to some strains of phosphate dissolving bacteria such as *Bacillus* can produce some organic acids as well as CO_2 these substances convert the insoluble forms of some nutrients like P into soluble forms, this led to increases of concentration-nutrients in the vicinity of.plant roots.and its availability in the soil solution as well as reduces.its fixation by soil factors. Also, these strains have the ability to produce some substances and enzymes that can promote growth and extension of the plant roots, this helps to increase the ability of the roots to absorb nutrients from the soil. These results are in agreement with those of (Abo-baker, 2017; Farrag and Bakr 2021). In addition, from the data in a Table (4) we found that, increase in the amounts of uptakes of N, P and K by wheat plants in most treatments whine applying rock phosphate bio-enriched by *Trichoderma harzianum* mixture combined with bacteria (*Bacillus megathirum or Bacillus polymyxa*). Li et al. (2015) found that the use of Trichoderma increased plant growth and enhanced nutrient uptake by plants and thus.promoted plant.growth was supported; However, due to the involvement of several mechanisms involved in Trichoderma-plant interactions, it was not possible to conclude that the mineral solubilization. by *Trichoderma* was primarily responsible for promoting plant growth, in addition, the improvements on the root system under *Trichoderma* affected which includes increases in lengths and volumes of root and the numbers of root tips, may have enabled the roots to maintain better contact with the minerals examined in this study these findings are of great significance for nutrient uptake when nutrients are scarce.it is one of the processes through which Trichoderma affects plant growth.

4. Conclusion

The application of rock phosphorus treated with *Bacillus polymyxa+Trichoderma harzianum* or *Bacillus megathirum+ Trichoderma harzianum* improve P release from rock phosphate and available of soil phosphorus. In addition, plant height, root lengths, dry matter content and N, P and K uptake of wheat plants compared to the other treatments. Also, in most treatments, all previous measurements were enhanced by applying phosphate rocks bio-enriched at the level 1.2 ton ha^{-1} compared to the same biological treatments at the level 2.4 ton ha^{-1} and superphosphate treatments. It was concluded that activation of rock phosphate via *Bacillus polymyxa+Trichoderma harzianum* or *Bacillus megathirum+ Trichoderma harzianum* increase the solubility of rock phosphate also, can be used as phosphate fertilizer. Finally, the production of phosphate fertilizers by the current method considered as promising alternative sustainable fertilizers.

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