e-ISSN: 2349-2759 p-ISSN: 2395- 1095

Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)

www.jipbs.com

Research article

Enhancing of rock phosphate solubility and plant availability in clay soil through inoculation of the rock with phosphate solubilizing fungal cultures and the effect of the amendment of soil with inoculated rock phosphate on *phaseolus vulgaris* plant growth

Hefnawy M. A., Gharieb M. M., Omima A. Eissa, Asmaa M. A. Ammar*

Botany Dept. Faculty of Science, Menoufia University, Egypt.

Key words: Phosphate availability in soil, rock phosphate solubilization, phosphate solubilizing microorganisms, crop production.

***Corresponding Author: Asmaa M. A. Ammar,** Botany Dept. Faculty of Science, Menoufia University, Egypt.

Abstract

Enhancing phosphate availability for plants stimulates early plant growth and hastens maturity. This study aimed to enhance solubility and plant availability of low soluble rock phosphate in clay soil by inoculation of the rock with phosphate solubilizing soil fungi. *A. niger* and *A. fumigatus* that showed the highest solubilizing ability among fungal species isolated from Egyptian soil. Rock phosphate was inoculated with Cultures of *A. niger* and *A. fumigatus* at different concentrations, results revealed that the best-solubilized phosphate value (63.5% & 50.9%) for *A. Niger* and *A. fumigatus*. Respectively was obtained at 30%w/v rock phosphate concentration and 15 days incubation period at 30ºC. The inoculated rock phosphate was applied as phosphate fertilizer to (common bean) *phaseolus vulgaris* plant, the amount of total phosphate and plant available phosphate and water soluble phosphate ofsoil were determined. Soil total dissolved salt and pH were measured along the life cycle of *phaseolus vulgaris* plant and compared to those of native soil, rock phosphate, and super phosphate. It was found that the addition of rock phosphate inoculated with *A. niger* culture considerably increased soil total phosphate (by 2.5times), plant available phosphate (by 5.25 times) when compared to native soil. Rock phosphate inoculated with culture of *A. fumigates* increased soil total dissolved salts by 62.307% greater than native soil; it was also found that rock phosphate inoculated with *A. niger* enhanced the growth of *phaseolus vulgaris* plant better than rock phosphate inoculated with *A. fumigatus* culture, native soil, rock phosphate and super phosphate respectively.

Introduction

In agricultural and crop production, phosphorus (P) is second only to nitrogen in importance as a fertilizer [3]. Agricultural soils are often rich in insoluble mineral phosphates but deficient in soluble orthophosphate.
The addition of phosphate fertilizers not only increases crop production but also to improve soil Phosphate status in order to avoid further soil degradation. In plants, phosphorus increases the strength of cereal straw promotes flower formation and fruit production, stimulates root development and also essential for seed formation [28].
Correction of Phosphorus deficiency is considered a major part of improving the fertility of acid soils. Rock phosphate is the primary raw material for producing phosphate fertilizers. Phosphate rock is recommended for application to acid soils where phosphorus is an important limiting nutrient for plant growth [10].
Direct application of phosphate rock (PR) has been shown to be a valuable source of nutrients in some conditions. The main obstacle associated with using directly applied ground phosphate rock is that the phosphate released is often unable to supply sufficient plant-available phosphorus for crop uptake [33]. Chemical fertilizers pose a health hazard and affect the microbial population in soil by degrading the physical structure of the soil leading to a lack of oxygen in the plant root zone besides being quite expensive and making high production. There is an urgent need for new types of phosphate fertilizers that are ergonomically effective and environmentally friendly. Plant and microbial-based mechanisms are low-cost, appropriate technologies to

enhance the solubilization and increase the agronomic effectiveness of phosphate rock [21].

Phosphate solubilizing microorganisms (PSM) could play an important role in supplying phosphate to plants in a more environmentally-friendly and sustainable manner [17].

A wide range of soil fungi is reported to solubilize insoluble phosphorous such as *A. niger* and *Penicillium sp.*, which are the most common fungi capable of phosphate solubilization [14,15]. Although large amounts of soluble phosphates are applied to soil as fertilizers, plants are able to use only a small portion of the applied phosphate and the remains are rapidly immobilized and become unavailable to plants.

The PSMs. play an important role in supplementing phosphorus to the plants, allowing a sustainable use of phosphate fertilizers [24]. *Aspergillus niger* and *Penicillium italicum* were isolated from the soil and rhizosphere of different plants, they showed high solubilizing index in agar plates. Also, they effectively solubilized tricalcium phosphate in Pikovskaya's liquid medium (PVK) they significantly increased dry matter and yield of soybean plants compared to the control soil [11]. *Aspergillus tubingensis* and *Aspergillus niger* were isolated from organically managed soil and tested for their ability to solubilize rock phosphate (RP), ferric phosphate and aluminum phosphate. The results of a field study conducted in two different seasons in organically managed soil proved that *A. tubingensis* and *A. niger* improved maize and wheat crop yield and soil fertility of organic farm when inoculated with RP fertilization as the Plant heights, shoot and root dry biomass and phosphorus (P) uptake in roots, shoots, and grains were significantly increased due to inoculation in both crops. Also, it was found that Organic carbon, P levels, and soil enzyme activities were significantly increased due to the inoculation [30].

It was observed that the treatment of pearl millet, brinjal, and tomato with *Penicillium sp.* conidial suspension treated for 6 h enhanced seed germination and seedling vigor. It also plays a role in root development, stalk and stem strength, flower and seed formation, maturity and production, crop quality and resistance to plant diseases [19].

The occurrence of rhizospheric phosphorous solubilizing microorganisms which play a key role in soil phosphorus dynamics and subsequent availability of phosphorus to plants [2].

Apot experiment was conducted under greenhouse conditionto assess the synergistic effect of AMF and PSB strains (*Coccus* DIM7 *Streptococcus* PIM6 and *Bacillus* sp. PIS7) on P solubility from RP and their successive uptake by maize (*Zea-mays*L)crop at the alkaline soil. It was indicated that the rhizo sphere interactions between AMF and PSB significantly promote RP mineralization in soil and improved all growth parameters including shoot (56%), root yield, height, N (80%) and Phosphate uptake by the maize plants as compared to control and single inoculation [8].

Experimental

Material and methods

Preparation of inoculated rock phosphate

A. niger and A. fumigatus among Nine fungal species isolated from Egyptian soil showed the best solubilization value of Egyptian rock phosphate ore. The optimum growth parameters for phosphate ore solubilization by both fungi were 28°C, 10 days incubation period, 1% ore concentration, initial pH 6.5-7, sucrose as a carbon source, $NaNO_3$ as a nitrogen source for *A. niger* and NH_4Cl for *A. fumigates* [28, 22]. Each of *A. Fumigates* and *A. niger* was grown at optimum solubilization conditions mentioned then cultures were filtered, centrifuged at 3000rpm for 15 min, the fungal growth was washed, culture filtrate was mixed with washed fungal growth. The culture mixtures for *A.niger and A. fumigate* were kept in sterilized capped glass jars for further work. Finely grounded and sieved rock phosphate was mixed in different concentrations (10%, 20%, 30%, 50% and 80% w/v) with culture mixtures in penta replicate set for *A. niger or A. fumigates* in 500ml *Erlenmeyer* flasks, incubated in a rotary shaker at 120 rpm and 30ºC for different incubation periods (0, 4, 8, 10, 15, 20 and 25 days).

The amount of soluble phosphate was determined colorimetrically in mixture filtrate by the Ascorbic acid method described by J. Murphy *et al.*[12], John F. Kopp *et al.*[13].

Reagents

Ammonium molybdate-antimony potassium tartrate solution: 8 g of ammonium molybdate (sigma) and 0.2 g antimony potassium tartrate (sigma) was dissolved in 800 mL of distilled water and diluted to 1 liter.

Ascorbic acid solution: 60 g of ascorbic acid was dissolved in 800 mL of distilled water and diluted to 1 liter then 2 mL of acetone was added. This solution is stable for two weeks.

Sulfuric acid, 11 N: 310 mL of conc. H_2SO_4 was slowly added to approximately 600mL distilled water. Cool and dilute to 1000 mL.

Sodium bisulfite ($NaHSO_3$) solution: 5.2 g of $NaHSO_3$ was dissolved in 100 mL of 1.0N H_2SO_4.

Stock phosphorus solution: 0.4393 g of predried (105°C for one hour) KH_2PO_4 was dissolved in distilled water and dilute to 1000 mL. 1.0 mL = 0.1 mg P.

Standard phosphorus solution: 100 mL of stock phosphorus was diluted solution to 1000 mL with distilled water. 1.0 mL = 0.01 mg P. A series of standards was prepared by diluting suitable volumes of standard or stock solutions to 100 mL with distilled water by which a standard curve was plotted (Figure1).

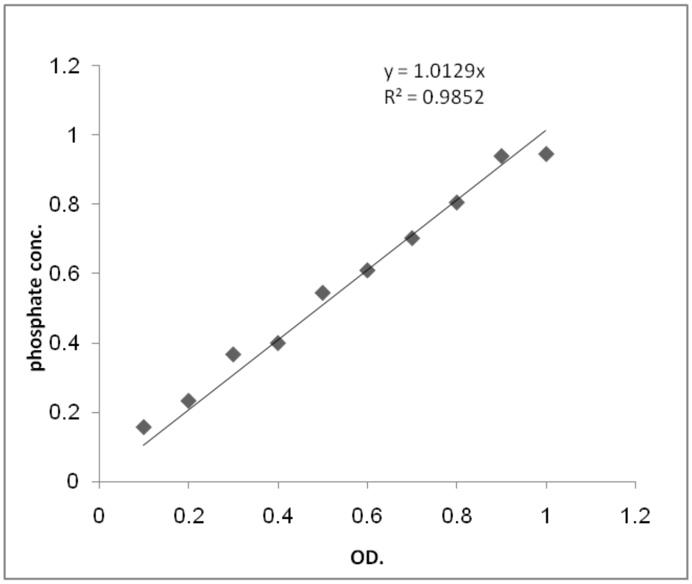

Figure 1. Phosphate standard curve for the ascorbic acid method.

Procedure

50.0 mL of digested sample was pipetted into an acid cleaned, dry 125 mL Erlenmeyer flask diluted to 50 mL. 1 drop of phenolphthalein indicator was added. If a red color develops, 5N sulfuric acid was added until the color disappears then 8.0 mL of combined reagent and mix thoroughly was add and allowed to stand at least 10 minutes (but not more than 30 minutes) for color development. Absorbance was measured at 880 nm using a reagent blank to zero the spectrophotometer. The reagent blank is made using 50 mL of distilled water.

Calculations

Determine the final concentration of the sample using the following equation: mg/L P = mg/L from the curve x 50 mL.

Plant experiment

Clay soil was obtained from the delta region (algharbia governorate) in Egypt ,air dried sieved and distributed in 5kg capacity plastic planting bags divided into 5 groups (inoculated rock phosphate 1 (with *A. fumigatus* culture), inoculated rock phosphate 2 (with *A. niger*cuther), native soil (No treatment), rock phosphate and super phosphate) by means of penta replicates.

Phaseolus vulgaris seeds

In these experiments, we used an early (53 days) and very productive round snap bean. Slender dark green pods about 5.5 inches in length which is Phaseolus vulgaris 'Bronco" or common bean obtained from the agriculture society in el gharbia governorate Egypt. The seeds were distributed with the rate of 5 seeds for each planting bag.

Soil analysis

Along the life cycle of *Phaseolus vulgaris* plant soil samples were collected and tested for the following:

Determination of total phosphate in soil samples

Soil samples were subjected to per-sulphate digestion to determine total phosphate method described by US EPA method [35].

Reagents

The stock acid solution 5.6M Sulfuric Acid: 310 mL of concentrated H_2SO_4 was diluted to 1 L with Double de-ionized water.

Working digestion acid solution: 12.8 g of ammonium per-sulfate (sigma) was dissolved in 32 mL of 5.6M H_2SO_4 in a 100-mL volumetric flask. Then it was diluted to mark with doubled-ionized water.

Procedures

Approx. 0250 g (~25 mg) dried ground soil sample was weighted and transferred to a capped tube adds 0.5 mL of working digestion acid to each tube. Vortex and cover with caps. Keep tubes loosely capped. The digestion tube sets were autoclaved for 1 hour (liquid cycle) at 121ºC and 15-20 psi. Tubes were removed from the autoclave, cooled, and securely capped. Then allow any particulate matter to settle over night. Then total phosphorus was colorimetrically determined in the clear supernatant using the ascorbic acid method.

Determination of plant available phosphate in soil samples

Olsen extracts of air-dried soil—The Olsen test (often referred to as the bicarbonate test) was developed to assay plant available phosphorus in alkaline soils of Colorado by S. R. Olsen [32]. The extracting solution is a 0.5M solution of weak alkaline sodium bicarbonate. Bicarbonate 1:20 extraction was shaken for 2h at 140 rpm let to stand filtration then centrifugation and the amount of plant available phosphate was colorimetrically determined using the ascorbic acid method.

Determination of water soluble phosphate in soil samples

1:10 soil slurry was prepared using 0.01M $CaCl_2$ solution shaking for 2h at 140 rpm then let to stand filtration then centrifugation and the amount of water-soluble phosphate were determined colorimetry using the ascorbic acid method [34].

Determination of pH and TDS in soil samples

1:10 soil slurry was prepared using distilled water shaking for 1h at 140 rpm then let to stand, filtration then centrifugation. 1000µS/cm at 25°C standard solution was prepared by (dissolving 0.2617g of KCl in 500 ml of H_2O). The conductivity meter was calibration with the prepared solution. The electrical conductivity in the filtrate was measured.

Electrical conductivity can be converted to salinity by multiplying the result of electrical conductivity by factor 0.264 [6].

Determination of soil pH

A fixed volume (12 mL) of dried and ground soil was weighed. 10 mL of soil is slurried with 20 mL of water, and after standing, the pH is measured in a (1:2 v/v slurry) using pH meter [5].

Results

Preparation of inoculated rock phosphate using the best solubilizing organism's cultures

The efficiency of both *A.niger* and *A. fumigatus* cultures (culture filtrate mixed with fungal propagules) grown in optimum conditions mentioned by Mohamed A *et al.,* [22] to solubilize rock phosphate was tested at different concentrations of rock phosphate at different incubation periods and it was revealed that the amounts of soluble phosphate increased considerably with increasing of rock concentration from 10% w/v to 30 %w/v (63.5 & 50.9 % for both *A. niger* and *A. fumigatus* cultures respectively) while it decreased with increasing rock concentration above 30% w/v to show the lowest solubilization values at the rock concentration 80% w/v (11& 19.7% for both *A. niger* and *A. fumigatus* cultures respectively) (Figure 2). On the other hand, the highest solubilization value obtained at 15 days of incubation at 28 C° for both of the tested organisms cultures (Figure 3). Data were subjected to analysis of variance (ANOVA TEST) Table (1).

Table 1. Test of Homogeneity of Variances
Soluble phosphate%

Test organism	Levene Statistic	df1	df2	Sig.
A. fumigatus.	6.282	4	29	0.001
A.niger	7.636	4	29	0.000

*significant when α>=0.05.

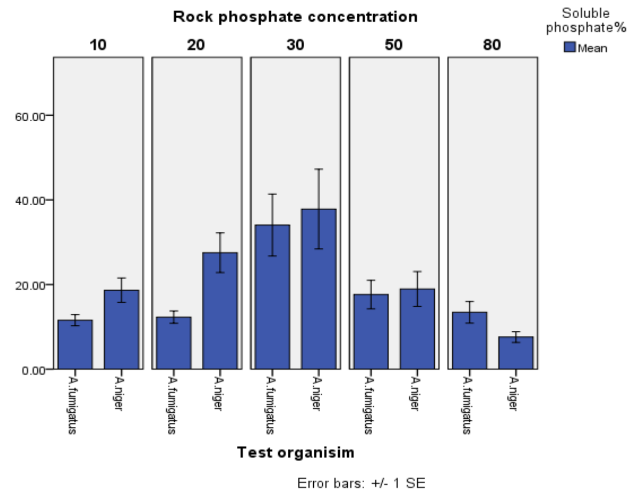

Figure 2. Solubilization of different concentrations of rock phosphate by *A. niger* and *A. fumigates.*

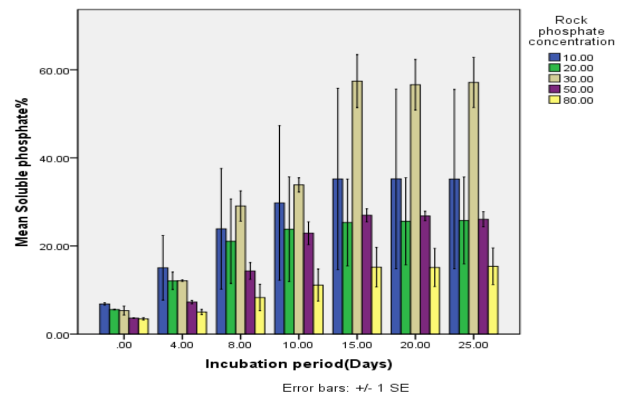

Figure 3. Solubilization of different concentrations of rock phosphate by *A. niger and A. fumigatus* at different incubation periods.

The effect of using inoculated rock phosphate as a phosphate fertilizer on soil total phosphate (TP), plant available phosphate (PA) and water soluble phosphate (wasp) in comparing to natural soil (no treatment), rock phosphate (RP) and superphosphate (SP) in the presence of phaseolus vulgaris plant

In this study 4 soil treatments was conducted and different forms of soil phosphate content during the life cycle of *phaseolus vulgaris* (common bean) plant was estimated for each treatment and compared to native soil it was found that using of rock phosphate inoculated with fungal cultures considerably increased soil total phosphate content in comparing to native soil by 3 times & 2.5 times for *A. fumigates* and *A. niger* cultures respectively**,** on the other hand soil total phosphate in case of inoculated rock phosphate increased by 1.4 &1.112 times greater than rock phosphate and increased by 2.3 & 1.9 times greater than super phosphate for *A. fumigates* and *A. niger* cultures respectively.

Results also revealed that the amount of plant available phosphate in soil increased in the case of using inoculated rock phosphate by 1.6 & 5.25 times greater than native soil, 1.04 & 3.5 times greater than rock phosphate and by 1.007 & 2.4 times greater than Superphosphate. As for water soluble phosphate, the using of super phosphate as a fertilizer to gave the highest water soluble phosphate value. (Figures 4, 5& 6). Data was subjected to analysis of variance (ANOVA) Tables (2, 3, 4 & 5).

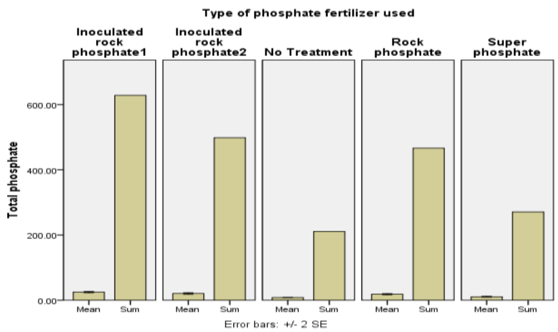

Figure 4. Effect of different phosphate treatments on total phosphate content of soil in the presence of *phasleousvulgaris* plant.

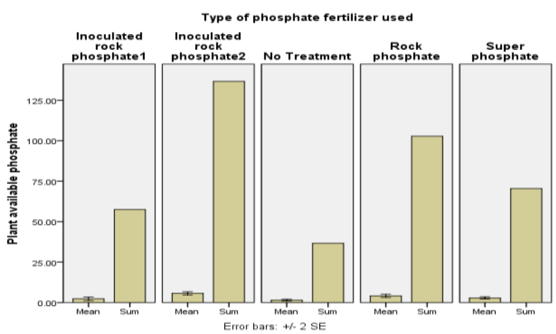

Figure 5. Effect of different phosphate treatments on plant available phosphate content of soil in the presence of *phasleous vulgaris* plant.

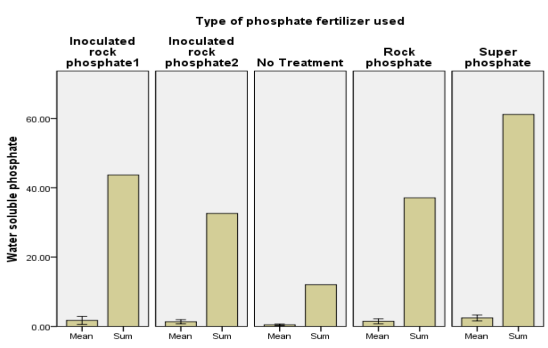

Figure 6. Effect of different phosphate treatments on water soluble the phosphate content of soil in the presence of *phasleous vulgaris* plant.

Table 2. Test of Homogeneity of Variances
Total phosphate

Levine Statistic	df1	df2	Sig.
3.943	4	119	0.005

*significant when α>=0.05

Table 3. Test of Homogeneity of Variances
Plant available phosphate

Levene Statistic	df1	df2	Sig.
11.653	4	119	0.000

*significant when α>=0.05.

Table 4. Test of Homogeneity of Variances
Water soluble phosphate

Levene Statistic	df1	df2	Sig.
23.782	4	119	0.000

*significant when α>=0.05.

The effect of different phosphate treatments on soil pH and total dissolved salts (TDS)

The results indicated that all of the tested phosphate treatments decreased soil pH comparing to native soil, the highest decreasing percentage obtained when super-phosphate was used as phosphate fertilizer the soil pH decreased from 8.444 in case of natural soil to 6.98 in case of super phosphate while inoculated rock phosphate and rock phosphate slightly decreased soil pH to 7.48 and 7.2 for inoculated rock phosphate and rock phosphate respectively (Figure 7).

On the other hand, all of the tested treatments increased soil TDS comparing to natural soil, where rock phosphate inoculated with *A. fumigates* culture showed the highest increase percentage 62.307% higher than natural soil, followed by rock phosphate inoculated with A. niger culture with 45.7% increase then came super phosphate and rock phosphate with 57.35% and 23.05% respectively higher than natural soil (Figure 8). Data were subjected to analysis of variance (ANOVA TEST) Tables (6&7).

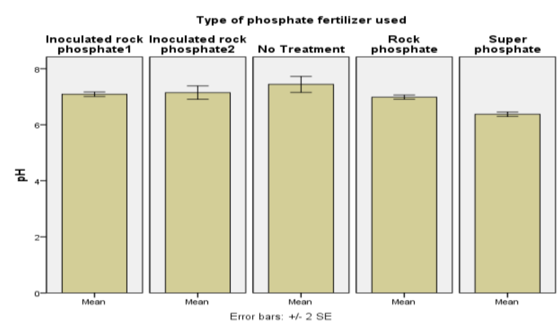

Figure 7. Effect of different phosphate treatments on soil pH in the presence of *phasleous vulgaris* plant.

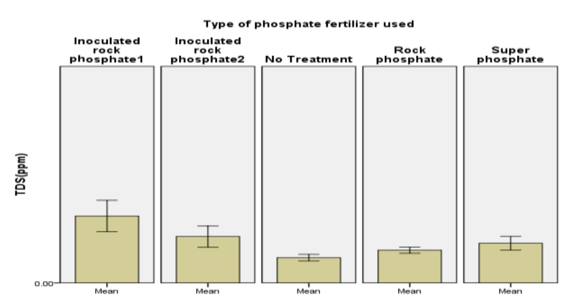

Figure 8. Effect of different phosphate treatments on soil TDS in the presence of *phasleous vulgaris* plant.

Table 5. ANOVA Table for the three measured phosphate forms

			Sum of Squares	df	Mean Square	F	Sig.
Total phosphate * Type of phosphate fertilizer used	Between Groups	(Combined)	4604.552	4	1534.851	74.432	0.000
	Within Groups		2474.501	119	20.621		
	Total		7079.053	123			
Plant available phosphate * Type of phosphate fertilizer used	Between Groups	(Combined)	200.425	4	66.808	8.240	0.000
	Within Groups		972.933	119	8.108		
	Total		1173.358	123			
Water soluble phosphate * Type of phosphate fertilizer used	Between Groups	(Combined)	48.443	4	16.148	4.308	0.001
	Within Groups		449.826	119	3.749		
	Total		498.269	123			

*significant when α>=0.05

Table 6. Test of Homogeneity of Variances pH

Levene Statistic	df1	df2	Sig.
2.631	4	119	0.038

*significant when α>=0.05

Table 7. Test of Homogeneity of Variances TDS (ppm)

Levene Statistic	df1	df2	Sig.
24.430	4	119	0.000

*significant when α>=0.05

Table 8. ANOVA test for plant growth parameters

			df	F	Sig.
Germination percentage * Type of phosphate fertilizer used	Between Groups	(Combined)	4	5.784	0.013
	Within Groups		119		
	Total		123		
Plant length (cm) * Type of phosphate fertilizer used	Between Groups	(Combined)	4	1.059	0.004
	Within Groups		119		
	Total		123		
No.of fruit * Type of phosphate fertilizer used	Between Groups	(Combined)	4	0.192	0.009
	Within Groups		119		
	Total		123		
No.of flowers * Type of phosphate fertilizer used	Between Groups	(Combined)	4	2.411	0.022
	Within Groups		119		
	Total		123		
Leaf area(cm2) * Type of phosphate fertilizer used	Between Groups	(Combined)	4	1.255	0.034
	Within Groups		119		
	Total		123		

*significant when α>=0.05

The effect of different phosphate treatments on growth parameters and seed germination of phaseolus vulgaris plant

Results revealed that using inoculated rock phosphate decreased the number of germinating seeds and germination percentage as a consequence by 33% & 10% for A. *fumigates* and *A. niger* cultures respectively while in cases of rock phosphate and super phosphate it decreased by 7% & 3% respectively comparing to native soil.

Obtained results proved that using of rock phosphate inoculated with *A. niger* culture increased phaseolus vulgaris plant height by 33.9%, 33.3%, 22.2%, and 22.6%, no. of fruits by 75%, 51%, 50.2% and 48%, no. of flowers by 43.75%, 45.6%, 43%and 42% as well as leaf area by 17%, 17.8%, 15% and 14.5% when compared to inoculated rock phosphate with *A. fumigatus*, natives oil, rock phosphate, and super phosphate respectively (Figure 9).

Data were subjected to analysis of variance (ANOVA TEST) Table (8).

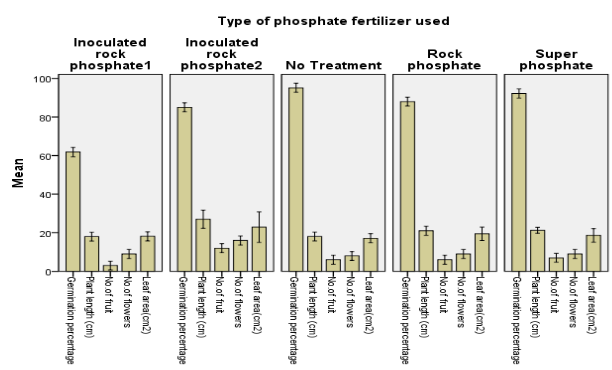

Figure 9. Effect of different phosphate treatments on seed germination and plant height no of flowers no. of fruits and leaf area of *phasleous vulgaris* plant.

Discussion

It is well-known phosphate fertilizers are very important for crop production. Chemical phosphate fertilizers are not enough for intensive and increasing agricultural lands. Therefore, an alternative and native phosphate sources should be utilized without chemical processing such as rock phosphate ores.

In this study results proved that the treatment of insoluble rock phosphate with efficient phosphate solubilizing fungal cultures before soil amendment enhanced phosphate solubility and plant availability in soil. Similar results obtained by E. Nahas *et al.* [7], N. Vassilev *et al.* [25], Nikolay *et al.* [26] that high carbon crop wastes have been suggested for use as fermentation substrates in the microbial solubilization of PR before application to the field. This method has the advantage of creating more optimal conditions for microbial organic acid production compared with conditions present in the soil.

It was proved that adding sugar beet (SB) (*Beta vulgaris*) waste, pre-cultured with *A. niger* in the presence of Moroccan PR, positively affected the growth of white clover (*Trifoliumrepens*) [27].

The pretreatment of PR with the inoculated SB waste significantly improved crop dry weight and shoot P content compared to plants given the same amount of untreated PR and SB waste. In another pot trial, using the same soil and PR, similar results was found in an experiment measuring the yield of alfalfa (*Medicago sativa* L. cv Aragon). When PR was pre-incubated with *A. niger* inoculated SB waste, alfalfa dry matter yield increased significantly compared to the treatment that received non-inoculated SB residue and PR [29].

Moroccan PR pretreated with *A. niger* inoculated olive cake-based medium, before application to greenhouse pots containing P-deficient calcareous soil [20]. It was found that white clover shoot dry weight and P content was significantly higher in the pots that received pretreated PR compared to the control that received untreated PR and olive cake waste. The authors stated that it appeared "the pre-incubation of the waste material is the key factor in the effectiveness of this system [18].

The combined treatment of SB waste, pretreated with A. *niger* in the presence of Moroccan phosphate rocks was studied. It was found that the fermented SB and RP mixture significantly increased the available P, total N, and extractable K contents of the soil, with the greatest increase, observed in available P content [16].

Results from this study indicated that soil amendment with inoculated rock phosphate as a phosphate considerably enhanced soil total phosphate, plant available phosphate and water soluble phosphate, which agreed with the results obtained by Ramesh Raliya *et al.,* [30] they studied the use of phosphate solubilizing microorganisms (*Klebsiella sp.* and *Aspergillus sp.*) to increase the solubility of rock phosphates, a slow release phosphorus (P) fertilizer. They indicated that total P and soluble P in soils treated with phosphate solubilizing microorganisms were significantly higher compared with soils solely applied with rock phosphate about 70 – 98% of P was released from rock phosphates in soils. Also available P within the range of 13 – 1289 ppm and 832 – 1441 ppm in soils treated with rock phosphates and soils treated with both rock phosphate and phosphate solubilizing microorganisms respectively. And they recommended the application of rock phosphates with phosphate solubilizing microorganisms should be for a sustainable agriculture practice as it is safe for the environment and reduces pollution caused by chemical fertilizers.

Similar results obtained when a greenhouse experiment was performed with *Bacillus subtilis* and *Pseudomonas putida*, with five levels of rock phosphate on corn plant they proved that pseudomonas with maximum fertilizer treatments and soil with medium P had highest dry matter [9].

Results of current study revealed that using of rock phosphate inoculated with A. niger as a phosphate culture significantly improved growth parameters of *phaseolus vulgaris* plant that agreed with the results obtained by Ahmed A. Khalil [1], Ramesh Raliya *et al.* [30], Hossein *et al.* [9], X. Yu *et al.* [34] as they studied the effect soil amendment with inoculation of rock phosphate with phosphate solubilizing microorganisms on different plants such as corn wheat, soybean bean and walnut it was proved that microbial inoculation of rock phosphate with phosphate solubilizing microorganisms increased plant yield, as well as phosphate up, take by the plant.

Conclusion

Results obtained from this study indicated that the inoculation of rock phosphate with cultures of phosphate solubilizing fungi significantly increased the amount of soluble phosphate released from the rock. The amendment of soil with inoculated rock phosphate considerably enhanced total phosphate, plant available phosphate as well as water soluble phosphate content of the soil and positively affected growth parameters of *phaseolus vulgaris plant* when compared to soil amended with non-inoculated rock phosphate, superphosphate, and native soil.

References

1. Ahmed A. Khalil: Significance of Some Soil Amendments and Phosphate Dissolving Bacteria to Enhance the Availability of Phosphate in Calcareous Soil. ISRN Soil Science 2013; 1-7.
2. A. E. Richardson, and R. J. Simpson: Soil Microorganisms Mediating Phosphorus Availability Update on Microbial Phosphorus. Plant Physiology Plant Physiology 2011; 156:989-96.
3. Alan H. Goldstein: Future Trends in Research on Microbial Phosphate Solubilization: One Hundred Years of Insolubility. Developments in Plant and Soil Sciences 2007; 91-96.
4. Association American Public Health, Association American Water Works: Federation Water Environment, Association American Public Health, and Analysis Committee on Standard Methods of Water 1992.

5. L. C. Blakemore, P. L. Searle, and B. K. Daly: Methods for Chemical Analysis of Soils. Lower Hutt, N.Z.: Dept. of Scientific and Industrial Research 1972.
6. Donald L. Sparks : Methods of Soil Analysis. Part3 America-Soil Science Society of Agronomy1996.
7. E. Nahas, D. A. Banzatto, and L. C. Assis: Fluorapatite Solubilization by Aspergillus Niger in Vinasse Medium. Soil Biology and Biochemistry 1990; 22(8):1097-1101.
8. Fazli Wahid, Muhammad Sharif, Siegrid Steinkellner, M. Azim Khan, K. B. Marwat, and S. A. Khan: Inoculation of ArbuscularMycorrhizal Fungi and Phosphate Solubilizing Bacteria in the Presence of Rock Phosphate Improves Phosphorus Uptake and Growth of Maize. Pakistan journal of botany 2016; 48:739-48.
9. Hossein Mirseyed, Hosseini A., Sara KhayamiA., Hossein Besharati B., and Sanam Bybordi A: Study of the effects of rock phosphate application with phosphate solubilizing bacteria on P availability for corn. 19th World Congress of Soil Science. Soil Solutions for a Changing World, Brisbane, Australia 2010.
10. Husnain, S. Rochayati, T. Sutriadi, A. Nassir, and M. Sarwani: Improvement of Soil Fertility and Crop Production through Direct Application of Phosphate Rock on Maize in Indonesia. PROENG Procedia Engineering 2014; 83: 336-43.
11. Iman M. El-Azouni: Effect of Phosphate Solubilizing Fungi on Growth and Nutrient Uptake of Soybean (Glycine max L.) plants. Journal of Applied Sciences Research 2008; 4(6): 592-598,
12. J. Murphy, and J. P. Riley: A Modified Single Solution Method for the Determination of Phosphate in Natural Waters. Analytica Chimia Acta 1962; 27: 31 – 36.
13. John F. Kopp, Gerald D. McKee: Methods for Chemical Analysis of Water and Wastes (Cincinnati; Washington, D.C.; Springfield, VA: Environmental Protection Agency, Environmental Monitoring and Support Laboratory1979.
14. M. A. Whitelaw: Growth Promotion of Plants Inoculated with Phosphate-Solubilizing Fungi. Advances in Agronomy 2000; 69:99-152.
15. M. A. Whitelaw, T. J. Harden, and K. R. Helyar: Phosphate Solubilisation in Solution Culture by the Soil Fungus Penicillium Radicum. Soil Biology and Biochemistry 1999; 31:655-65.
16. M. M. Alguacil, F. Caravaca, R. Azcón, J. Pera, G. Díaz, and A. Roldán: Improvements in Soil Quality and Performance of Mycorrhizal(CistusAlbidus L). Seedlings Resulting from Addition of Microbially Treated Sugar Beet Residue to a Degraded Semiarid Mediterranean Soil. SUM Soil Use and Management 2003; 19: 277-83.
17. M. S. Khan, and A. Zaidi: Synergistic Effects of the Inoculation with Plant Growth-Promoting Rhizobacteria and an Arbuscular Mycorrhizal Fungus on the Performance of Wheat. Turkish Journal of Agriculture and Forestry 2007; 31:355-62.
18. M. Vassileva, N. Vassilev, and R. Azcon: Rock Phosphate Solubilization by Aspergillus Niger on Olive Cake-Based Medium and Its Further Application in a Soil-Plant System. World Journal of Microbiology and Biotechnology 1998; 14: 281-84.
19. MahadevamurthyM., Thriveni M Channappa, Manjula Sidappa, Mythrashree S Raghupathi, Amruthesh K Nagaraj: Isolation of phosphate solubilizing fungi from rhizosphere soil and its effect on seed growth parameters of different crop plants. Journal of Applied Biology & Biotechnology 2016; 4(06):022-026.
20. Maria Vassileva, Rosario Azcon, Jose-Miguel Barea, and NikolayVassilev: Application of an Encapsulated Filamentous Fungus in Solubilization of Inorganic Phosphate. Journal of Biotechnology1998; 63: 67-72.
21. Melissa M. Arcand, and Kim D. Schneider: Plant- and Microbial-Based Mechanisms to Improve the Agronomic Effectiveness of Phosphate Rock: A Review. Anais da Academia Brasileira de Ciências 2006; 78:791-807.
22. Mohamed A. Hefnawy Mohamed M. GhariebOmima A. EissaAsmaa M. Ammar: Evaluation and optimization of rock phosphate and tri-calcium phosphate solubilization by some soil fung. Egypt. J. Exp. Biol. (Bot.) 2009; 5(0): 75-84.
23. Monitoring Environmental, and Laboratory Support: Technical Additions to Methods for Chemical Analysis of Water and Wastes'. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory 1982.
24. N.Pradhan, and L.B. Sukla: Solubilization of inorganic phosphates by fungi isolated from agriculture soil. African Journal of Biotechnology 2005; 5(10): 850-854.
25. N. Vassilev, M. T. Baca, M. Vassileva, I. Franco, and R. Azcon: 'Rock Phosphate Solubilization by Aspergillus Niger Grown on Sugar-Beet Waste Medium. Appl Microbiol Biotechnol Applied Microbiology and Biotechnology 1995; 44: 546-49.
26. Nikolay Vassilev, Irena Franco, Maria Vassileva, and Rosario Azcon: Improved Plant Growth with Rock Phosphate Solubilized by Aspergillus Niger Grown on Sugar-Beet Waste. Bioresource Technology 1996; 55:237-41.
27. Nikolay Vassilev, Maria Vassileva, MassimilianoFenice, and Federico Federici: Immobilized Cell Technology Applied in Solubilization of Insoluble Inorganic Rock Phosphates and P Plant Acquisition.BITE</cja:jid>Bioresource Technology 2001;79:263-71.
28. O. Sharma, P.Bambawale, O. M.Gopali, J. B. Bhagat, S. Yelshetty, S. Singh, S. K. Anand, R. Singh: Field guide Mung bean and Urd bean. The government of India, Department of agricultural and co-operation 2011; NCIPM, ICAR, New Delhi, India.
29. R. Rodriguez, N. Vassilev, and R. Azcón: Increases in Growth and Nutrient Uptake of Alfalfa Grown in Soil Amended with Microbially-Treated Sugar Beet Waste. Applied Soil Ecology 1999; 11: 9-15.
30. Ramesh Raliya, Jagadish Chandra Tarafdar, and Pratim Biswas: Enhancing the Mobilization of Native Phosphorus in the Mung Bean Rhizosphere Using Zno Nanoparticles Synthesized by Soil Fungi. Journal of Agricultural and Food Chemistry 2016; 64: 3111-18.
31. S. R. Olsen: Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate, Washington, D. C. United States Department of Agriculture 1954; 19.
32. S.H. Norman., Chien, Luis I. Prochnow, and Robert P. Mikkelsen: Agronomic Use of Phosphate Rock for Direct Application Better Crops. 2010; 4:94.
33. V. J. G. Houba, E. J. M. Temminghoff, G. A. Gaikhorst, and W. van Vark: Soil Analysis Procedures Using 0.01 M Calcium Chloride as Extraction Reagent. Communications in soil science and plant analysis 2000; 31:1299-396.
34. X. Yu, X. Yu, T. H. Zhu, and X. Liu: Walnut Growth and Soil Quality after Inoculating Soil Containing Rock Phosphate with Phosphate-Solubilizing Bacteria. Science Asia Science Asia 2014; 40:21-27.
35. US EPA method 600: Methods for Determination of Inorganic Substances in Environmental Samples, EPA/600/R-93/100. 1993.