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Response of *Rhizobium*-Faba Bean Symbiosis System to Rhizobacterial Inoculation and Arbuscular Mycorrhizal Fungi under Graded Levels of Natural Rock Phosphate in Sandy Soil

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ABSTRACT

Two field trials were conducted during two winter growing seasons of 2016/2017 and 2017/2018, using drip irrigation system. The target of this study was to evaluate the response of *Rhizobium*-faba bean symbiosis system to rhizobacterial inoculation (*Serratia marcescens* and *Bacillus megaterium*) and AM-fungi under graded levels of natural rock phosphate (15,30 and 45 kg P_2O_5/fed).The experimental design was arranged in a completely randomized block design with four replicates. The number and dry weight of nodules, N_2-ase activity, plant height, dry weight of shoot and their contents of N, P and K ,the number of pods plant^{-1} and the number of seeds pod^{-1}as well as 100-seed weight, the biological yield, seed yield and seed crude protein percentage were evaluated. The study was also concerned with the impacts of such microbial inocula and rock phosphate on dehydrogenase and phosphatase activities in soil rhizosphere. The obtained results elicited that the uninoculated plants were poorly nodulated. While, inoculating faba bean seeds with efficient *Rhizobium* exerted great improvement in nodulation status and N_2-ase activity. The microbiological properties of rhizosphere soil, which expressed by dehydrogenase enzyme activity and the activity of phosphatases displayed higher response to applied biofertilizers. In fact, the splendid effect was observed with the use of inoculation approach (mixture of *Rhizobium*, *Serratia*, *Bacillus* and AM-fungi), which caused promotive impression in nodulation, the activity of rhizosphere soil enzymes, plant growth aspects and all faba bean yield charactersin relative to other combinations or plants inoculated with *Rhizobium* only. Irrespective of inoculation, there is an increase in nodulation status, all vegetative growth characters, the activity of rhizosphere soil enzymes and faba bean yield with increasing natural rock-phosphate rate from 60 to 180 kg/fed(15 to 45 kg P_2O_5/fed).Hence, data confirmed the superiority of treatment comprising (*Rhizobium* conjugated mixture of PGPR's and AM-fungi)in combination with 30 or 45 kg P_2O_5/fed, which surpassed the other tested combinations and caused a significant augmentation in all studied faba bean parameters. While, inoculation with *Rhizobium* combined with PGPRs or AM-fungi and fertilized with 45 kg P_2O_5/fed came in the second rank. Obtained findings displayed a considerable evidence that inoculation of faba bean seeds with *Rhizobium* combined with a mixture of rhizobacteria may revealing a good practice for improving faba bean growth and yield characters and leading to healthier food, particularly when this practice supported by effective AM-fungi and natural rock phosphate (30 kg P_2O_5/fed).

Keywords: Faba bean (*Vicia faba* L.), *Rhizobium leguminosarum*, *Serratia marcescens*, *Bacillus megaterium*, Arbuscular mycorrhizal fungi (AM-fungi),Rock-phosphate and Sandy soil.

INTRODUCTION

Faba bean (*Vicia faba* L.) is a member of leguminous family which widely grown in winter season and contain a high amount of proteins and carbohydrates (Sepetoğlu, 2002). It is also economical lyre presents a strategic crop in terms of its income to the farmers. Furthermore, high nutrients, i.e. nitrogen, contents make it a green manure for soil fertility, human nutrition as a vital source of vegetarian protein, animal feeding as well as other industrial objectives. However, there was a considerable decline in faba bean cropping in Egypt. Thus, the cultivated area should be increased by expending in the newly reclaimed soil and cultivation of highly yield cultivars throughout application of best agricultural management including the soil mineral fertilization along with bio fertilization (El-Gizawy and Mehasen, 2009 and Ghazi, 2017).

Biologically fixed nitrogen by *Rhizobium*-legume symbiosis is efficiently share forest ablish and clean the agricultural environment in a sustainable sound (Jensen and Hauggaard, 2003). Inoculation of legumes with effective and efficient competitive strains of *Rhizobium* in newly soils has been recommended. So that, an efficient biological nitrogen fixation could be consider as one of the main factors that should be available to cultivate legumes and to increase the fertility of newly reclaimed soils (Badawi *et al.*, 2011 &Nikfarjam and Aminpanah, 2015).

The fertilization with phosphorus (P) has various obstacles although it is found in abundant amount within cultivated soils as inorganic and organic forms. It is recognized to playa highly role in the growth and metabolism of leguminous plants and it considered the most important fertilizer needed for maximizing yield. Phosphorus is a constituent of nucleic acids and considered as high strong energy compounds, stimulates cell division and metabolic processes as well as enhances root growth, nodulation and N_2-fixation (Zaidi *et al.*, 2009 and Sharma *et al.*, 2012).However, the immobilization of phosphorus through the processes of sorption and precipitation with other cations in the soils lead to increase the cost with low efficiency of soil P fertilization in the agro-ecosystems (Garg and Bahl, 2008 and Ghazi, 2017).Rock phosphates (apatite) are fertilizers amply recommended for soils with high Pfixation capacity because other more soluble sources are quickly fixed. However, rock-phosphates are extremely insoluble, particularly in alkaline soils, and a little more reactivity is always desired (Akintokum *et al.*, 2003; Vassilev *et al.*, 2006 and Vega, 2007). Phosphate solubilization process that potentially mediated by phosphate solubilizing microorganisms (PSMs) are widely occur in the cultivated soils and has a vital role in stimulate the availability of accumulated phosphates taking in mind the environment health as well as sustainability aspects. Such process that taken place by PSMs could also share into improve the growth of plants by many pathways other than P solubilization including the production of phytohormones and antagonism toward soil borne pathogens (Khan *et al*., 2014).

Plant growth promoting rhizo bacteria "PGPRs" are numerous microorganisms that could potentially colonize the plant roots and reveal the ability in order to improve the

plant development as well as their productivity viamany exerted beneficial strategies (Das and Singh, 2014). Such strategies included in the direct enhancement of the plant growth could be represented by fixation of atmospheric nitrogen, siderophores production, availability of nutrients and release of phytohormones (Kloepper, 2003;Yang *et al.,* 2009and Santoyo *et al.,* 2016). However, there are indirect strategies could be exerted by mean of PGPRs through biological restriction of phytopathogenic microorganisms or stimulation of self-defense by host (Kloepper*et al.,* 2004andLugtenberg and Kamilova, 2009).

Recently, the fertilization using beneficial microorganisms to improve availability of phosphorus as well as micronutrients along with the improvement of their uptake has become a vital tool in crop cultivation. In this concern, *Bacillus megaterium*, which also referred as "phosphate dissolving bacteria (PDB)" was used as biofertilizer for improving P solubilization, in turn, absorption and producing antimicrobial agents against deleterious microorganisms(Kloepper, 2003 and Gururani *et al.,* 2013). Also, *Serratia marcescens* is a species of Gram-negative bacterium which characterized by production of antagonistic substances, i.e. extracellular enzymes, for degradation of chitinolytic comp on entmainly comprises pathogenic fungal cell walls and in ternpromotes the plant growth(De Queiroz and De Melo, 2006),biologically controlling plant pathogens(Tilak *et al.,* 2006 and Verma *et al.,* 2010) and availability of phosphates (Tripura *et al.,* 2007 and Badawi *et al.,* 2011).

Arbuscular mycorrhiza (AM)are continuously interacting with a wide range of soil microorganisms including PGPR, mycorrhiza helper bacteria and deleterious bacteria. Their interactions can have important implications in agriculture (Miransari, 2013). AM-fungi are known to be ubiquitous in agricultural soils and are believed to enhance P nutrition of plants by scavenging the available P due to the large surface area of their hyphae, and by their high affinity P uptake mechanisms. There are also documents of organic acid production by AM that could solubilize the insoluble mineral phosphates (Gyaneshwar *et al.,* 2002).Many benefits have been resulted because of the symbiosis between AM-fungi and roots of hosted plants and widely spread in the natural environment including the improvement of nutrition and enhancement of nitrogen fixation (Antunes *et al.,* 2006),biological control of soil borne pests as well asstimulate the resistance to drought conditions (Liu *et al.,* 2012 and Hashem *et al.,* 2014).

Seed inoculation with some biofertilizers such as *Rhizobium, Bacillus megaterium,* arbuscular mycorrhiza and *Serratia* promoted the plant growth and yield of legume crops. Beneficial responses due to interaction of PGPRs with rhizobia have been reported previously (Verma *et al.,* 2010 and Badawi *et al.,* 2011). However, AM-fungi and rhizobia are two of the most important plant symbionts.

Mycorrhiza sbene fit the host through mobilization of phosphorus from nonlabile sources, whereas *Rhizobium* fixes N (Aysan and Demir, 2009 and Tajini*et al.,* 2012).There were several cases in which AM-fungi and PGPR coinoculations supported each other in terms of the improvement of plant growth and nutrient uptake (Bisht *et al*., 2009; Bhromsiri and Bhromsiri,2010 & Liu *et al.,* 2012). They showed a positive and synergistic interaction between mycorrhizae and associated microorganisms that can improve plant nutrition, growth, reduce plant disease, and they are usually more effective when added together than alone. In this concern, results of Miransari, 2013 showed that the utilization of poorly soluble sources of phosphate were improved as a result of the synergistic interaction between AM-fungi and PDB.

This investigation was carried out to study the response of *Rhizobium*-faba bean symbiosis system, growth and productivity to rhizo bacterial inoculation and AM-fungi under different levels of natural phosphorus in sandy soil.

MATERIALS AND METHODS

Two field trials were performed at the field of the Environmental Studies & Research Institute (ESRI), Sadat City University, Egypt, during winter seasons of 2016/2017 and 2017/2018 under drip irrigation conditions in order to study the response of *Rhizobium*-faba bean symbiosis system, growth and productivity to rhizo bacterial inoculation and AM-fungiunder different levels of phosphate fertilization. The influence of such microbial inoculation and rock phosphate on dehydrogenase and phosphatase activities in soil rhizosphere also studied.

Soil:

The soil of experiment was sampled from the top 20 cm layer of field, air-dried and sieved through a 2 mm screen. The main physiochemical characters of the experimental soil are shown in Table (1).

Table 1. Some physiochemical characters of the soil

Property	Values	
	Season 2016/2017	Season 2017/2018
Particle size distribution :		
Sand (%)	88.60	88.50
Silt(%)	4.80	4.60
Clay (%)	6.60	6.90
Texture grade	Sandy	Sandy
$CaCO_3$ (%)	1.90	1.82
Saturation percent (S.P %)	21.30	21.10
pH (1:2.5,soil: water suspension)	7.63	7.70
E.C (dS m^{-1}, in the paste extract)	1.82	1.73
Soluble cations and anions (meq L^{-1}):		
Ca^{++}	5.30	5.41
Mg^{++}	4.92	4.42
Na^{+}	5.82	5.33
K^{+}	2.14	2.20
$CO^{=}_3$	0.00	0.00
HCO^{-}_3	5.82	5.61
Cl^{-}	4.70	4.56
$SO^{=}_4$	7.66	7.19
Organic matter (%)	0.30	0.33
Total soluble N (mg kg^{-1})	15.55	16.82
Available-P (mg kg^{-1})	6.25	6.70
Available-K (mg kg^{-1})	66.40	72.50
*DTPA-extractable (mg kg^{-1}):		
Fe	1.10	1.14
Mn	0.32	0.34
Zn	0.38	0.36
Cu	0.17	0.15

*** Di-ethylene tri-amine penta acetic acid**

Microbial cultures:

Rhizobium leguminosarum bv. *Viciae* (mixture of ARC 221 and ICARDA 441),rhizo bacterial, namely *Serratia marcescens* (strain WW4)and *Bacillus megaterium* var. *phosphaticum* (local isolate) and Arbuscular mycorrhizal fungi were kindly provided by Microbiol. Dept., Soils, Water and Environ. Res. Instit., Agricultural Research Center (ARC), Giza, Egypt.

Inoculants preparation:

Rhizobium was cultured in a yeast extract mannitol (YEM)broth medium (Vincent, 1970), while *Serratia* and *Bacillus* were grown in King's medium B (Atlas, 1995).

Cultures were incubated at 28°C for three days on a rotary shaker until early log phase had been developed to 10^9 viable cell ml^{-1}.Mixed spores of Arbuscular mycorrhizal fungi genera (*Glomus*, *Gigaspra* and *Acaulospora*) were prepared after propagation and mixed with sterilized peatmoss as a carrier (200 spore/g).

Experimental design:

The experimental design was arranged in a completely randomized block design with four replicates with a plot area of 13.5 m^2 including 6 rows 3m in length and 75cm in width. The following treatments were conducted:

1- Uninoculated (received recommended NPK).
2- Inoculated with *Rhizobium* (*Rh.*) + 15 kg P_2O_5/fed.
3- (*Rh.*) + 30 kg P_2O_5/fed.
4- (*Rh.*) + 45 kg P_2O_5/fed.
5- (*Rh.*) +*Serratia marcescens* (*S.*) + 15 kg P_2O_5/fed.
6- (*Rh.*) + (*S.*) + 30 kg P_2O_5/fed.
7- (*Rh.*) + (*S.*) + 45 kg P_2O_5/fed.
8- (*Rh.*) + Arbuscular mycorrhizal fungi (AM-fungi) + 15 kg P_2O_5/fed.
9- (*Rh.*) + (AM-fungi) + 30 kg P_2O_5/fed.
10- (*Rh.*) + (AM-fungi) + 45 kg P_2O_5/fed.
11- (*Rh.*) + *Bacillus megaterium* (*B.m*) + 15 kg P_2O_5/fed.
12- (*Rh.*) + (*B.m*) + 30 kg P_2O_5/fed.
13- (*Rh.*) + (*B.m*) + 45 kg P_2O_5/fed.
14- (*Rh.*) + (*S.*) + (AM-fungi) + (*B.m*) + 15 kg P_2O_5/fed.
15- (*Rh.*) + (*S.*) + (AM-fungi) + (*B.m*) + 30 kg P_2O_5/fed.
16- (*Rh.*) + (*S.*) + (AM-fungi) + (*B.m*) + 45 kg P_2O_5/fed.

Seeds:

Seeds of faba bean (*Vicia faba*, cv. Giza 40) were kindly supplied by Food Legume Research Program, Field Crops Research Institute, ARC, Giza, Egypt.

Fertilization:

Organic fertilizer (compost) at a rate of 10m^3/fed kindly supplied by Soil, Water and Environ. Res. Inst. (SWERI), ARC, Giza, Egypt, was added to the experimental soil 10 days before sowing. The main traits of used mature compost are shown in Table (2).

All treatments received potassium sulphate (48% K_2O) at the rate of 50 kg/fed (24 kg K_2O/fed).All inoculated treatments received 20 Kg N/fed in the form of ammonium sulphate (20.6% N) at sowing as an activator dose for bacteria, while the uninoculated treatment received 40 kg N/fed, which applied in three equal split doses after 15, 25 and 35 days from sowing. Phosphorus fertilizer was added to the uninoculated treatment as calcium superphosphate (15% P_2O_5) during soil preparation nat a rate of 200 Kg/fed (30 kg P_2O_5/fed). All inoculated treatments fertilized with rock phosphate (25% P_2O_5), which broadcasted and incorporated into the soil 10 days before sowing at the rates of 15, 30 and 45 kg P_2O_5/fed equal 60, 120 and 180 kg rock phosphate/fed, respectively.

Table 2. Characterization of the used compost

Property	Value
Bulk density (kgm^{-3})	544.0
Water holding capacity (%)	189.4
pH (1:10 watery extract)	7.10
EC (dS/m)	2.95
Organic-C %	26.85
T-Nitrogen %	1.61
C/N ratio	16.68
Total-P (%)	1.12
Total-K (%)	1.43
Soluble-N (mg kg^{-1})	665.4
Available-P (mg kg^{-1})	216.9
Available-K (mg kg^{-1})	715.2
*DTPA-extractable (mg kg^{-1}):	
Fe	189.9
Mn	39.2
Zn	46.1
Cu	5.9
Total bacteria ($cfug^{-1}$)	8 x 10^7
Total fungi ($cfug^{-1}$)	5 x 10^6
Total actinomycetes ($cfug^{-1}$)	3.6 x 10^6
**Dehydrogenase activity (mg TPF/100 g)	168.4
***Germination test of cress seeds (%)	86.0

***Di-ethylene tri-amine penta acetic acid.**
****2,3,5 Tri-Phenyl-Formazan.**
*****Cress seeds incubated for 48 hr.**

Inoculation:

Seeds of faba were inoculated with gamma irradiated vermiculite-based inoculants of each bacterium (for a mixture of two *Rhizobium* strains and tested PGPR)at a rate of 600g inoculum/60 kg seeds. Mixed spores of AM-fungi were prepared after propagation, mixed with sterilized peatmoss as a carrier (200 spore/g), and then applied as a seed coating for surface sterilized faba bean seeds. The inoculants mixed with a sticker such as Arabic gum and uniformly coated on the seeds (70 spore/seed) and then air dried for 2 hours before planting.

Seeds of faba bean were sown into the hills on 1st and 5rd November in 2016 and 2017 growing seasons, respectively, and then drip irrigation took place immediately. The growing faba bean plants were thinned to specify the plant density and the crop was kept clean by hand weeding two and three weeks after sowing.

Analyses:

The characterization of the used soil and compost was done as descripted by Piper (1950), Page *et al.* (1982), Iglesias-Jimenez & Perez-Garcia (1989) and Pare *et al.*, 1997.Nitrogenase enzyme activity was executed as described by Hardy *et al* (1973). DHA-ase activity was assayed calorimetrically according to Page *et al.* (1982). Phosphatase enzyme activity was determined according to procedure described by Tabatabai (1982).

The nitrogen, phosphorus and potassium were determined in the dried and digested plant materials according Page *et al.*, 1982. Seed crude protein% was

expressed as a product of nitrogen % and 6.25 according to A.O.A.C (1990).

Analysis of variance (ANOVA) was performed according to Snedecor and Cochran (1980) using LSD at level of 0.05 for differences comparison among the means.

RESULTS AND DISCUSSION

Faba bean nodulation status and N_2-ase activity:

Nodulation originated on the faba bean roots and N_2-ase activity as affected by inoculation with *Rhizobium* either singly or combined with tested rhizobacteria (*Serratia marcescens* and *Bacillus megaterium*) and/or AM-fungi under different levels of natural phosphate in sandy soil are presented in Table (3). Uninoculated plants were poorly nodulated due to poor establishment of native rhizobia in experimental soils. Hence, the presence of native rhizobia of faba bean in the experimental soil is of inadequate number, having a low efficiency of nitrogen fixation. Inoculating seeds with efficient *Rhizobium* exerted great improvement in nodulation status and N_2-ase activity in both seasons, indicating the necessity of using effective strains of *Rhizobium* to achieve a good nodulation, especially in areas where indigenous nodulation has been found to be inadequate. Badawi *et al.* (2011) & Siczek and Lipiec (2016) reported this observation. However, the combined application of *Rhizobium* conjugated with tested rhizobacteria or AM-fungi showed a significant predictable improvement in nodulation and N_2-ase activity of faba bean roots in relative to the uninoculated plants or plants inoculated with *Rhizobium*. Obviously, mixed inoculation treatment (*Rhizobium* + PGPR+ AM-fungi) surpassed the other treatments in both seasons. These results may be due to that biofertilizers produce some growth regulators, which may improve the nodulation status by hormonal stimulation besides N_2-fixation (Tajini *et al.,* 2012 and Byan and El-Shimi, 2014).

Table 3. Nodulation status of faba bean plants as affected by bacterial inoculation and arbuscular mycorrhizal fungi under different rates of rockphosphate

Treatments		Number of nodules/plant		Dry weight of nodules (mg/plant)		N_2-ase activity (µmol C_2H_4/g d.wt nodules/hr)	
		2016/2017	2017/2018	2016/2017	2017/2018	2016/2017	2017/2018
Uninoculated (Recom. NPK)		19.67	21.67	258.09	263.85	5.36	4.94
Rhizobium (*Rh.*)	15-P	33.67	42.67	339.98	352.15	37.47	34.50
	30-P	40.00	46.33	397.43	438.17	41.16	37.93
	45-P	48.67	52.00	443.80	449.54	48.63	47.10
Rh. + *Serratia* (S.)	15-P	41.67	50.33	435.06	467.12	39.27	36.15
	30-P	50.33	56.67	453.86	468.01	45.03	41.45
	45-P	56.33	61.67	463.99	487.17	53.62	49.38
Rh. + (AM-fungi)	15-P	44.67	49.67	454.77	458.19	37.60	34.62
	30-P	54.67	56.67	465.17	471.05	50.38	46.38
	45-P	61.67	58.33	477.06	483.21	54.48	54.40
Rh. + *Bacillus* (*B.m*)	15-P	46.33	52.00	446.99	458.53	40.31	36.43
	30-P	54.33	59.00	471.33	466.10	46.70	42.97
	45-P	62.67	62.00	479.74	478.95	56.69	52.14
Rh. + *S.* + (AM fungi) + *B.m*	15-P	50.00	55.33	453.57	474.38	48.63	44.77
	30-P	59.67	65.00	474.03	487.48	53.00	48.75
	45-P	66.00	67.00	487.14	491.53	67.29	61.89
LSD at 5%		3.986	4.484	17.250	7.920	6.210	4.340

Uninoculated treatment received 40 kg N/fed, 30 kg P_2O_5/fed (as superphosphate 15% P_2O_5) and 24 kg K_2O/fed.
All inoculated treatments received 20 kg N/fed and 24 kg K_2O/fed.
Rock phosphate (25% P_2O_5) was used as a source of phosphorus levels (15, 30 and 45 kg P_2O_5/fed) for all inoculated treatments.
***Rh.*:*Rhizobium leguminosarumS.*:*Serratia marcescens* AM-fungi: Arbuscular mycorrhiza *B.m*:*Bacillus megaterium*.**

Irrespective of inoculation, the results in Table (3) showed that phosphorus fertilization improved the nodulation status and N_2-ase activity in both seasons. The highest number and dry weight of nodules and N_2-ase activity were obtained by adding 30 or 45 kg P_2O_5/fed as rockphosphate. It is clear that the legumes responded well to rockphosphate and this reflects in producing strong root systems, increasing nodulation and biological N_2-fixation.

Hence, it is known that P is the most limiting factor for N_2 fixation by *Rhizobium*-legume symbiosis (Aboel-Soud *et al.,* 2003 and Mohammed, 2004).These results might be attributed to phosphorus is an essential element for root development and stimulate cell division as well as required for the normal functioning of nitrogen fixing bacteria and has a favorable effect on the number and weight of the effective nodules formation on the root system. This finding is congruent with the investigation of (Knany *et al.,* 2004 and Zarrin *et al.,* 2007).Recently, Divito and Sadr as (2014) and Mouradi *et al.* (2018) added that nodules are highly sensitive to nutrient deficiency, with the hypothesis that plants grown under P-deficiency tend to reduce their nodule number rather than nodule mass, in this manner they facilitate oxygen diffusion into the nodule to assure effective N_2-fixing.

The data in Table (3) revealed that all inoculated treatments in the presence of different natural phosphorus levels (15, 30 or 45 kg P_2O_5/fed) were superior to uninoculated treatment. The higher nodulation status and N_2-ase activity was obtained due to application of 30 or 45 kg P_2O_5/fed, with relatively higher values in the case of faba bean inoculated with mixed inoculum (*Rhizobium* + rhizobacteria + AM-fungi) compared with other tested

combinations. The difference between the two phosphorus levels did not reach the level of significance. The associative action of such biofertilizers and phosphorus levels yielded the highest values of nodules number (59.67 and 66.00 plant^{-1}); nodules dry weight (474.03 and 487.14 mg plant^{-1}) and N_2-ase activity (53.00 and 67.29µmol C_2H_4/g d.wt noduleshr^{-1}) in the first season, respectively. During the second season, the corresponding higher values of nodules number were (65.00 and 67.00 phant^{-1}), nodules dry weight (487.48 and 491.53mg plant^{-1}) and N_2-ase activity (48.75 and 61.89µmol C_2H_4/g d.wt nodules hr^{-1}), respectively. These results could be explained as increasing phosphorus level up to 30 or 45 kg P_2O_5/fed, AM-fungi and rhizobacteria may enhance the *Rhizobium* bacteria to form more nodules due to increasing the level of supply in available form of nutritional elements. The promoting effects on nodulation and symbiotic performance might occur through the integration between the various mechanisms offered by mixing more than efficient rhizobacteria and AM-fungi, which act to enhance root proliferation and provide more infection sites to rhizobia. Many investigators confirmed the stimulating effect of PGPR and AM-fungi in creating a favorable habitat for improving nodulation pattern and biological N_2-fixation (Aysan and Demir, 2009; Badawi *et al.*, 2011; Tajini *et al.*, 2012; Miransari, 2013).

Dehydrogenase and phosphatase activities:

In general, the measurement of soil enzymes activity is a good index of soil quality and can be used as indicative of the biological activity or biochemical process. DHA enzyme has often been used as a parameter to evaluate the overall microbial activity in soil. However, efficient acquisition and utilization of ++phosphorus requires ubiquitous class of enzymes known as phosphatases. They play an important role in the production, transport and remobilization of available phosphorus.

In this context, rhizosphere soil enzymes (dehydrogenase and phosphatase activities) as affected by inoculation with *Rhizobium* either individually or interacting with tested PGPRs and AM-fungi under different levels of natural phosphorus in sandy soil are presented in Table (4). All tested enzymes were significantly affected by different treatments under study and behaved in a similar manner as in N_2-ase activity. Irrespective of phosphate levels, a significant increase in DHA-ase and phosphatase (acid and alkaline) activities was observed upon in oculation. The uninoculated treatment showed lower values of DHA-ase activity (6.90 and 7.49 µg TPF/g dry soil/day), alkaline phosphatase (30.45 and 32.42 µg P/g dry soil) and acid phosphatase (25.18 and 24.68µg P/g dry soil) in both seasons, respectively. Rhizobial inoculation increased the rate of DHA-ase and phosphatase activities. Siczek and Lipiec (2016) showed that *Rhizobium* inoculation induced a significant increase in a majority of enzymatic activities in the rhizosphere throughout the vegetative period of faba bean. However, the maximal rates of DHA-ase activity, alkaline phosphatase and acid phosphatase were obtained by a *Rhizobium* conjugated mixture of PGPR's and AM-fungi treatment. The other tested treatments (*Rhizobium* conjugated with AM-fungi or with PGPR's) came in the second rank. This might have been due to increased microbial and root activities. In fact, the microbiological properties of rhizosphere soil, which expressed by dehydrogenase enzyme activity and the activity of phosphatases displayed higher response to applied biofertilizers. These results are in conformity with those of Hashem *et al.* (2014)and Vafadar *et al.*(2014) who confirmed that dual inoculation of PGPR and AM-fungi led to higher biological N_2 fixation (BNF), DHA-ase and phosphatase(acid and alkaline) activities.

Table 4. Effect of bacterial inoculation, arbuscular mycorrhizal fungi and different rates of rockphosphate on dehydrogenase and phosphatase activities in soil rhizosphere after 60 days from sowing

Treatments		Dehydrogenase activity (µgTPF/g dry soil /day)		Alkaline phosphate (µg P/g dry soil)		Acid phosphate (µg P/g dry soil)	
		2016/2017	2017/2018	2016/2017	2017/2018	2016/2017	2017/2018
Uninoculated (Recom. NPK)		6.90	7.49	30.45	32.42	25.18	24.68
Rhizobium (*Rh.*)	15-P	11.93	13.00	40.30	44.40	31.10	31.60
	30-P	14.48	15.70	48.90	53.60	36.20	38.00
	45-P	15.70	17.11	49.84	53.91	44.40	45.40
Rh. + *Serratia* (S.)	15-P	12.33	13.50	44.30	53.80	34.80	37.50
	30-P	14.93	16.20	48.30	56.50	38.90	42.80
	45-P	15.90	17.26	52.50	56.90	46.60	50.00
Rh. + (AM-fungi)	15-P	12.72	13.80	52.80	57.90	39.00	38.20
	30-P	14.94	16.20	61.00	64.30	49.10	52.80
	45-P	16.20	17.55	69.20	69.80	56.40	56.70
Rh. + *Bacillus* (*B.m*)	15-P	12.60	13.70	49.20	57.20	42.90	44.80
	30-P	15.07	16.40	57.00	60.20	53.20	59.20
	45-P	16.30	17.66	61.40	66.30	60.10	61.90
Rh. + *S.* + (AM-fungi) + *B.m*	15-P	16.69	18.10	52.20	56.10	58.40	53.40
	30-P	17.20	18.70	55.80	61.40	52.00	61.10
	45-P	17.92	19.47	61.90	71.90	60.30	67.10
LSD at 5%		1.181	1.278	4.180	6.300	10.920	2.143

Uninoculated treatment received 40 kg N/fed, 30 kg P_2O_5/fed (as superphosphate 15% P_2O_5) and 24 kg K_2O/fed.
All inoculated treatments received 20 kg N/fed and 24 kg K_2O/fed.
Rockphosphate (25% P_2O_5) was used as a source of phosphorus levels (15, 30 and 45 kg P_2O_5/fed) for all inoculated treatments.
***Rh.*:*Rhizobium leguminosarumS.*:*Serratia marcescens* AM-fungi: Arbuscular mycorrhiza *B.m*:*Bacillus megaterium*.**

Irrespective of inoculation, the highest dehydrogenase and phosphatase activities were obtained under phosphorus levels (30 or 45 kg P_2O_5/fed as rock phosphate) in both seasons. Šarapatka (2002) reported that the carbon, total nitrogen and phosphorus content could serve as a basis for increasing both biological and enzymatic soil activities. However, it is often assumed, that inorganic fertilizers had relatively less effect on soil enzymes activity, particularly DHA-ase activity, than organic fertilizers (Macci *et al*., 2012).It is known that phosphatase activity is strongly regulated by P supply and production of phosphatase may play an important role in the regulation of P supply (Olander and Vitousek, 2000& Fouda, 2017). However, phosphorus deficiency often enhances extracellular phosphatase activity from plant roots, fungi and other microorganisms. Hence, there are a negative correlation between the available phosphorus content and both acid and alkaline phosphatase activity. The result substantiates the findings of Boudanga *et al*.(2015).

Among the enzymes, DHA-ase showed the little bit different path during application natural phosphorus levels combined with biofertilizers. It varied from 11.93 to 17.92 µg TPF/g dry soil/day in the first season and ranged from 13.00 to 19.47 µg TPF/g dry soil/day in the second one. Alkaline phosphatase ranged from 40.30 to 69.20 µg P/g dry soil and from 44.40 to 71.90 µg P/g dry soil in both seasons, respectively. While, acid phosphatase varied from 31.10 to 60.30 µg P/g dry soil and from 31.60 to 67.10 µg P/g dry soil, respectively. However, the results clearly evident that the synergy of using 30 or 45 kg P_2O_5/fed and inoculation with *Rhizobium* either individually or combined with PGPRs or AM-fungi in increasing the activity of dehydrogenase and phosphatase, relative to the uninoculated treatment or inoculated treatments fertilized with 15 kg P_2O_5/fed. Treatment comprising (*Rhizobium* + PGPRs + AM-fungi) in combination with 30 kg P_2O_5/fed confirmed their synergistic interaction to enhance the activity of rhizo sphere soil enzymes. For instance, dehydrogenase enzyme activity (which is considered the reliable indicator of global biological activity in soil) was increased by 149.28 and 149.67% and the increase in alkaline phosphatase were 83.25 and 89.39% , while the increase in acid phosphatase were 106.51 and 147.57 % over that obtained under uninoculated treatment in both seasons, respectively. Apparently, rhizosphere phosphatase and dehydrogenase activity tends to be higher because of increased microbial numbers in the rhizosphere and the excretion of plant root enzymes. These enzyme activities associated with higher availability and uptake of nutrients, particularly phosphorus in plants (Šarapatka, 2002 and Dotaniya *et al.,* 2014).These results are in the same line with previous studies confirming a positive impact of rhizobia with PGPR and AM-fungi activities on soil quality by increasing the activity of soil enzymes (Prasad *et al.,* 2012**;** Othman and Tamimi 2016& Mouradi *et al.,* 2018).

Plant growth:

The plant growth characters including; plant height, shoot dry weight and its N, P and K accumulation as affected by *Rhizobium* inoculation with different PGPR's and AM-fungi under graded levels of natural phosphorus are given in Table (5).

Table 5. Effect of bacterial inoculation and arbuscular mycorrhizal fungi on plant height, shoot dry weight and shoot N,P and K contents of faba bean plants grown under different rates of rockphosphate in sandy soil

Treatments		Plant height (cm)		Shoot dry weight (g/plant)		Shoot N-content (mg/plant)		Shoot P-content (mg/plant)		Shoot K-content (mg/plant)	
		2016/2017	2017/2018	2016/2017	2017/2018	2016/2017	2017/2018	2016/2017	2017/2018	2016/2017	2017/2018
Uninoculated (Recom. NPK)		55.06	51.78	19.98	20.36	608.30	597.80	74.50	80.10	215.80	220.10
Rhizobium (Rh.)	15-P	32.85	30.60	13.62	11.56	496.22	482.70	55.40	59.40	132.80	143.70
	30-P	33.15	30.59	14.37	13.45	524.82	527.10	61.80	67.00	144.70	156.80
	45-P	37.68	33.92	15.99	15.35	543.11	605.90	63.89	70.51	164.47	173.54
Rh. + Serratia (S.)	15-P	46.27	42.00	15.49	14.58	495.96	538.20	56.80	65.50	171.30	186.50
	30-P	52.04	48.69	16.70	17.15	591.65	642.30	76.80	74.60	185.40	200.70
	45-P	56.67	52.63	21.53	22.14	624.20	677.88	75.90	82.20	258.70	280.50
Rh. + (AM-fungi)	15-P	41.85	40.90	14.50	13.20	511.62	555.40	67.70	64.70	169.90	183.80
	30-P	50.91	49.99	18.40	19.24	604.78	656.80	78.80	85.10	212.70	266.20
	45-P	53.66	52.40	23.06	26.54	626.80	681.22	101.50	110.00	300.50	326.20
Rh. + Bacillus (B.m)	15-P	49.39	50.34	13.34	14.26	485.53	538.70	62.30	67.00	178.20	166.60
	30-P	53.02	57.26	16.66	15.46	555.72	603.40	71.40	76.80	228.30	247.40
	45-P	58.27	61.14	20.87	19.78	612.70	664.96	81.90	88.40	290.30	333.00
Rh. + S. + (AM-fungi) + B.m	15-P	49.82	54.12	18.61	19.04	548.44	595.60	67.60	72.80	256.60	275.30
	30-P	53.96	59.67	23.58	26.78	634.37	688.70	84.20	90.90	332.50	361.10
	45-P	59.28	64.48	25.75	27.08	660.50	719.80	106.80	111.40	424.80	461.60
LSD at 5%		7.550	6.880	1.930	2.110	32.430	17.590	8.570	5.130	91.950	35.410

Uninoculated treatment received 40 kg N/fed, 30 kg P_2O_5/fed (as superphosphate 15% P_2O_5) and 24 kg K_2O/fed.
All inoculated treatments received 20 kg N/fed and 24 kg K_2O/fed.
Rockphosphate (25% P_2O_5) was used as a source of phosphorus levels (15, 30 and 45 kg P_2O_5/fed) for all inoculated treatments.
***Rh.*:*Rhizobium leguminosarumS.*:*Serratia marcescens* AM-fungi: Arbuscular mycorrhiza *B.m*:*Bacillus megaterium*.**

The present study clearly demonstrated that the uninoculated treatment (received a full dose of N,P and K) has been superior to *Rhizobium* inoculation treatment in all growth characters. As previously mentioned, bacterization of faba bean seeds with rhizobia combined with tested rhizobacteria and/or AM-fungi/fed can stimulate all plant growth aspects through enhancing processes such as nutrients uptake and controlling plant pathogens. These results were true in the two studied seasons. Accordingly, co-inoculation strategy seemed to be more valuable, with relatively surpassing of mixture treatment comprised (*Rhizobium* + *Serratia* + *Bacillus* + AM-fungi).This could be due to the essential role of *Rhizobium* in enhancing plant growth and N_2-fixation, and then it was magnified as a result of co-inoculation with the mixture of PGPRs and/or AM-fungi as reported by (Zaidi *et al.,* 2009;Tajini *et al.,* 2012;Vafadar *et al.,* 2014&Kumar and Verma, 2017). They confirmed that the nitrogen fixers, phosphate-solubilizing fungi and PGPR when inoculated together colonized the rhizosphere and improve plant growth promotion by triggering plant growth hormones, enhance the nutritional capacity of the plants and confer stress tolerance in plants.

Irrespective of inoculation, the results in Table (5) indicated that there is an increase in all vegetative growth characters with increasing phosphorus rate from 15 to 45 kg P_2O_5/fed. For instance, top dressing sandy soil with 30 or 45 kg P_2O_5/fed as rockphosphate increased all vegetative growth characters, relative to plants fertilized with 15 kg P_2O_5/fed. Such effects may be due to that phosphorus encourages the growth of the root system, nodulation and the fixation and utilization of N as well as its role in enhancing photosynthesis, carbohydrates metabolism and protein synthesis and this in turn increased the number of metabolites synthesized by the plants resulting increasing dry weight of its organs. These results are confirmed by those reported by Mohammed (2004),Byan and El-Shimi (2014) and Boudanga *et al.* (2015).Recently, Fouda (2017)added that supplied faba bean plants with P-fertilization might have improved and developed a good root system of plant and the capacity of root to absorb more N,P and K accordingly their contents increased.

The results in Table (5) elicited that faba bean seeds inoculated with *Rhizobium*in combination with any level of natural phosphorus showed lower values for all studied plant growth aspects compared to other inoculated or uninoculated treatments. It is clear that fertilizing with rockphosphate combined with tested biofertilizers showed a significant augmentation in all studied characters, particularly in the case of faba bean inoculated with *Rhizobium* and a mixture of rhizobacteria and AM-fungi, which surpassed the other tested combinations or recommended treatment. The associative action of rockphosphate and such biofertilizer treatment confirmed their synergisticinter action in both seasons. In this context, co-inoculation with *Rhizobium* and PGPRs or AM-fungi combined with 45kg P_2O_5/fed or mixture inoculation treatment (*Rhizobium* conjugated mixture of PGPR's and AM-fungi) combined with 30 or 45 kg P_2O_5/fed caused a significant increase in all plant growth parameters over the single inoculation with *Rhizobium* fertilized with 45 kg P_2O_5/fed. The corresponding increases attained in plant height ranged from 42.41 to 57.32 % and from 54.48 to 90.09 % and those of shoot dry weigh ranged from 30.52 to 61.04 % and from 28.86 to 76.42%, in both seasons, respectively. While, increases in shoot N-uptake ranged from 12.81 to 21.61% and from 9.75 to 18.80%, shoot P-content ranged from18.80 to 67.16 % and from 16.58 to 57.99% and those of shoot K-content ranged from 57.29 to 158.28% and from 61.63 to 165.99%, respectively. It is evident that the applied phosphorus together with biofertilizers achieved many of the beneficial effects that are more attributed to one or more of PGP-related properties indicating an increase in root biomass and provides it with more branching and larger surface area, and then indirectly enhanced nutrient uptake capacity. These results are in conformity with those of Artursson *et al.* (2006), Shinde *et al.* (2008), El-Gizawy and Mehasen (2009), Lugtenberg and Kamilova (2009)and Elkoca *et al.* (2010)who confirmed the synergistic effects of phosphorus combined with PGPRs and AM-fungi on the vegetative growth characters and its nutrient contents. They added that the plant growth consistently increased due to a wide variety of mechanisms such as increase inphytohormones production, the activities of enzymes and suppression of pathogens by producing antibiotics and siderophores.

Faba bean yield parameters:

Faba bean yield parameters as affected by co-inoculation with rhizobia and tested rhizobacteria and/or AM-fungi under graded levels of natural phosphorus are present in Table (6). Results elicited that the response of all investigated yield parameters is in parallel to the vegetative growth stage.

Irrespective of phosphorus addition, data showed again, an improvement in all faba bean yield parameters due to the stimulatory effect of inoculation with *Rhizobium* combined with rhizobacteria or AM-fungi. However, the promotive effect was further enhanced due to dual inoculation with both rhizobacteria and AM-fungi in relative to other combinations or plants inoculated with *Rhizobium* only.These increases in yield parameters as a function of inoculation may explain their prominent roles to their colonized plants in the improvement of N_2-fixation performance, supplying with growth promoting substances and increasing the nutrient status in the rhizosphere, which reflected on enhancing nutrient uptake, plant growth and consequently enhanced yield parameters. Many studies have confirmed that combined inoculation with *Rhizobium* and rhizobacteria and/or AM-fungi improved most of the studied yield and yield attributes of legumes (El-Habbasha *et al.,* 2007;Shinde *et al.,* 2008; Verma *et al.,* 2010; Badawi *et al.,* 2011&Rakha and El-Said, 2013).

Data in Table (6) revealed that number of pods plant^{-1}, number of seeds pod^{-1} and 100-seed weight increased significantly with increasing application rate of phosphorus from 15 to 45 kg P_2O_5/fed in both growing seasons. These increases may due to phosphorus is considered as high strong energy compounds, stimulate cell division and metabolic processes as well as enhances root growth, nodulation, N_2-fixation and faba bean yield characters. The promoting effect of phosphorus on yield

components of faba bean has been reported by many investigators(Knany *et al.*, 2004; El-Habbasha *et al.*, 2007;Rakha and El-Said, 2013& Fouda, 2017).They found that the number of pods plant^{-1},100-seed weight and the number of seeds pod^{-1} of faba bean significantly enhanced as the P applied increased. They added that phosphorus plays a vital role in flower formation and fruit set and in processes such as sugar and starch utilization, photosynthesis, cell division and nodule formation.

At a given interaction treatments, the data in Table (6) displayed that the existence of rockphosphate in conjugation with *Rhizobium* and rhizobacterial co-inoculation or AM-fungi tended to enhance the faba bean yield parameters as compared to the plants inoculated with *Rhizobium* only. The results exerted that the synergy between plant growth promoting rhizobacteria (PGPR) and AM-fungi increased the fertilization efficiency. However, results ratify that the highest values of faba bean yield parameters were attained in the case of combination among the highest levels of rockphosphate (30 and 45 kg P_2O_5/fed) and *Rhizobium* in the presence of rhizobacterial inoculation and AM-fungi compared to all other combinations. The difference between the two higher rockphosphate doses could not reach the level of significance. The highest number of pods plant^{-1} (22.67 and 24.00), number of seeds pod^{-1} (4.33 and 4.33) and hundred seed weight (g) (83.50 and 84.70), in the first season, occurred when the crop was amended with 30 or 45 kg P_2O_5/fed along with *Rhizobium*, PGPRs and AM-fungi inoculation. In the second season, the highest number of pods plant^{-1}were (23.00 and 24.67), while number of seeds pod^{-1}were (4.56 and 4.70) and those of hundred seed weight (g) were (83.60 and 85.20). Other combination treatments fertilized by 45 kg P_2O_5/fed and the recommended treatment came at the second rank. It is evident that such combined treatment may act to improve sandy soil quality through affecting its chemical and biological features, production of specific activator compounds that have the ability to enhance nutrient and water availability leading to boost the nodulation,N_2-fixation and productivity of the faba bean. The result substantiates the findings of many researchers (El-Habbasha *et al.*, 2007; El-Gizawy and Mehasen, 2009;Rakha and El-Said, 2013 and Metwali *et al.*, 2015)who found the superiority of plants which treated with phosphorous (30 or 45kg P_2O_5/fed) and inoculated with some biofertilizers such as (*Rhizobium*, *Bacillus megaterium*, AM-fungi and *Serratia*) in improving the number and dry weight of podsplant^{-1}, number of seedsplant^{-1} and 100-seed weight.

Table 6. Effect of bacterial inoculation and arbuscular mycorrhizal fungi on number of pods plant^{-1}, number of seeds pod^{-1} and 100-seed weight of faba bean plants grown under different rates of rockphosphate in sandy soil

Treatments		Number of pods/ plant		Number of seeds/ pod		100-seed weight (g)	
		2016/2017	2017/2018	2016/2017	2017/2018	2016/2017	2017/2018
Uninoculated (Recom. NPK)		19.00	19.67	3.22	3.50	71.83	74.41
Rhizobium (*Rh.*)	15-P	16.33	17.00	3.00	3.30	67.60	66.60
	30-P	17.67	18.30	3.22	3.70	68.20	68.10
	45-P	17.67	18.33	3.33	3.70	71.40	69.50
Rh. + *Serratia* (S.)	15-P	19.33	20.70	3.33	4.00	78.70	77.20
	30-P	20.67	22.70	3.67	4.22	81.40	80.20
	45-P	22.70	23.33	3.44	4.30	82.80	83.90
Rh. + (AM-fungi)	15-P	17.33	18.30	3.67	4.28	76.20	69.73
	30-P	18.67	19.30	3.33	4.30	76.39	74.80
	45-P	20.00	20.67	4.00	4.61	79.00	78.00
Rh. + *Bacillus* (*B.m*)	15-P	17.00	18.30	3.33	3.70	78.40	77.10
	30-P	18.33	18.70	3.67	3.78	82.33	79.70
	45-P	21.00	21.67	4.33	4.00	81.00	83.70
Rh. + *S.* + (AM-fungi) + *B.m*	15-P	21.33	22.00	3.67	4.30	81.70	81.90
	30-P	22.67	23.00	4.33	4.56	83. 50	83.60
	45-P	24.00	24.67	4.33	4.70	84.70	85.20
LSD at 5%		1.300	1.740	0.960	1.150	4.670	9.480

Uninoculated treatment received 40 kg N/fed, 30 kg P_2O_5/fed (as superphosphate 15% P_2O_5) and 24 kg K_2O/fed.
All inoculated treatments received 20 kg N/fed and 24 kg K_2O/fed.
Rockphosphate (25% P_2O_5) was used as a source of phosphorus levels (15, 30 and 45 kg P_2O_5/fed) for all inoculated treatments.
***Rh.*:*Rhizobium leguminosarumS.*:*Serratia marcescens* AM-fungi: Arbuscular mycorrhiza *B.m*:*Bacillus megaterium*.**

Faba bean yield and seed crude protein:

Biological yield, seed yield and seed crude protein of *Rhizobium*-faba bean symbiosis along the two consecutive seasons, as affected by application of rockphosphate and inoculation with PGPRs and AM-fungi are given in Table (7).Results elicited that the response of such yield characters in both seasons parallel to the vegetative growth stage. It is evident that all biofertilization treatments affected faba bean yield and seed crude protein.

In another meaning, when seeds co-inoculated with rhizobia and any of the tested rhizobacteria and/or AM-fungi the faba bean yield and seed crude protein are magnified in the two investigated seasons. However, the splendid effect was observed with the use of inoculation approach (mixture of *Rhizobium*, *Serratia*, *Bacillus* and AM-fungi), which caused promotive impression in all

faba bean yield characters in relative to other combinations or plants inoculated with *Rhizobium*. These positive results could be ascribed to the promotive effects of such biofertilizers, which contains beneficial rhizobacteria, AM-fungi, acted as plant growth promoting by adding some nutrients to the soil, which stimulate root development leading to enhanced vegetative growth as well as improve overall health of plant and consequently the productivity.

Similar results were reported by other investigators (Bisht *et al.,* 2009; Massoud and El-Batanony, 2009;Bhromsiri and Bhromsiri, 2010 and Metwali *et al.,* 2015).They found that the triple inoculation with *Rhizobium*, AM-fungi and associative bacteria, which supported each other, occasionally produces positive effects and develop activities involved in plant growth promotion and plant protection, which in turn profoundly enhanced the productivity of crops.

Table 7. Effect of bacterial inoculation and arbuscular mycorrhizal fungi on biological yield, seed yield and seed crude protein of faba bean plants grown under different rates of rockphosphate in sandy soil

Treatments		Biological yield (kg/fed)		Seed yield (kg/fed)		Seed crude protein (%)	
		2016/2017	2017/2018	2016/2017	2017/2018	2016/2017	2017/2018
Uninoculated (Recom. NPK)		2394.96	2298.32	962.18	966.39	23.09	24.30
Rhizobium (*Rh.*)	15-P	2004.20	2042.02	886.55	882.35	22.60	23.60
	30-P	2142.86	2155.46	907.56	924.37	23.00	23.70
	45-P	2197.48	2184.87	920.17	936.97	23.10	23.90
Rh. + *Serratia* (S.)	15-P	2121.85	2142.86	1004.20	1029.41	24.00	24.00
	30-P	2462.18	2394.96	1029.41	1053.62	24.10	24.70
	45-P	2554.62	2726.89	1058.82	1092.44	24.37	24.75
Rh. + (AM-fungi)	15-P	2235.29	2184.87	1054.62	1130.25	23.68	23.90
	30-P	2264.71	2378.15	1084.03	1134.45	24.00	24.40
	45-P	2478.99	2521.01	1239.50	1302.52	24.00	24.47
Rh. + *Bacillus* (*B.m*)	15-P	2079.83	2142.86	957.98	1012.61	23.90	24.50
	30-P	2243.70	2352.94	966.39	1050.42	24.08	24.50
	45-P	2352.94	2453.78	1016.81	1055.82	24.40	24.71
Rh. + *S.* + (AM-fungi) + *B.m*	15-P	2113.45	2310.92	1180.67	1176.47	24.90	25.90
	30-P	2592.14	2752.10	1301.68	1347.90	25.45	26.00
	45-P	2700.69	2817.14	1407.56	1470.59	25.90	26.26
LSD at 5%		119.750	79.410	185.760	211.540	0.451	0.407

Uninoculated treatment received 40 kg N/fed, 30 kg P_2O_5/fed (as superphosphate 15% P_2O_5) and 24 kg K_2O/fed.
All inoculated treatments received 20 kg N/fed and 24 kg K_2O/fed.
Rockphosphate (25% P_2O_5) was used as a source of phosphorus levels (15, 30 and 45 kg P_2O_5/fed) for all inoculated treatments.
Rh.*:*Rhizobium leguminosarumS.*:*Serratia marcescens* AM-fungi: Arbuscular mycorrhiza *B.m*:*Bacillus megaterium

Irrespective of inoculation, data recorded in Table (7) show clearly that phosphate levels affected faba bean yield and seed crude protein. Meanwhile, results evident that the synergy of using higher doses of phosphorus (30 and 45 kg P_2O_5/fed), relative to the plants treated with 15 kg P_2O_5/fed. The positive effect of phosphorus fertilization might be due to its favorable effects in stimulating nodulation and the growth of the root system, consequently increasing the efficiency of the roots in absorbing various nutrients enhance the vegetative growth and necessary for building proteins and other compounds as well as has a measurable impact on crop quality and yield. Indeed, the soil P deficiency is one of the most significant abiotic factors limiting crop productivity. These results stand in accordance with those obtained by Abd Alla (2002), Ahmed and El-Abagy (2007), El-Habbasha *et al*. (2007)&Nik farjam and Aminpanah (2015).They found a positive linear response of faba bean yield to phosphorus fertilization due to its role in enhancing metabolic processes. Byan and El-Shimi (2014),Fouda (2017) and Mouradi *et al.* (2018)added that legumes respond well to rockphosphate, which reflect on strong root systems, increased nodulation, and good growth; report less fungal problems as well as increased crop production and protein of faba bean seeds.

Data regarding faba bean yield (estimated by biological yield and seed yield) and seed quality (estimated by seed crude protein) showed a significant interaction between natural phosphate levels and biofertilization treatments (Table 7). Data confirmed the superiority of mixture inoculation treatment combined with adding 30 or 45 kg P_2O_5/fed, which surpassed the other tested treatments. Such treatments recorded the greatest biological yield (2592.14 and 2700.69 kg/fed) and (2752.10 and 2817.14 kg/fed) and those of seed yield (1301.68 and 1407.56 kg/fed) and (1347.90 and 1470.59 kg/fed), while seed crude protein recorded (25.45 and 25.90%) and (26.00 and 26.26%) in both seasons, respectively. Other combination treatments and the recommended treatment came at the second rank. In this context, co-inoculation with *Rhizobium* and PGPRs or AM-fungi combined with 45 kg P_2O_5/fed or mixture inoculation treatment (*Rhizobium* conjugated mixture of PGPR's and AM-fungi) combined with 30 or 45 kg P_2O_5/fed caused a significant increase in all faba bean yield characters over the single inoculation with *Rhizobium* fertilized with 45 kg P_2O_5/fed. Such interaction treatments caused an increase in biological yield kg/fed ranged from 7.07 to 22.90 % and from 12.31 to 28.94 %, while seed yield kg/fed ranged from 10.50 to 52.97% and from 12.68 to 56.95%, and those of seed

crude protein ranged from 3.90 to 12.12% and from 2.38 to 9.87% in both seasons, respectively. In fact, PGPRs and AM-fungi have been shown to greatly improve the productivity and quality of many legumes, when they co-inoculated with rhizobia. Hence, the promotive effect of *Rhizobium* might be magnified by the presence of rhizobacteria, AM-fungi and applied rockphosphate, which they act to enhance pods weight plant^{-1} resulting from a number of pods plant^{-1} as well as the plant growth promoters could stimulate plant growth, absorption of nutrients and their efficiency, the metabolism of photosynthates and bio-protection against phytopathogens.

Artursson *et al.* (2006), Ahmed and El-Abagy (2007), El-Habbasha *et al.* (2007),Bisht *et al.* (2009), Miransari (2013) & Rakha and El-Said (2013) confirmed the above results. They reported that seed inoculation with nitrogen fixers and phosphate solubilizing microorganisms combined with phosphate fertilizer caused the synergistic effect of alleviating the adverse effects of soil stresses on plant growth, produce plant hormones, increase the solubility of different nutrients by producing different enzymes as well as interact with AM fungi and improve the productivity and quality of many legumes.

In conclusion, there is considerable evidence that the bacterization of faba bean seeds with *Rhizobium* combined with a mixture of rhizobacteria exerted considerable improvement in nodulation status, all growth aspects and yield characters, particularly when this practice supported by effective AM-fungi. Moreover, the combination between such biofertilization approach and natural rockphosphate (30 kg P_2O_5/fed) may be acting as a good practice for improving the most growth and yield characters and leading to healthier food, particularly under sustainable agricultural systems. Further studies are necessary for more data that are conclusive by using different faba bean varieties in multi-locations under different soil conditions to confirm the results for successful large-scale use and to reach the level of recommendation.

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استجابة العلاقة التكافلية للفول البلدى والريزوبيوم للتلقيح بالرايزويكتيريا وفطر الميكوريزا تحت مستويات متدرجة من الصخر الفوسفاتى الطبيعى فى الأراضى الرملية

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أجريت تجربتين حقليتين بمزرعة معهد الدراسات والبحوث البيئية (ESRI) ،جامعة مدينة السادات،مصر،خلال الموسمين الشتويين ٢٠١٧/٢٠١٦ و٢٠١٨/٢٠١٧، باستخدام نظام الريب التنقيط. كان الهدف من هذه الدراسة هو تقييم استجابة العلاقة التكافلية للفول البلدى والريزوبيوم للتلقيح بالرايزبكتيريا (سراتيا مارسينس وباسيلس ميجاتيريم) وفطر الميكوريزا تحت مستويات متدرجة من الصخرالفوسفاتي الطبيعي (١٥ ، ٣٠ ، ٤٥ كجم فو٢أ٥/فدان) تحت ظروف الأراضى الرملية. وقد كان تصميم التجربة هى قطاعات كاملة العشوائية مع وجود أربع مكررات. وتم تقديرالأعداد والاوزان الجافة للعقد الجذرية،نشاط أنزيم النيتروجينيز، ارتفاع النباتات،الوزن الجاف للمجموع الخضرى ومحتواه من النيتروجين والفوسفور والبوتاسيوم، وكذلك عدد القرون لكل نبات وعدد البذور لكل قرن و وزن الـ١٠٠ بذرة،المحصول البيولوجى،محصول البذور و نسبة البروتين الخام للبذور. وكانت الدراسة معنية أيضًا بتأثيرات اللقاحات الميكروبية والصخر الفوسفاتى على نشاط انزيم الديهيدروجينيز والفوسفاتيز لريزوسفير التربة.أظهرت النتائج أن النباتات الغيرملقحة اعطت اقل قيم لحالة التعقيد (الأعداد والأوزان الجافة للعقد الجذرية)ونشاط أنزيم النيتروجينيز.في حين أدى تلقيح بذورالفول البلدى بالريزوبيوم الى تحسن كبيرفي حالة التعقيد ونشاط انزيم النيتروجينيز. كما أظهرت الخصائص الميكروبيولوجية لريزوسفير التربة،والتي يعبرعنها بنشاط إنزيم الديهيدروجينيز و نشاط الفوسفاتيز،استجابةعالية للقاحات الحيوية المضافة. وقد لوحظ التأثير الجيد والفعال لاستخدام معاملة التلقيح المشتركة (خليط من الريزوبيوموالسراتيا والباسيلس فى وجود فطر الميكوريزا)، والتي أدت الى تشجيع وتحسين حالة التعقيد ،زيادة معدل أختزال الأستيلين بواسطة العقد الجذرية ونشاط إنزيمات ريزوسفير التربة وجميع القياسات الخضرية والمحصول وبعض مكوناته وذلك مقارنة بمعاملات التلقيح المشتركة الأخرى او بالنباتات الملقحة بالريزوبيوم فقط .بصرف النظرعن التلقيح ،كان هناك زيادة في حالة التعقيد ،وكل قياسات المرحلة الخضرية ،ونشاط إنزيمات ريزوسفير التربة وجميع قياسات المحصول مع زيادة معدل اضافة صخر الفوسفات الطبيعى من ٦٠ الى ١٨٠ كجم/فدان (من ١٥ الى ٤٥ كجم فو٢أ٥/فدان). وبالتالي،أكدت النتائج تفوق معاملة التلقيح المشتركة (مزيج من الريزوبيوم والرايزوبكتيريا فى وجود فطر الميكوريزا) مع التسميد بـ٣٠أو٤٥كجم فو٢أ٥/فدان،والتي تفوقت على جميع المعاملات المشتركة الأخرى المختبرة وتسببت في زيادة معنوية في جميع قياسات الفول البلدى المختبرة.بينما جاءت معاملات التلقيح المشتركة بالريزوبيوم مع الرايزوبكتيريا او مع فطر الميكوريزاوالمسمدة بـ ٤٥كجم فو٢أ٥/فدان في المرتبة الثانية .أكدت هذه الدراسة على وجود أدلة كبيرة لأهمية تلقيح بذورالفول البلدى بالريزوبيوم مع خليط الرايزوبكتيريا (سراتيا مارسينس وباسيلس ميجاتيريم) والتى أدت الى زيادة كفاءة عملية التلقيح, وهذا أنعكس على تحسين حالة التعقيد وعملية تثبيت الأزوت الجوى والنمو وانتاجية الفول البلدى تحت ظروف التربة الرملية،لاسيماعندما تكون هذه الممارسة مدعومة بفطر الميكوريزا الفعال.علاوة على ذلك، فأن الجمع بين نهج التسميد الحيوي واستخدام الصخرالفوسفاتى الطبيعى بمثابة عمل جيد لتحسين النمو والأنتاجية وتؤدي الى الحصول على غذاء صحى وأمن، لا سيما في ظل النظم الزراعية المستدامة. ومع ذلك، فإن هذه النتائج هي في حاجة لتكرارها باستخدام اصناف مختلفة من الفول البلدى في مواقع متعددة وتحت ظروف الأراضي المختلفة حتى يمكن التوصية باستخدامها.