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Effects of Co-Inoculation of *Rhizobium* and Plant Growth-Promoting Rhizobacteria on Common Bean (*Phaseolus vulgaris*) Yield, Nodulation, Nutrient Uptake, and Microbial Activity under Field Conditions

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Abstract: Rhizobacteria are promising as biotechnological methods to improve soil fertility and legume production under field conditions. A field experiment, organized in a randomized complete block design with three replications, was conducted to evaluate the effects of inoculation with plant growth-promoting rhizobacteria (PGPR) in combination with *Rhizobium* on nodulation, growth, yield components, nutrient and protein content of common bean (*Phaseolus vulgaris* cv. Nebraska) as a model legume plant. The effect of co-inoculation of *Rhizobium* and PGPR on soil microbial activity and availability of soil N, P, and Fe was also investigated. Three PGPR strains (*Micrococcus agilis* KMe7, *Serratia marcescens* BM1 and *Pseudomonas fluorescens* TW2) were selected based on their ability to produce indole acetic acid and siderophores as well solubilization of inorganic phosphate. Generally, the results obtained from this study indicated that the co-inoculation of *Rhizobium* with each of the three tested PGPR significantly increased (P <0.05) the nodulation (nodule number and nodule dry weight), root and shoot dry weight as compared to *Rhizobium*-inoculated alone and uninoculated control plants at 70 days after sowing. The uptake of N, P, K, and Fe in shoot tissues increased significantly in the co-inoculation treatment and plant growth was enhanced as compared with the single inoculation treatment and uninoculated control. Co-inoculation with *Rhizobium* and PGPR significantly increased soil dehydrogenase activity compared with single inoculation. Additionally, co-inoculation of *Rhizobium* and PGPR also had a positive effect on the composition of the rhizosphere microbial populations and significantly increased populations of bacteria, phosphate solubilizing bacteria and siderophores producing bacteria as compared with individual inoculation. The results show that the dual inoculation of PGPR and *Rhizobium* significantly increased common bean yield and yield components (pods per plant, seeds per pod, and 100-seed weight as well as straw, seed, and biological yields) compared with single inoculation and uninoculated control. Results showed that the best co-inoculation treatment was *Rhizobium* + *Pseudomonas fluorescens* with relative increase of seed, straw, and biological yield reaching 19.6, 35.4 and 22.2%, respectively, over the *Rhizobium* alone treatment. Finally, co-inoculation significantly increased the percentage of protein in common bean seeds as compared with single inoculation or uninoculated control. These results indicate that co-inoculation with PGPR and *Rhizobium* had a positive effect on common bean nodulation, growth, soil nutrients status, nutrients uptake, and yield. The present study concluded that PGPR can be used to promote legume plants in Egypt and to increase soil fertility when it is inoculated with symbiotic N_2-fixing bacteria.

Keywords: *Rhizobium*; PGPR; nutrient uptake; soil fertility; *Phaseolus vulgaris*; Dehydrogenase

INTRODUCTION

The new green revolution, also known bio-revolution, aims to maintain increased crop yields by reducing the amounts of chemical fertilizers inputs and replacing them with microbial inoculants (Besset-Manzoni *et al.,* 2018). The symbiosis between legume and rhizobia is one of the best relationships between crops and phytomicrobiome. In particularly in Egypt, legumes are primary source of protein in vegetarian diets, hence it is important to increase their yields and maintain soil fertility using microbial inoculants (Stajkovic *et al.,* 2011). Leguminous plants also contain several important species that are used all over the world for food and fodder. They can meet their own nitrogen necessities through nitrogen fixation in symbiosis with soil bacteria collectively known as rhizobia. Many agricultural systems depend on symbiotic relationships between leguminous plants and rhizobia. On leguminous plants, these rhizobia create root nodules and transform atmospheric N_2 into a form that plants can use. Therefore, a key strategy in sustainable agriculture is the use of efficient rhizobial strains as biofertilizers to increase the production of legumes (Babu *et al.,* 2015). However, environmental factors such as low numbers of rhizobia in the soil, high temperature, salinity, low clay content, and increased concentrations of heavy metals and pH conditions that are harmful to the survival of rhizobia in the soil, frequently cause a threat to the production of legumes (Denton *et al.,* 2013).

Common bean (*Phaseolus vulgaris* L.) is among the most important legume crops in Egyptian agriculture and is considered an important edible food legume for direct human consumption in the world. It provides protein (15%) and caloric requirement (30%) to the world's population and represents 50% of the grain legumes consumed worldwide (FAO, 2021). Although this legume has been grown for many years in the Nile delta and Nile valley, little is known about the population diversity of symbiotic N_2-fixing rhizobia specific to this crop (Moawad *et al.,* 1998). According to the statistics of the Egyptian Ministry of Agriculture in 2011, the area devoted for dry seed yield was about 21033 feddans, which is producing a total of about 29634 tons, with an average yield of 1.336 tons fed^{-1} (Moghazy, 2014). However, common bean is

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considered a poor fixer of atmospheric N compared with other crop legumes, and generally responds poorly to inoculation with *Rhizobium* under field conditions. Thus, enhancement of nodulation and N_2-fixation is a major goal in breeding projects of common bean.

To increase the availability of nitrogen in sustainable agriculture production systems, co-inoculation with rhizobia and plant growth-promoting rhizobacteria (PGPR) can be used to enhance symbiotic nitrogen fixation. Several studies, for instance Hungria *et al.* (2013), Sánchez *et al.* (2014), and Kumari *et al.* (2020), have demonstrated that co-inoculation of PGPR and rhizobia enhance nodulation, nitrogen fixation, and yield of several legumes, including common bean. Recently, the combined inoculation of plant growth promoting strains to improve the nodulation and nitrogen fixing potential of the inoculated rhizobial strains has received considerable attention. In this regard, under greenhouse conditions using unsterile soil, Mishra *et al.* (2011) found that co-inoculation of rhizobacteria (*Pseudomonas* spp., PGERs17 and NARs1) with *R. leguminosarum*-PR1 significantly improved the number of nodules, fresh weight, leghaemoglobin, chlorophyll content, physiologically available Fe content, and nutrients uptake (N, P, K, Zn, and Fe) in lentil over *R. leguminosarum*-PR1 alone and uninoculated control.

PGPR is very important soil bacteria for increasing the availability of nutrients in plant rhizosphere. They have a high capacity to fix nitrogen (N), sequester iron (Fe), solubilize inorganic phosphate (P), and generate 1-aminocyclopropane 1-carboxylate (ACC) deaminase. Additionally, they directly promote plant growth by altering effect the level of plant hormones (indole acetic acid, indole acetamide, gibberellic acid and cytokinins). It has been reported that 'helper' PGPRs enhance numerous legume-rhizobia symbioses (Vessey, 2003). PGPR have also been shown to enhance the production of flavonoid-like compounds in roots of several legumes and/or stimulating the host legume to produce more flavonoid signal molecules (Bai *et al.,* 2003). However, most of the research has been conducted in the controlled conditions laboratory and in the greenhouse. More studies are required to evaluate the applicability of these findings in the field and to improve co-inoculation techniques for application in agricultural activities. Therefore, the general hypothesis for this study was that the inoculation of legumes in combination with other PGPR strains such as *Micrococcus agilis* and *Serratia marcescens* will led to an increase nodulation, N_2-fixation, nutrient uptake, and plant biomass as compared to inoculation with *Rhizobium* alone. This study evaluated how three local PGPR strains combined with *Rhizobium* affected nodulation, yield, yield components and nutrient uptake under field conditions for common bean. The effect of co-inoculation on soil microbial activity was also investigated to monitor changes in soil quality under different treatments.

MATERIALS AND METHODS

Rhizobacterial strains and their characteristics:

Three rhizobacterial strains were used in this study, *Micrococcus agilis* KMe7, *Pseudomonas fluorescens* TW2, and *Serratia marcescens* BM1. The first two strains were isolated from the rhizosphere of melon and wheat grown in Ismailia, Egypt, whereas *Serratia marcescens* BM1 was isolated from the rhizosphere of maize grown in a highly saline soil (EC 18.9 dS m^{-1}) in Port-Said, Egypt. These rhizobacterial strains were selected based on their previously demonstrated ability to produce indole acetic acid (IAA) and siderophores, and to solubilize tricalcium phosphate as inorganic insoluble phosphate (Abd El-Azeem *et al.,* 2007a). Briefly, *Pseudomonas fluorescens* TW2 had the highest P solubilization activity (109.6 mg P l^{-1}) of all strains, and *Serratia marcescens* BM1 demonstrated the highest IAA production in the presence of L-tryptophan (25.4 mg l^{-1}). All tested strains had ability to produce siderophores except *Serratia marcescens* BM1. The single inoculation with tested rhizobacteria (*Micrococcus agilis* KMe7, *Serratia marcescens* BM1 and *Pseudomonas fluorescens* TW2) significantly increased seed yield of faba bean by 23.5, 24.8 and 26.8% under greenhouse pot experiment in a previous study (Abd El-Azeem *et al.,* 2007b). *Rhizobium leguminosarum* strain was obtained from Microbiology Department, Agricultural Research Center, Cairo, Egypt.

Bacterial strains preparation and seed inoculation:

The cultural medium used to grow the rhizobacterial strains was tryptic soy broth (composed of Tryptone, 15g; Soybean peptone (Soytone), 5g; NaCl, 5g; Distilled water, 1000 ml) (Starr *et al.,* 1981). The strains were incubated at 28 °C for 3-5 days and the viable counts were recorded. *Rhizobium* strain was cultivated in yeast extract mannitol broth medium (composed of mannitol, 10g; K_2HPO_4 0.5g; $MgSO_4.H_2O$ 0.2g; NaCl 0.1g; yeast extract, 0.5g; calcium carbonate 0.01g; distilled water, 1000 mL; pH 7.0). For rhizobial growth, the strain was incubated at 28°C for 3 days and the viable counts were recorded (Weaver *et al.,* 1994). Before sowing, common bean seeds were surface sterilized with ethanol (70%) for 30 s, then by solution (2%) for 3 min, and residues were removed by washing seeds with sterilized distilled water. Before cultivation, the appropriate quantity of seeds was mixed with liquid bacterial suspension and Arabic gum (1%). Seeds were dried in the shade and then manually sown. The inoculation dose was composed of 10^8 colony forming unit (cfu) $seed^{-1}$; therefore, the co-inoculated treatments received a 10^8 cfu $seed^{-1}$ of each strain.

Experimental design and treatments:

A field experiment was conducted at the Experimental Farm of the Faculty of Agriculture, Suez Canal University, Ismailia, Egypt using common beans (*Phaseolus vulgaris cv. Nebraska*) as model legume plant. The sowing date was 15th February during 2017 season. Seeds of common bean were obtained from the

Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. The experiment was composed of four treatments as follows: 1) uninoculated control; 2) Inoculation with *Rhizobium* alone (R); 3) co-inoculation with R and *Micrococcus agilis* KMe7 (R+M); 4) co-inoculation with R and *Serratia marcescens* BM1; and 5) co-inoculation with R and *Pseudomonas fluorescens* TW2. All treatments (the uninoculated control or the inoculated or co-inoculated treatments) did not receive any dose of mineral fertilizers. The experimental design was organized as randomized complete block design (RCBD) with three replicates giving 15 experimental units. Plot dimensions were 5m × 5m and each plot contained 3 rows of 5m length and 70cm width. Two inoculated seeds were cultivated in each hole on one side of the ridge with a 25cm distance between holes and 60cm for row spacing, then the plants were thinned to one after three weeks. Before cultivation, three soil samples were collected from the experiment site to analyze physical and chemical properties. Before being analyzed, the soil samples were air-dried, crushed, sieved (2mm) and analyzed for some selected physical and chemical properties (Gee and Bauder, 1986; Sparks *et al.,* 1996), the average values of soil properties are shown in Table (1).

Soil, plant sampling and analysis:

Plant samples were randomly collected per plot at flowering stage of common bean (after 70 days from cultivation), to evaluate some growth parameters (shoot and root dry weight) and nodulation (number and dry weight of nodules per plant). The total N, P, K and Fe were evaluated in shoots. The plant harvest was also performed at maturity stage by harvesting a central area of each plot. The number of pods per plant, number of seed per pod, seed and straw yields, biological yield and 100-seed weight were recorded. Harvest index (%) was also calculated by 100 × (economic yield/biological yield), where economic yield (kg ha^{-1}) equal seed yield only (kg ha^{-1}).

In the laboratory, shoots were separated from roots, and the roots were washed carefully, and nodules were removed from roots and dried for nodulation evaluation. Shoot and root were oven-dried at 70 °C for 3 days and their designated weight was recorded. The content of N, P, K and Fe in the shoot were evaluated by digestion method (Jackson, 1973). The total N content in seeds was determined, and the percentage of protein was calculated.

Soil samples were collected from each plot for the determination of available N, P, and Fe. Available N was extracted using KCl (2M) and was determined using the Kjeldahl method (Bremner, 1996) Available P was extracted using sodium bicarbonate solution (0.5M, pH adjusted to 8.5). The P in the extraction solution was measured spectrophotometrically using the molybdenum-blue method (Jackson, 1973). Available Fe was extracted using diethylenetriaminepentaacetic acid (DTPA) solution and DTPA-extractable Fe was measured by an atomic absorption spectrophotometer (Lindsay and Norvell, 1978). Soil pH was measured electrometrically using calibrated glass electrode.

Table (1): Some selected physical and chemical properties of the soil used in this study

Property	Unit	Value
Sand	%	71.6
Silt	%	10.3
Clay	%	18.1
Textural class	-	Sandy loam
$CaCO_3$	g kg^{-1}	41.2
pH†	-	7.76
EC_e‡	dSm^{-1}	1.59
Soluble cations‡:		
Ca^{2+}	meq l^{-1}	7.52
Mg^{2+}	meq l^{-1}	3.82
Na^{+}	meq l^{-1}	3.71
K^{+}	meq l^{-1}	0.85
Soluble anions‡:		
HCO_3^{-}	meq l^{-1}	1.0
Cl^{-}	meq l^{-1}	3.8
SO_4^{-2}	meq l^{-1}	11.1
Available P	mg kg^{-1}	8.90
Available N	mg kg^{-1}	11.9
Available Fe	mg kg^{-1}	4.39
Total N	g kg^{-1}	0.54
Organic carbon	g kg^{-1}	10.7

† In soil water suspension (1:2.5)
‡ In soil saturated paste

Determination of soil microbial activity:

To evaluate the effects of co-inoculation of tested rhizobacterial strains and *Rhizobium* on soil microbial activities, rhizosphere samples were collected at flowering stage (after 70 days from cultivation). Total count of bacteria, siderophore producers and phosphate-solubilizing bacteria were evaluated. Specifically, the numbers of total bacteria were counted on tryptic soy agar using agar plate dilution method (Pepper and Gerba, 2009). Phosphate-solubilizing bacteria were counted on a National Botanical Research Institute phosphate agar medium (NBRIP): (glucose 10g, $Ca_3(PO_4)_2$ 5g, $MgCl.H_2O$ 5g, $MgSO4.7H_2O$ 0.25g, KCl 0.2g, $(NH_4)_2SO_4$ 0.1g, Agar 15g and distilled water 1000 mL (Nautiyal, 1999). The colonies that formed a halo clear zone around it were counted as an indication of the capability of these bacteria for phosphate solubilization. Siderophore producing bacteria were counted using Chrome Azurol S (CAS) agar medium that contained the blue dye complex of CAS, Fe^{3+} and HDTMA (Alexander and Zuberer, 1991). The glassware was rinsed with 6 N HCl for 24 h then washed with de-ionized water to get rid of iron contamination because of the production of siderophores occur under iron deficient conditions.

The activity of dehydrogenase enzyme was measured colorimetrically using an UV-visible spectrophotometer according to the method described by Tabatabai (1994). In brief, one gram of soil was

mixed with 1-mL Tris buffer, 50-µL glucose solution (10g L^{-1}), and 0.2-mL 2,3,5-triphenyltetrazolium chloride, followed by a 24 h incubation at 37 °C. Then, the soil was extracted with 10-mL methanol and the triphenyl formazan concentration in this extract was determined using an UV spectrophotometer at a wavelength of 485 nm.

Statistical analysis:

The studied variables were subjected to analysis of variance (ANOVA), using s significant of $P < 0.05$. Means of treatment were compared using Duncan's Multiple Range Test as Post Hoc significant test (SPSS 26.0 for windows (Lead Technology, Inc)). Bivariate analysis between studied variables was analyzed using Pearson correlation.

RESULTS AND DISCUSSION

Nodulation and plant growth:

Generally, the results in this study revealed that the tested plant growth promoting rhizobacterial strains did not have any negative effects on common bean plant growth (root and shoot dry weights) and nodulation. Table 2 shows that co-inoculation of the rhizobia strain with each of three tested PGPR significantly increased (P <0.05) the nodulation (nodule number and nodule dry weight) of common bean compared to those inoculated only with rhizobia strain and uninoculated control plants under field conditions. Surprisingly, the co-inoculation with *Pseudomonas fluorescens* or *Serratia marcescens* with rhizobia strain significantly increased both nodule number (NN) and nodule dry weight (NDW) compared to *Rhizobium* alone and uninoculated control plants. However, co-inoculation of rhizobia strain with *Micrococcus agilis* increased NN and NDW but these increases were not significant. These findings indicated that different PGPR vary in their ability to increase nodulation when co-inoculated with the rhizobia strain. The highest values of NN (28.3 plant^{-1}) and NDW (64.3 mg plant^{-1}) were observed in plants that were inoculated with *Pseudomonas fluorescens* and rhizobia strain (Table 2). These results are in accordance with those obtained by Bai *et al.* (2003), Sivaramaiah *et al.* (2007), Rajendran *et al.* (2008), Kumar *et al.* (2016) and Leite *et al.* (2022). In this respect, Pastor-Bueis *et al.* (2021) reported that the co-inoculation with *Rhizobium leguminosarum* bv. *Phaseoli* and *Pseudomonas brassicacearum* subsp. *Neoaurantiaca* enhances nodulation and nodule functions as compared with *Rhizobium* alone. Specifically, the co-inoculation increased tendency for enhanced nodule biomass by 25% in average and N-fixed by more than 20%. Stajkovic *et al.* (2011) observed the existence of a positive interaction between *Rhizobium* and *Pseudomonas* sp. or *Bacillus* sp. under *in vitro* and *in vivo* conditions. Figueiredo *et al.* (2008) reported that co-inoculation of *R. tropici* and *P. polymyxa* improved nodulation and nitrogen fixation in common bean. Moreover, the production of indole acetic acid by three tested PGPR has been reported to promote the secretion by the crop's roots of nod-gene-inducing flavonoids, enhancing nodulation by rhizobia (Pastor-Bueis *et al.,* 2021). In addition, PGPR that have activity of ACC-deaminase can also enhance the nodulation by rhizobia, this may be attributed to the decreasing of the endogenous level of ethylene's precursor and resulting reduction of ethylene in the plant roots. Ethylene prevents the early stages of nodulation by controlling the threshold concentration of Nod factor necessity for initiation of nodule (Subramanian *et al.,* 2015).

Co-inoculation of rhizobia strain and tested PGPR recorded higher root, shoot dry weights and whole plant as compared to single inoculation with rhizobia strain and uninoculated control plants (Table 2). However, this was dependent on the specific *rhizobium*-PGPR combination. In this regard, co-inoculation with *Rhizobium* plus *Pseudomonas fluorescens* recorded a significantly (P <0.05) higher root dry weight (2.48 g plant^{-1}), shoot dry weight (16.7 g plant^{-1}) and whole plant (19.2 g plant^{-1}) compared to inoculation with *Rhizobium* alone (1.22, 10.3 and 11.5 g plant^{-1}, respectively). However, there was no significant difference between *Rhizobium* + *Micrococcus agilis* or *Rhizobium* + *Serratia marcescens* and rhizobia alone for root dry weight, whereas there was no significant difference between *Micrococcus agilis* and *rhizobium* alone for shoot dry weight and whole plant. Co-inoculation had a better performance of common bean growth under field conditions. There is some evidence that these positive responses of common bean growth to three rhizobacterial strains inoculation could partially be attributed to the strains exhibit several plant growth-promoting properties. These characteristics include the synthesis of indole acetic acid, siderophores and insoluble phosphates solubilization. *Pseudomonas fluorescens* that proved to give the highest of root and shoot dry weight, it has the three above-mentioned traits (Abd El-Azeem *et al.,* 2007a). The interaction between beneficial bacteria and legume plants is known to be influenced by the presence of PGPR in the rhizosphere that is known to promote shoot and root growth, root hair development, plant hormone regulation, nitrogen fixation, the solubilization of minerals and the suppression of pathogens, as well as root colonization (Babu *et al.,* 2015). Indole acetic acid is crucial for plant growth and development. It is recognized to enhance plant cell elongation, cell division, and tissue differentiation. In this regard, Hungria *et al.* (2013) reported that PGPR mechanisms (hormone modulation) is a vital factor for increasing root growth and productivity because of positive effects of the co-inoculation of *Rhizobium tropici* and *Azospirillum brasilense*.

Table (2): Effect of co-inoculation of three plant growth promoting rhizobacterial strains with *Rhizobium* on nodulation and some growth parameters of field-grown common bean at flowering stage (after 70 days from cultivation)

Treatment	Nodule number plant^{-1}	Nodule weight mg plant^{-1}	Root weight g plant^{-1}	Shoot weight g plant^{-1}	Whole plant g plant^{-1}
Uninoculated control	10.8±0.67^{a}	30.3±0.9^{a}	0.60±0.08^{a}	6.06±0.38^{a}	6.66±0.41^{a}
***Rhizobium* alone**	14.3±0.33^{b}	42.3±3.3^{b}	1.22±0.05^{b}	10.3±0.54^{b}	11.5±0.59^{b}
Rhizobium* + *Micrococcus agilis	17.0±0.88^{b}	48.0±1.2^{b}	1.49±0.07^{b}	11.6±0.29^{bc}	13.1±0.36^{bc}
Rhizobium* + *Serratia marcescens	23.7±0.58^{c}	60.7±1.2^{c}	1.70±0.23^{b}	12.4±0.2^{c}	14.1±0.11^{c}
Rhizobium* + *Pseudomonas fluorescens	28.3±1.20^{d}	64.3±2.7^{c}	2.48±0.23^{c}	16.7±0.75^{d}	19.2±0.98^{d}

Notes: Values are the means of three replicates ± standard error. Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple-range test.

Shoot N, P, K and Fe content:

Co-inoculation of common bean with rhizobia strain and tested PGPR was found to positively influence shoot N, P, K and Fe contents (Table 3). Regarding shoot N content, seeds bacterized with *Rhizobium* and *Pseudomonas fluorescens* significantly increased shoot N content as compared to single inoculation with *Rhizobium* and uninoculated control. This treatment was the best mixed inoculation compared to other PGPR strains and gave the highest shoot N content (593 mg plant^{-1}). However, the co-inoculation with *Rhizobium* and *Micrococcus agilis or Serratia marcescens* led to a significant increase of shoot N content as compared to *Rhizobium* alone, and no significant difference were observed between these treatments. As expected, the inoculation with *Rhizobium* alone also significantly increased Shoot N content as compared to uninoculated control.

For shoot P content, the combined inoculation with rhizobia strain with *Serratia marcescens or Pseudomonas fluorescens* significantly increased shoot P content as compared to single inoculation with *Rhizobium* or uninoculated control. However, the co-inoculation of *Rhizobium* and *Micrococcus agilis* increased shoot P content but this increase was not significant when compared with *Rhizobium* alone. Overall, the inoculation either single or combination with tested bacteria significantly increased shoot P content as compared to uninoculated control plants. The maximum shoot P content (41.3 mg plant^{-1}) was obtained when seeds were bacterized with *Rhizobium* and *Pseudomonas fluorescens,* and the relative increase was 72.8% compared to *Rhizobium* alone.

The trend of results for shoot K and Fe contents was similar with the different inoculation treatments. For instance, the combined inoculation with *Rhizobium* and *Serratia marcescens or Pseudomonas fluorescens* significantly increased shoot K and Fe contents as compared to *Rhizobium* alone or uninoculated control plants. No significant difference was observed in shoot K or Fe content when seeds were bacterized with *Serratia marcescens* or *Micrococcus agilis* with *Rhizobium*. These findings indicated that the type of PGPR strain was a limited factor in this result. The maximum values were 325 and 2.65 mg plant^{-1} for shoot K and Fe, respectively, when seeds were bacterized with mixed inoculation (*Rhizobium* plus *Pseudomonas fluorescens*).

The results in this study indicated that the combined inoculation with each of the three PGPR and *Rhizobium* highly enhanced the uptake of N, P, K, and Fe in common bean shoots. These findings are supported by the fact that these strains exhibited multiple growth-promoting traits, mainly auxin production, which enhanced root growth and increased nutrient uptake from the soil (Rolfe *et al.,* 1997; Abd El-Azeem *et al.,* 2007a). In addition, these PGPR strains increased the availability of P in the rhizosphere of common bean, because of ability of these strains for P solubilization (Abd El-Azeem *et al.*, 2007a). Another explanation is that PGPR strains enhanced microbial activity in the rhizosphere, especially bacteria that produce siderophores, which regulate Fe availability in the rhizosphere. In such circumstances siderophores producing bacteria can provide Fe to plants under Fe stress or Fe limitation. There has been earlier research that suggests plant roots have receptors or channels that can uptake ferric (ferric-siderophores complexes) in their rhizosphere. Plant ferric reductase is also responsible for converting ferric to ferrous form inside the plant. For instance, the potential of siderophores-producing bacteria to improve Fe availability to plants was also reported by some workers (Bar-Ness *et al.,* 1992; Rroco *et al.,* 2003; Sharma *et al.,* 2003). Positive correlation coefficients were observed between total bacterial populations and shoot N ($r = 0.899^{**}$), P ($r = 0.0.874^{**}$), K ($r = 0.888^{**}$), and Fe ($r = 0.911^{**}$) contents. In this study, the positive correlations were observed between the availability of nutrients in soil and the uptake these nutrients by common bean tissues. Additionally, positive correlations were observed between available N, P and Fe in the soil and the shoot's N, P and Fe contents, with correlation values of 0.909^{**}, 0.858^{**}, and 0.892^{**}, respectively.

Similar results were obtained by Stajkovic *et al.* (2011), who observed that the co-inoculation with *Pseudomonas* sp. or *Bacillus* sp. with *Rhizobium* increased shoot dry weight, N and P contents in

common bean plants. In this context, Mishra *et al.* (2011) suggested that co-inoculation of rhizobacteria and *Rhizobium leguminosarum*-PR1 significantly increased nodule number, fresh weight, leghaemoglobin, physiologically available Fe content and nutrients uptake (N, P, K and Fe) over *Rhizobium leguminosarum*-PR1 alone and uninoculated control. Co-inoculation in this study increased nodulation and this improve may have resulted in increased N content in the shoots. These results are also supported by observed positively person correlation coefficients at (P = 0.01) between nodule number and nodule dry weight and shoot N (r = 0.923** and 0.880**), P (r = 0.892** and 0.951**), K (r = 0.926** and 0.931**) and Fe (r = 0.878** and 0.819**) contents, respectively. In addition, the inoculation with PGPR in this study led to an increase of plant growth (root system) that can be attributed to increased absorption of nutrients from the soil, which subsequently increased the nutrient use efficiency and plant uptake. This result can be confirmed by the presence of positive correlation coefficients at (P = 0.01) between common bean root dry weight and shoot nutrients uptake N, P, K and Fe, with correlation coefficient values of 0.938**, 0.915**,0.915** and 0.952**, respectively.

Table (3): Effect of co-inoculation of three plant growth promoting rhizobacterial strains with *Rhizobium* on shoot N, P, K and Fe content (mg plant^{-1}) of field-grown common bean at flowering stage (after 70 days from cultivation)

Treatment	N	P	K	Fe
Uninoculated control	149±12.6^{a}	12.0±0.69^{a}	122±10.4^{a}	0.86±0.07^{a}
***Rhizobium* alone**	308±18.3^{b}	23.9±0.82^{b}	208±17.0^{b}	1.42±0.15^{b}
Rhizobium* + *Micrococcus agilis	425±8.10^{c}	26.4±2.83^{b}	229±6.23^{bc}	1.58±0.10^{bc}
Rhizobium* + *Serratia marcescens	495±9.80^{c}	34.0±2.60^{c}	259±7.21^{c}	2.01±0.11^{c}
Rhizobium* + *Pseudomonas fluorescens	593±60.3^{d}	41.3±1.66^{d}	325±14.6^{d}	2.65±0.30^{d}

Notes: Values are the means of three replicates ± standard error. Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple-range test.

Soil available nutrients:

Table (4) shows that the inoculation with *Rhizobium* + *Pseudomonas fluorescens* resulted in significant reductions in the soil pH values compared to *Rhizobium* alone or uninoculated control. The obtained pH reduction reached a maximum of 0.32 pH unit in the plants treated with *Rhizobium* + *Pseudomonas fluorescens*. The combined inoculation with Rhizobium and *Micrococcus agilis* or *Serratia marcescens* also led to a decrease in soil pH value, but these decreases were not significant. Moreover, the *Rhizobium* alone significantly decreased soil pH by 0.17 pH unit as compared to uninoculated control plants. This may be related to the ability of these bacterial strains to produce organic acids (*i.e.* lactic, oxalic, citric, succinic, acetic and formic acids) in the rhizosphere. Among their functions are the solubilization and acquisition of essential nutrients such as P as well as rhizospheric bacterial chemotaxis (Macias-Benitez *et al.,* 2020). In addition, organic acids have different abilities to lower soil pH, and their behavior may be explained by differential chemical interactions between them and soil components (Macias-Benitez *et al.,* 2020). According to Nautiyal *et al.* (2000), soil microorganisms that decrease pH during their growth are efficient P solubilizers and increase plant P availability.

Table (4) indicates that co-inoculation of *Rhizobium* and PGPR strains significantly increased the levels of available P and DTPA-extractable Fe in the soil as compared to the single inoculation with *Rhizobium*. However, the differences in levels of P and Fe between combined inoculation and *Rhizobium* alone were not always significant. The highest levels of P and Fe were recorded in the soil treated with *Rhizobium* + *Pseudomonas fluorescens,* and the values reached 14.4 and 7.43 mg kg^{-1}, respectively. The co-inoculation with *Rhizobium* + *Micrococcus agilis or Rhizobium* + *Serratia marcescens* significantly increased the level of available P in the soil, whereas inoculation only with *Rhizobium* + *Micrococcus agilis* resulted in no significant increase of available Fe as compared to *Rhizobium* alone.

A similar significant increase in available N was observed after co-inoculation with *Rhizobium* and each of three PGPR strains, compared to *Rhizobium* alone or uninoculated control (Table 4). Seeds bacterized with *Rhizobium* + *Pseudomonas fluorescens* resulted in the highest amount of available N (18.1 mg kg^{-1}), followed by *Rhizobium* + *Serratia marcescens* (17.0 mg kg^{-1}), then followed by *Rhizobium* + *Micrococcus agilis* (16.8 mg kg^{-1}) compared to *Rhizobium* alone and uninoculated control (15.3 and 12.7 mg kg^{-1}, respectively). Moreover, significant inverse correlation coefficients (P < 0.01) were found between the values of soil pH and the levels of N (r = -0.911**), P (-0.896**) and Fe (-0.869**) in the soil (Table 4). These findings indicated that the availability of these nutrients in the soil depended partially on the changes in soil pH resulted from the inoculation with either *Rhizobium* or PGPR strains.

Table (4): Effect of co-inoculation of three plant growth promoting rhizobacterial strains with *Rhizobium* on soil available N, P, Fe (mg kg^{-1}) and pH values of field-grown common bean at flowering stage (after 70 days from cultivation)

Treatment	pH (1:2.5)	AP	AN	DTPA-Fe
Uninoculated control	7.61±0.007[a]	9.13±1.20[a]	12.7±0.37[a]	4.76±0.53[a]
***Rhizobium* alone**	7.44±0.009[b]	11.4±0.17[b]	15.3±0.19[b]	5.72±0.51[b]
Rhizobium* + *Micrococcus agilis	7.35±0.029[bc]	12.8±0.57[c]	16.8±0.32[c]	6.02±0.54[b]
Rhizobium* + *Serratia marcescens	7.36±0.009[bc]	13.0±0.41[c]	17.0±0.67[cd]	6.52±0.63[c]
Rhizobium* + *Pseudomonas fluorescens	7.29±0.049[c]	14.4±0.14[d]	18.1±0.19[d]	7.43±0.88[d]
r		-0.896**	-0.911**	-0.869**

Notes. Values are the means of three replicates ± standard error. Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple-range test. r; Correlation coefficient between the values of soil pH and levels of available P, N and Fe.
** Correlation is significant at the 0.01 level (2-tailed)

Soil microbial activity:

Table (5) presents a quantitative analysis of the soil microbial community in terms of total bacteria (TB), phosphate solubilizing bacteria (PSB) and siderophores producing bacteria (SPB). Because of its high sensitivity to nutrient and water shortages in the soil, microbial populations are frequently used as an indicator for measuring soil fertility. Co-inoculation of *Rhizobium* and PGPR strains significantly increased the TB, PSB and SPB populations in the rhizosphere of common bean and the significance was proportional to the type of PGPR strain. For instance, TB showed a significant increase in the rhizosphere for plants treated with PGPR strains, TB reached 158.6×10^5, 172.4×10^5, and 310.2×10^5 CFU g^{-1} for mixed inoculation with *Micrococcus agilis* or *Serratia marcescens* or *Pseudomonas fluorescens* and *Rhizobium*, respectively (vs. 89.62×10^5 CFU g^{-1} for *Rhizobium* alone, and 55.15×10^5 CFU g^{-1} for the uninoculated control).

Regarding PSB populations, the combined inoculation of *Rhizobium* and *Pseudomonas fluorescens* significantly increased the population of PSB as compared to *Rhizobium* alone, and the relative increase reached 108%. Similarly, PSB counts was increased with the co-inoculation with *Rhizobium* and *Micrococcus agilis or Serratia marcescens*, however, these increases were not significant when compared to single rhizobia strain. Inoculation with either *Rhizobium* alone or combined with bacterial strains significantly increased the PSB population as compared to uninoculated control plants.

The results revealed that the mixed inoculation with *Serratia marcescens or Pseudomonas fluorescens* with *Rhizobium* led to a significant increase in SPB populations in the rhizosphere of common bean compared to *Rhizobium* alone. The highest populations of SPB reached 17.93×10^4 CFU g^{-1} for combined inoculation of *Pseudomonas fluorescens* + *Rhizobium*, followed by *Rhizobium* + *Serratia marcescens* (15.86×10^4 CFU g^{-1}) compared to single inoculation with *Rhizobium* (8.96×10^4 CFU g^{-1}). However, no significant difference was observed between Rhizobium alone and *Rhizobium* + *Micrococcus agilis.* The results showed that inoculations (*Rhizobium* alone or with each of PGPR strains) significantly enhanced soil biological activities in the rhizosphere of common bean. These findings were consistent with those reported by Ju *et al.* (2020), who found that co-inoculation with PGPR strain (*Paenibacillus mucilaginosus*) and *Rhizobium* strain (*Sinorhizobium meliloti*) improved biological activities by altering soil environmental factors such as pH and nutrients status as well as soil enzyme activity to restore the abundance and diversity of the rhizosphere bacteria.

Table (5): Colony forming unit (CFU) of total bacteria, phosphate solubilizing bacteria and siderophores producing bacteria in the rhizosphere of common bean after inoculation with *Rhizobium* alone and with plant growth promoting rhizobacteria after 70 days from cultivation

Treatments	CFU × 10^5 g^{-1} dry soil		
	Total bacteria	Phosphate solubilizing bacteria	Siderophores producing bacteria
Uninoculated control	55.15[a]	5.515[a]	4.826[a]
***Rhizobium* alone**	89.62[b]	8.962[b]	8.962[b]
Rhizobium* + *Micrococcus agilis	158.6[c]	10.34[b]	11.72[b]
Rhizobium* + *Serratia marcescens	172.4[c]	11.72[b]	15.86[c]
Rhizobium* + *Pseudomonas fluorescens	310.2[d]	18.61[c]	17.93[c]

Values are means of three replications ± S.E
Values followed by the same letters within a column indicate no significant difference at a 0.05 significance level, determined by Duncan's multiple-range test (n = 3)

Similarly with microbial populations, soil enzyme activity are important to assess soil microbial activity, especially intracellular enzyme such as dehydrogenase. Fig. (1) shows that co-inoculation of *Rhizobium* and tested PGPR significantly increased the activity of dehydrogenase in the rhizosphere of common bean as compared to *Rhizobium* alone or uninoculated control plants. The maximum activity of dehydrogenase (6.90 mg TPF kg^{-1} soil h^{-1}) was recorded in *Rhizobium* plus *Pseudomonas fluorescens* treatment as compared to single *Rhizobium* inoculation (4.02 mg TPF kg^{-1} soil h^{-1}). Similarly, the combined inoculation of *Micrococcus agilis* or *Serratia marcescens* with *rhizobium* led to a significant increase of dehydrogenase activity in the rhizosphere as compared to *rhizobium* alone or uninoculated control plants. There was no significant difference between rhizobium alone and co-inoculation of rhizobium and *Micrococcus agilis* (Fig. 1). Moreover, the inoculation with single *Rhizobium* strain also significantly increased the activity of dehydrogenase compared to uninoculated control. From previous results, all three tested PGPR strains led to a significant increase (P <0.05) of dehydrogenase activity when combined with rhizobia strain. These results indicate positive interactions with indigenous microorganisms and inoculated bacteria in the soil rhizosphere. Similar results were reported by Mader *et al.* (2011), who found that the addition of PGPR increased the activity of dehydrogenase and therefore enhanced soil quality. Soil dehydrogenase activity is intracellular enzymes and is thus considered to reflect the total viable microbial population and microbiological activity. It is used as indicator for soil microbial activity and is affected by numerous factors such as soil type and pH. Dehydrogenase activity values in the present study were much greater than those in the control treatment, indicating a considerable increase in viable cells because of inoculation (Babu *et al.,* 2015).

Based on the statistical analysis of the microbial activity data, significant positive correlations were found between the activity of dehydrogenase and total bacterial populations ($r=0.892^{**}$), and populations of phosphate solubilizing bacteria ($r=0.898^{**}$). Conversely, a significant inverse correlation was obtained between dehydrogenase activity and values of soil pH ($r=-0.745^{**}$).

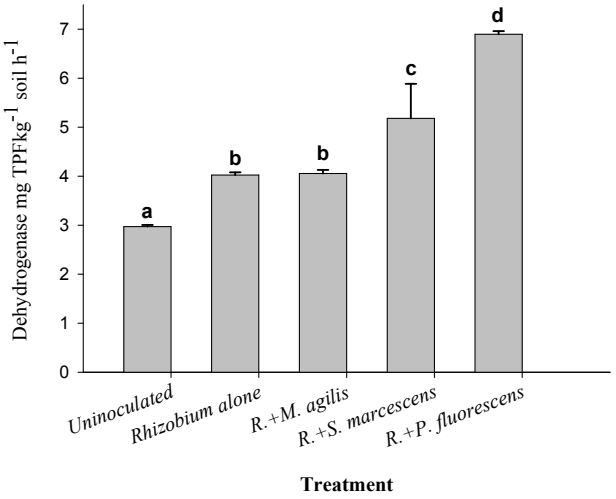

Fig. (1): Effect of co-inoculation of three plant growth promoting rhizobacterial strains with *Rhizobium* on specific activity of dehydrogenase in the rhizosphere of common bean at flowering stage (after 70 days from cultivation)
Error bars represent the standard error of the mean (n = 3)
The same letters in the Figure indicate no significant difference at a 0.05 significance level, determined by Duncan's multiple-range test (n = 3) *M. agilis; Micrococcus agilis, S. marcescens; Serratia marcescens, P. fluorescens; Pseudomonas fluorescens*

Yield, yield components and protein content:

Overall, inoculation with rhizobia strain either alone or in combination with each of three PGPR, significantly increased common bean yield and yield components as compared to single inoculation with *Rhizobium* and uninoculated control. The best co-inoculation treatment was *Rhizobium* + *Pseudomonas fluorescens* that significantly increased number of pods per plant, number of seeds per pod, and 100-seed weight by 80.6, 23.7 and 9.9%, respectively, over *Rhizobium* alone (Table 6). Moreover, the combined inoculation of *Micrococcus agilis or Serratia marcescens* with *Rhizobium* also significantly increased these parameters as compared to *Rhizobium* alone. However, no significant differences were observed between some combined inoculations. When compared with uninoculated control, the *Rhizobium* alone significantly increased number of pods per plant, number of seeds per pod, and 100-seed weight. In this study, the uninoculated control plants deliver some details about the effects of single inoculation with *Rhizobium* and the indigenous *Rhizobium* population. No significant difference between *Rhizobium* alone or in combination with tested PGPR were found for the harvest index.

Results also indicate that, the inoculation with *Rhizobium* and *Micrococcus agilis or Serratia marcescens or Pseudomonas fluorescens* resulted in significant increase in straw and biological yield when compared to the corresponding *Rhizobium* alone and uninoculated control. These findings are in concord with those obtained by Yadegari *et al.* (2008) who investigated the influence of co-inoculation with PGPR (*Pseudomonas fluorescens* P-93 or *Azospirillum lipoferum* S-21) and *Rhizobium* on yield and yield components of common bean (*Phaseolus vulgaris* L.) in a field trial. They concluded that *Rhizobium* and PGPR co-inoculation significantly increased pods per plant, seeds per pod, 100-seed weight, total dry matter, seed yield and protein content. In this regard, in Egypt, Massoud *et al.* (2009) found that mixed inoculation from arbuscular mycorrhizal fungi, symbiotic *Rhizobium* sp., *Azospirillum* sp. and *Bacillus circulans* increased plant height, number of branches, number of nodules per plant, and fresh yield of common beans, when compared with control plants. Additionally, rhizobacterial strain *Pseudomonas fluorescens* was proved to be the best inoculant among the three tested PGPR strains in the field experiment of the present study, which was evident from the increasing of seed, straw, and biological yields. The percentage of increase in seed, straw, and biological yield by microbial inoculants (*Rhizobium* + *Pseudomonas fluorescens*) were found to be 19.6, 35.4 and 22.2%, respectively, over the *Rhizobium* alone. This result agreed with those reported by Yadegari *et al.* (2008), who found that co-inoculation with *Rhizobium* and *Pseudomonas fluorescens* P-93 gave the highest seed yield, number of pods per plant, weight of 100 seed, seed protein yield, number seeds per pod, and seed protein yield. In addition, *Pseudomonas fluorescens* used in this study had not only the ability of the production of indole acetic acid and siderophores, but also enhanced solubilization of inorganic phosphates, leading altogether to exhibit a more important combined effect on the crop yield. Furthermore, several species of *Rhizobium* are known to enhance the growth and yield of several crops through P solubilization (Yasmeen and Bano, 2014).

Fe is required for several living soil microorganisms to grow. Unfortunately, it predominates as an insoluble form (Fe^{3+}) that cannot be assimilated by soil microorganisms. In this study, PGPR strains were found to produce siderophores, facilitating the ferric form of the plant, and leading to higher yields and growth. The results agree with those of Stajkovic *et al.* (2011), who found an increase in *Phaseolus vulgaris* yield and growth when the two siderophore-producing bacteria (*Bacillus* and *Pseudomonas*) were combined with *Rhizobium*.

Table (6): Effect of co-inoculation of three plant growth promoting rhizobacterial strains with *Rhizobium* on yield and yield components of field grown common bean at maturity stage

Treatment	No. of pods plant^{-1}	No. of seeds pod^{-1}	100-seed weight (g)	Seed yield (kg h^{-1})	Straw yield (kg h^{-1})	Biological yield (kg h^{-1})	Harvest index (%)
Uninoculated control	5.41^{a}	2.71^{a}	46.72^{a}	2152.9^{a}	2013.2^{a}	4166.1^{a}	51.7^{a}
***Rhizobium* alone**	6.59^{b}	3.34^{b}	50.23^{b}	3302.3^{b}	2961.9^{b}	5910.1^{b}	53.3^{ab}
Rhizobium* + *Micrococcus agilis	8.32^{c}	4.01^{c}	52.77^{c}	3478.4^{b}	3025.2^{c}	6477.4^{c}	54.22^{ab}
Rhizobium* + *Serratia marcescens	8.43^{c}	4.02^{c}	54.73^{d}	3515.4^{b}	3270.7^{c}	6503.6^{c}	54.70^{b}
Rhizobium* + *Pseudomonas fluorescens	11.9^{d}	4.13^{c}	55.2^{d}	3949.9^{c}	4009.1^{d}	7220.5^{d}	55.78^{b}

Regarding protein content in common bean seeds, the results shown in Fig. (2) indicated that the percentage of protein in seeds increased significantly as the inoculation was dual with *Rhizobium* and PGPR strains. All tested PGPR strains with *Rhizobium* showed high increases of seed protein when compared to uninoculated control. For instance, all PGPR strains led to effective increases in the percentage of protein in the common bean seeds were *Pseudomonas fluorescens*, *Serratia marcescens*, and *Micrococcus agilis* when mixed with *Rhizobium*. No significant difference between tested PGPR strains when mixed with *Rhizobium* were observed. However, the single inoculation with *Rhizobium* significantly increased the protein in seed as compared to uninoculated control plant.

The effect of combined inoculation with *Rhizobium* and PGPR significantly increased common bean yield, yield components and protein content in seeds. These results indicated the potential of using rhizobia and PGPR as inoculants, replacement of expensive and environmentally unfriendly fertilizers. Analysis of Pearson correlation coefficients between yield and yield components data and some parameters measured in the present study showed the impact of these parameters on yield of common bean (Table 7). According to the observed results, the nodulation and root dry weight are the parameters most related to straw yield. Furthermore, positive highly significant correlation coefficients (r) were found between the uptake of N, P, K and Fe and the common bean yield components. Soil microbial activity (in terms of total bacteria and the activity of dehydrogenase) and the availability of nutrients in soil were also highly correlated with yield and yield components. These results indicated that the inoculation of common bean seeds with *Rhizobium* alone or in combination with tested PGPR significantly increased abovementioned parameters. The presence of PGPR strains together with *Rhizobium* increased the abovementioned parameters over *Rhizobium alone*.

Table (7): Pearson linear correlation coefficients between yield and yield components with various parameters determined in this study

Yield components	NN	NW	Root DW	Shoot DW	Shoot nutrients uptake				Availability of nutrients			Dehy	Total Bacteria
					N	P	K	Fe	N	P	Fe		
No. of pods plant^{-1}	0.898**	0.836**	0.948**	0.966**	0.927**	0.885**	0.927**	0.925**	0.833**	0.847**	0.924**	0.895**	0.971**
No. of seeds pod^{-1}	0.781**	0.797**	0.850**	0.814**	0.919**	0.798**	0.807**	0.831**	0.888**	0.880**	0.828**	0.690**	0.795**
100-seed weight (g)	0.906**	0.951**	0.911**	0.865**	0.942**	0.915**	0.923**	0.866**	0.933**	0.942**	0.934**	0.814**	0.833**
Seed yield (kg h^{-1})	0.829**	0.816**	0.750**	0.852**	0.792**	0.795**	0.832**	0.806**	0.838**	0.831**	0.885**	0.772**	0.835**
Straw yield (kg h^{-1})	0.941**	0.904**	0.898**	0.879**	0.913**	0.892**	0.879**	0.900**	0.800**	0.822**	0.886**	0.902**	0.844**
Harvest index (%)	0.474	0.541*	0.401	0.500	0.527*	0.446	0.553*	0.312	0.723**	0.637*	0.497**	0.403	0.473
Protein %	0.849**	0.920**	0.822**	0.864**	0.901**	0.849**	0.897**	0.802**	0.883**	0.902**	0.850**	0.749**	0.783**

**Correlation is significant at the 0.01 level

*Correlation is significant at the 0.05 level

NN: nodule number plant^{-1}, NW: nodule weight (mg plant^{-1}), Root DW: Root dry weight (g plant^{-1}), shoot DW: shoot dry weight (g plant^{-1}), Dehy: dehydrogenase activity (mg TPFkg^{-1} soil h^{-1})

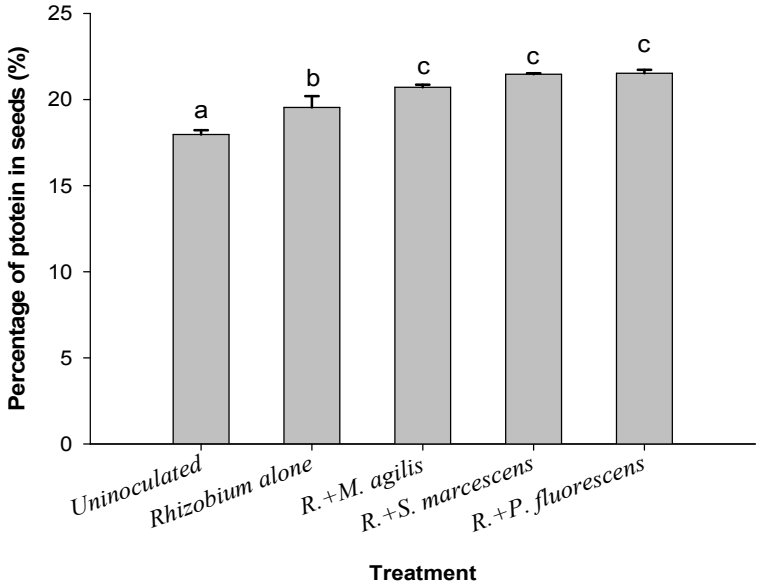

Fig. (2): Effect of co-inoculation of three plant growth promoting rhizobacterial strains with *Rhizobium* on percentage of protein in common bean seeds.

Error bars represent the standard error of the mean (n = 3)

The same letters in the Figure indicate no significant difference at a 0.05 significance level, determined by Duncan's multiple-range test (n = 3) *M. agilis; Micrococcus agilis, S. marcescens; Serratia marcescens, P. fluorescens; Pseudomonas fluorescens*

CONCLUSIONS

This study is an attempt to answer the question whether the addition of plant growth promoting rhizobacteria with *Rhizobium* will increase the growth and production of the common bean plant under the Egyptian soil conditions as compared with *Rhizobium* alone. Therefore, this study evaluated the efficiency of co-inoculation of *Rhizobium* and three PGPR strains on common bean yield, yield components, nodulation, nutrients uptake and soil available nutrients compared to single inoculation with *Rhizobium*. This study also examined the changes of some microbiological and biochemical properties in the rhizosphere of common bean treated with single and dual inoculation. Our results indicated that all three tested PGPR when combined with *Rhizobium* led to an increase in the nodule number, yield and yield components than the single inoculated and uninoculated control plants. In addition, co-inoculation treatment had a positive effect on rhizosphere microbial activities, namely, the population of bacteria, phosphate solubilizing bacteria, siderophores producing bacteria and the activity of dehydrogenase over single inoculation. Moreover, the availability of P, N and Fe, and the content of N, P, K and Fe in shoot tissues significantly increased as compared to *Rhizobium* alone. However, in some cases, no significant differences were observed between single and combined inoculation. The best results in this study were observed in *Pseudomonas fluorescens* + *Rhizobium* treatment, suggesting that the type of PGPR strain is an important factor in *Rhizobium*-PGPR interactions. According to the results of this study, co-inoculating *Rhizobium* and PGPR has a synergistic effect on common bean growth and yield while also improving soil fertility. *Rhizobium* biofertilizers may be more effective when PGPR is used with them for common bean production.

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تأثير التلقيح المشترك للريزوبيوم وبكتيريا الجذور المنشطة لنمو النبات على محصول الفاصوليا والتعقيد وامتصاص العناصر والنشاط الميكروبي تحت ظروف الحقل

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تعتبر بكتيريا الجذور من التقنيات الحيوية الواعدة لتحسين خصوبة التربة وإنتاج البقوليات تحت الظروف الحقلية. أجريت تجربة حقلية بتصميم القطاعات الكاملة العشوائية بثلاثة مكررات، لتقييم تأثير التلقيح المزدوج بالريزوبيوم وبكتيريا الجذور المنشطة لنمو النبات على نمو وتكوين العقد الجذرية ومكونات المحصول ومحتوى العناصر والبروتين لنبات الفاصوليا الجافة (صنف نبراسكا) كنموذج لنبات بقولي. كما تم دراسة تأثير هذا التلقيح المزدوج على النشاط الميكروبي وصلاحية العناصر الغذائية بالتربة (النتروجين والفوسفور والحديد). تم اختيار ثلاث سلالات من بكتيريا الجذور المنشطة لنمو النبات PGPR (*Serratia marcescens* BM1- *Micrococcus agilis* KMe7 - *Pseudomonas fluorescens* TW2) بناءً على قدرتها على إنتاج حامض الخليك والسيدروفورس وإذابة الفوسفات غير العضوي غير الذائب. أشارت النتائج إلى أن التلقيح المزدوج بالريزوبيوم والسلالات البكتيرية الثلاثة أدى إلى زيادة معنوية في عدد العقد الجذرية ووزنها الجاف والوزن الجاف للمجموع الخضري والجذري بالمقارنة بالتلقيح المفرد بالريزوبيوم أو النباتات غير الملقحة بعد ٧٠ يومًا من الزراعة. كما أدى التلقيح المزدوج إلى زيادة امتصاص النبات بالمجموع الخضري لعناصر النتروجين والفوسفور والبوتاسيوم والحديد بالمقارنة بالتلقيح المفرد بالريزوبيوم أو النباتات غير الملقحة، كما ازداد معنوياً نشاط أنزيم Dehydrogenase في التربة بالمقارنة بالتلقيح المفرد. بالإضافة إلى تلك النتائج، أدى التلقيح المزدوج إلى تأثير إيجابي على نشاط التجمعات الميكروبية في منطقة الجذور، وزيادة معنوية في أعداد البكتيريا والبكتيريا المذبة للفوسفات والبكتيريا المنتجة للسيدروفورس بالمقارنة بالتلقيح المفرد. كما أوضحت النتائج أن التلقيح المزدوج بـ PGPR و*Rhizobium* أدى إلى زيادة معنوية في محصول الفاصوليا ومكونات المحصول (عدد القرون لكل نبات، عدد البذور لكل قرن، ووزن الـ ١٠٠ بذرة وكذلك محصول القش والبذور عند المقارنة بالتلقيح الفردي بالريزوبيوم والنباتات غير الملقحة. أوضحت النتائج أيضاً أن أفضل النتائج المتحصل عليها هي معاملة *Rhizobium + Pseudomonas fluorescens* حيث بلغت نسبة الزيادة في محصول البذور والقش والمحصول الكلى ١٩.٦ و٣٥.٤ و٢٢.٢٪ على التوالي عن معاملة التلقيح بـ *Rhizobium* فقط. أخيرًا، أدى التلقيح المشترك إلى زيادة معنوية في نسبة البروتين في بذور الفاصوليا مقارنةً بالتلقيح الفردي أو النباتات غير الملقحة. لذا توصى الدراسة باستخدام بكتيريا الجذور لتنشيط النباتات البقولية في مصر وزيادة خصوبة التربة عندما يتم تلقيحها ببكتيريا تثبيت النتروجين تكافلياً.

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