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# ENHANCED FABA BEAN GROWTH BY COMBINED INOCULATION WITH *Rhizobium* STRAINS AND *Pseudomonas fluorescens* PF-23932 STRAIN AS A PLANT GROWTH PROMOTING RHIZOBACTERIA

**El Sayed, A. I.; A. S. El–Sanosy and M.A. Nassef**
**Microbiology Department, Soil, Water and Environmental Institute, Agriculture Research Center, Giza, Egypt.**
**E-mail: Dr.ahmedie@yahoo.com**

## ABSTRACT

Response of faba bean (*Vicia faba* L.) to co-inoculation with *Rhizobium leguminosarum* bv. *viciae* strains as a plant growth promoting and *Pseudomonas fluorescens* with half recommended doses of NPK was studied. *Rhizobium* strain RL-12612 showed significantly increased in Indole acetic acid (IAA) produced in complete and minimal medium supplemented with tryptophan in relation to bacterial strains. Whereas, PF-23932 showed significantly increased in IAA produced in minimal medium supplemented with tryptophan. A field experiment was conducted at Tag El-Ezz Research Station, Dakahlia Governorate, Egypt during two seasons 2014 to 2015. Co-inoculation with *rhizobium* strains and *Pseudomonas fluorescens* PF-23932 improved nodulation parameters during two seasons above the uninoculated plants. All treatments showed significant increase in root dry weight at 60 days plant-old above uninoculated plants during two seasons. Whereas, all treatments appeared significant increase in plant height in 2014 season. The result revealed high significant increase in the number of pods per plant in both seasons when the plants inoculated with RL-12612 strain and co-inoculated *rhizobium* strains with *Pseudomonas fluorescens* PF-23932 above uninoculated plants at 150 days-old among two seasons. RL-207 and co-inoculation with *Pseudomonas fluorescens* PF-23932 play an important role to improve chlorophyll b at 60 days plant-old among first seasons. All treatments appeared significant increase in NPK content at 60 days plant-old above uninoculated plants among two seasons except potassium content in second season .

**Keywords:-**Combined inoculation, IAA production, Nodulation efficiency, *Pseudomonas*, *Rhizobium*, *Vicia faba.*

## INTRODUCTION

Legumes are used for a wide variety of purposes mainly as human food and animal feed. In addition to being rich in protein, legumes are also high in bone building minerals and vitamins essential for good health (FAO, 1984). Faba bean (*Vicia faba* L.) is one of the most important legume crops in Egypt. It is grown mostly to fulfill food and feed requirements for human and animal consumption. Seeds of faba bean are rich in protein (26:28%) and some other compounds. An increased production and consumption of legumes is highly desirable taking into account the high nutritional value and the beneficial health effects (Morsy *et al*. 2005).

Nitrogen (N) is the most significant yield-limiting element in many agricultural production systems (Reeve *et al*., 2010). Nitrogen is an essential constituent of proteins, nucleic acids, some carbohydrates, lipids, and many metabolic intermediates involved in synthesis and transfer of energy molecules (Davis, 1980).Chemical fertilizers are the most expensive energy element (Brkić *et al*. 2004). Chemical fertilizers are generally used to supply essential nutrients to the soil–plant system throughout the world. However, the prices, availability, and the environmental concerns of chemical fertilizers especially the N fertilizers are real issues of today's agriculture. Therefore, there is an urgent need to find alternative strategies that can ensure competitive crop yields, provide environmental safety, and protection while maintain long term ecological balance in agro-ecosystem (Majeed *et al*., 2015).

Biological nitrogen fixation, one of the most important soil processes, is developed by means of microorganisms. Use of microbial inoculants or plant growth- promoting rhizobateria (PGPR) for the enhancement of sustainable agricultural production is becoming a more widely accepted practice in intensive agriculture in many parts of the world to avoid environmental pollution resulted from chemical fertilizers (Kumar *et al*., 2014).

A special role in biological nitrogen fixation is attributed to symbiosis between nodule bacteria of the genus *Rhizobium*, *Bradyrhizobium* and legumes. Rhizobia are the most studied PGPR for their potential to fix $N_2$ in the leguminous plants (Gage, 2004). Burns and Hardy (1975) reported that *Rhizobium* and *Bradyrhizobium* fixed about 175 × 106 tons of nitrogen per year. It is many times higher amount of nitrogen in comparison with the world annual production of nitrogen fertilizers being 30 × 106 tons (Postgate, 1982). The $N_2$-fixing capability of rhizobia varies greatly up to 450 Kg N/ha among host plants species and bacterial strains (Stephens and Rask, 2000).

Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and/or indirectly. In last few decades a large array of bacteria including species of *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus and Serratia* have reported to enhance plant growth (Glick, 1995). *Pseudomonas fluorescens* is adapted to survival in soil and colonization of plant roots. This applies also to the particular case of biocontrol agents from this species. The microbial inoculants that are used in agriculture include biofertilizers, biocontrol agents and plant growth promoting rhizobacteria (Kiely *et al*., 2006). PGPR-mediated mechanisms to enhancement plant growth and yields in many crops. They are produce ACC deaminase to reduce the level of ethylene in the roots of the developing plants and thereby increasing the root length and growth. They are produced hormones like indole acetic acid (IAA), gibberellic acid and cytokinins; asymbiotic nitrogen fixation; solubilization of mineral phosphates and mineralization of other nutrients and control of phytopathogenic microorganisms (Cassan *et al*., 2009).

Simultaneous inoculation of legumes with *Rhizobium* and free-living rhizobacteria belonging to the genera *Pseudomonas* has been reported to give positive effects on nodulation for several legumes (Oliveira *et al*. 1997).

Therefore, this study was devoted to investigating the synergistic interaction of *Rhizobium* and *Pseudomonas fluorescens* for improving faba bean growth and yield.

## MATERIALS AND METHODS

**Materials**

**Bacterial strains and growth conditions:**

Bacterial strains used in this study are listed in Table 1, which including their references, as well as, their designations.

**Table1. Bacterial strains used in this study.**

| Strains | Source or Reference | Designation |
|---|---|---|
| *Rhizobium leguminosarum* (12612) | IAM culture collection, Univ. of Tokyo, Japan. | RL- 12612 |
| *Rhizobium leguminosarum* (3841) | Kindly provided by Prof J P W Young, Department of Biology, University of Yourk , UK. | RL-3841 |
| *Rhizobium leguminosarum* (ARC 207) | Agric. Res. Center, Dept. of Microbiology, Giza, Egypt. | RL -207 |
| *Pseudomonas fluorescences* (NRRL B-23932) | National Center for Agriculture Utilization Research, USA. | PF-23932 |

**Media:** *Rhizobium* strains were grown at 28 C in yeast extract-mannitol medium (YEM) according to Vincent (1970). It was supplemented with 0.1 $mgml^{-1}$ L-tryptophan for IAA assay.

**Yeast extract-mannitol-congo red agar (YMCRA):** This medium was used to checked rhizobium strains according to Pattison and Skinner (1974).

**Kings Medium:** This medium was used for the maintenance of *Pseudomonas* strains according to Merck (1994). It was supplemented with 0.5g tryptophan for determining IAA in *Pseudomonas* strains according to Ahmad *et al.* (2005).

**Minimal Medium:** This medium supplemented with 0.5g tryptophan to be used for IAA assay according to Balassa (1963).

**M9 minimal medium:** This medium used as a minimal medium for IAA determination in *Pseudomonas* strains according to Leveau and Lindow (2005).

**Soil analysis:** Soil samples representing the studied location (Tag El-Ezz Research Station, Dakahlia Governorate, Egypt) were analyzed before cultivation process for some physico-chemical properties (Table 2) according to Page *et al*. (1982) and Klute (1986).

**Table 2. Chemical and physical characteristics of experimental soil used in this study.**

| Soil characteristics | Value | |
|---|---|---|
| | 2013-2014 | 2014-2015 |

| | | |
|---|---|---|
| Mechanical analysis (%) | | |
| Course sand % | 3.77 | 3.52 |
| Fine sand % | 30.65 | 31.32 |
| Silt % | 30.42 | 40.23 |
| Clay % | 30.16 | 25.11 |
| Texture class | Clay loamy | Clay loamy |
| Physical and chemical analysis | | |
| pH | 8.9 | 7.6 |
| Organic matter % | 1.45 | 1.32 |
| E.C dsm$^{-1}$ (1: 5) extract | 1.78 | 1.65 |
| Available macro and micro-nutrients | | |
| N mg kg$^{-1}$ | 21 | 24 |
| P mg kg$^{-1}$ | 7.92 | 7.21 |
| K mg kg$^{-1}$ | 312.5 | 215.21 |
| Ca meq/ 100g | 2 | 1.77 |
| Mg meq/ 100g | 1 | 0.965 |
| Na meq/ 100g | 5.7 | 4.3 |
| K meq/ 100g | 0.2 | 0.12 |
| $SO_4^{--}$ meq/ 100g | 1.9 | 1.85 |

**Plants :** Faba bean (*Vicia faba L.*) seeds from Giza 843 were used. Seeds were obtained from Agronomy Res. Institute, Agric. Res. Centre, Ministry of Agric., Giza.

**Methods**

**Preparation of inoculum**

Yeast extract mannitol (YEB) was prepared in conical flasks and autoclaved at 121$^{o}$C. This broth was then inoculated with *Rhizobium* strains and incubated at 28$^{o}$ C for three days. However, *Pseudomonas* strain was inculated in conical flasks containing King's B medium according to Merck (1994). The flasks shaking at 150 rpm for 48 hrs at room temperature (25 ± 2°C). Inoculants were applied in soil with 2 mL pure culture/pot (1 × 10$^{12}$ cfu mL$^{-1}$).

**IAA-detection with the Salkowski Colorimetric Technique**

Production of IAA in the supernatant was assayed using the PC method, as described by Pilet and Chollet (1970). This method was shown to be most sensitive and most specific (Glickmann and Dessaux, 1995).

**Field experiments**

A field experiment was designed and conducted at Tag El-Ezz Research Station, Dakahlia Governorate, Egypt, during the winter seasons of 2013-2014 to 2014-2015. This experiment was conducted in a randomized complete block design with three replicates. Ten treatments were used as follows:

1- Uninoculated plants (control)
2- 100%N+100%P (recommended doses).
3- 50% N+100% p (recommended doses).
4- RL- 12612+ 50% N +100 %P.
5- RL-3841+ 50% N +100 %P.
6- RL- 207+ 50% N +100 %P.
7- PF-23932 + 50% N +100 %P.

8- RL- 12612 + PF-23932 + 50% N+100 %P.
9- RL-3841+ PF-23932 + 50%N+100%P.
10- RL-207 + PF-23932 + 50% N +100%P.

Nitrogen and phosphorus fertilizers were applied to the control treatment at the rate of 15 kg/fed ammonium sulphate (20.5%N) and 25150 kg/fed superphosphate (15.5% $P_2O_5$), respectively. These additions were as recommended by Egyptian Ministry of Agriculture.

**Plant sampling**

Plants were harvested in two stages 60-days-plant old and harvesting time.

**Nodulation**

Nodules developed on the plants were counted after 60-days-plant old of inoculation. Three plants from each replicate were removed and washed carefully with tap water. Nodule number were counted, dried and weighted according to Novak *et al.* (2004). Average weight of nodule (AWON) was estimated according to Pereira *et al.* (1989).

**Vegetative Traits**

Different parts of plants (shoots and roots) at 60-days-plant-old were oven dried at 70°C until reached to a constant mass and then turned immediately to weight. Plant height was measured when the plants became to blooming at harvest time by centimeters from the first leaf to the apex. Leaf area/plant was determined using leaf fresh weight method according to Beadle (1993). At harvesting time total NPK content, number of pods/plants and weight of one hundred seed were determined.

**Plant mineral content analysis**

Oven – dried samples of roots, shoots and seeds were powdered for estimation of total N, P and $K^+$. Estimation of total N was done by kjeldahl's method, while the total P content was measured by vanado – molybdate yellow color method (Papakosta and Gagianas, 1991). Total K+ were measured by flame photometric method (Ryan *et al*., 2001).

**Photosynthetic Pigments:**

Chlorophyll concentration (chl. a, b, total and carotene per g tissue) in plant leaves was extracted in 80% methanol. The pigments were determined spectrophotometrically after stored the extracted solution for twenty four hours in a refrigerator and calculated according to the Lichtenthaler and Wellburn (1983).

**Total protein assay**

Protein was extracted from seeds at harvesting time by dilute alkaline hydrolysis. Then proteins in the supernatants were quantified by the Coomassie Brilliant Blue procedure for protein determination (Bradford, 1976) was used to determine protein concentration . However, Bovine serum albumin ranging in concentrations from 0 to 100 µg/ml was used as the standard from the standard curve.

**Free amino acids assay**

Free amino acids were determined in plant leaves at 60-days-plant-old according to the method of Lee and Takahashi (1966).

**Determination of total phenolic content**

Total phenolics were determined using the Folin±Ciocalteau reagent according to Singleton & Rossi (1965).

**Statistical analysis:**

Data were subjected to the analysis of variance according to Snedecor and Cochran (1955). Least significant difference (L.S.D.) was used to compare between means if the F-test was significant maintained for further experimentation.

## RESULTS AND DISCUSSION

**Indole-3-acetic acid production**

Indole acetic acid (IAA) production is a major property of rhizosphere bacteria that stimulate and facilitate plant growth (Mohite, 2013). In the present investigation bacterial strains were evaluated based upon quantitative estimation of IAA in complete and minimal medium in presence of tryptophan to selected the efficient ones to be used as a plant growth promoting biofertilizers. As shown from the results presented in Fig1, RL-12612 showed significantly increased in IAA produced in complete and minimal medium in relation to other strains. Whereas, PF-23932 showed significant production of IAA produced in minimal medium. IAA production varied between 27.18 to 7.89 in complete medium and 2.3 to 7.01 in minimal medium. This result agreed with Mohite (2013), who found that five rhizobacterial isolates preferred tryptophan for production and the maximum production was found in the medium amended with 0.1% tryptophan. Garge *et al*. (2015) found that all *Rhizobium* isolated from the root nodules of berseem produced IAA after 72 h, however their production varied significantly. Williams and Signer (1990) found that alfalfa symbiont *Rhizobium meliloti* produces indole-3-acetic acid in a regulated manner when supplied with exogenous tryptophan. The strains differ in their growth and production of IAA on different carbon and nitrogen sources (Sridevi and Mallaiah 2007). Indole acetic acid (IAA) is one of the most physiologically active auxins and common product of L-tryptophan metabolism by several microorganisms including rhizobia (Mandal *et al*. 2007).

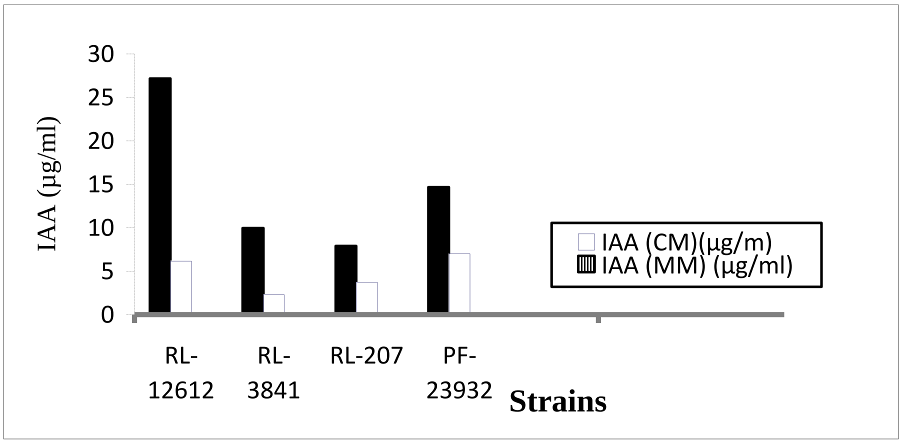


**Figure1. Qualitative assessment of IAA production by *R. leguminosarum strains* and *Pseudomonas fluorescences*.**

**Symbiotic effectiveness**

As shown from the results presented in the Table 3, co-inoculation (RL-12612+ PF-23932, RL-3841+ PF-23932 and RL-207+ PF-23932) improved nodulation parameters during two seasons above the uninoculated plants. *Rhizobium* strains (RL-3841 and RL-207 appeared significant increase in nodules number developed per plant in the second season above the uninoculated plants. Whereas, *Rhizobium* strains (RL-12612, RL-3841) appeared significant increase in nodules dry weight per plant during two seasons and nodulation index in the second season. On the other hand, *rhizobium* strain RL-207 and co-inoculation (RL-207 + PF-23932) appeared significant increase in average weigh of nodule above the uninoculated plants. These results agreed with Fatma *et al*.(2012), who found that the combination of AM fungi and *Rhizobium tropici* induced a significant increase in nodulation in common bean (*Phaseolus vulgaris* L.). Smith and Zhang (1999) reported that inoculating soybean with preinduced *Bradyrhizobium japonicum* improved soybean nodulation. Thilak *et al.* (2006) found that *Rhizobium* can also affect the growth and nitrogen fixation in pigeon pea by enhancing the occupancy of introduced *Rhizobium* in the nodules of the legume. The results indicated that nodulation index of *Rhizobium* strains with *Pseudomonas putida* was higher than that of *Rhizobium.*

**Table 3. Means of nodulation parameters in faba bean plants inoculated with *Rhizobium*, *Pseudomonas.***

| Treatments | Nodules developed /plant | | Nodule DW† (mg/plant) | | Average weight of nodule (mg) | | Nodulation index | |
|---|---|---|---|---|---|---|---|---|
| | I | II | I | II | I | II | I | II |
| Uninoculated plants | 2 | 4 | 0.004 | 0.005 | 0.003 | 0.001 | 0.074 | 0.052 |
| Full dose of N | 5 | 3 | 0.005 | 0.009 | 0.001 | 0.002 | 0.037 | 0.063 |
| Half dose of N | 6 | 3 | 0.067 | 0.017 | 0.011 | 0.005 | 0.567 | 0.147 |
| RL-12612 | 18 | 3 | 0.106 | 0.155 | 0.006 | 0.079 | 0.728 | 1.559 |
| RL-3841 | 17 | 9 | 0.136 | 0.163 | 0.008 | 0.020 | 0.907 | 1.429 |
| RL-207 | 20 | 13 | 0.060 | 0.092 | 0.026 | 0.044 | 0.484 | 0.879 |
| PF-23932 | 2 | 2 | 0.002 | 0.004 | 0.0001 | 0.0003 | 0.016 | 0.037 |
| RL-12612+ PF-23932 | 24 | 13 | 0.137 | 0.179 | 0.005 | 0.014 | 1.140 | 1.203 |
| RL-3841+ PF-23932 | 30 | 17 | 0.189 | 0.189 | 0.007 | 0.011 | 1.749 | 1.594 |
| RL-207+ PF-23932 | 18 | 13 | 0.184 | 0.155 | 0.012 | 0.012 | 1.274 | 1.143 |
| F-test | NS | * | * | * | * | NS | * | ** |
| LSD | | | | | | | | |
| 0.05 | | 3.69 | 0.08 | 0.10 | 0.01 | | 0.61 | 0.92 |
| 0.01 | | 5.43 | 0.11 | 0.14 | 0.01 | | 0.84 | 1.27 |

**NS,*,** = Insignificant differences , significantce at 0.05 and 0.01 probability levels, respectively.**
**†= Dry weight.**
**I,II = Growing seasons of 2013/2014 and 2014/2015, respectively.**

The data presented in Table 4 showed that plants inoculated with Rl-3841+PF-23932 showed significant increase in root dry weight above plants fertilized with recommended dose. However, all inoculum were expressed significant increase in shoot dry weight above uninoculated plants. Whereas, all inoculum appeared significant increase in plant height in the second season. This may be due to environmental condition of this year which stimulated more production of indole-3-acetic acid (IAA) leading to play a significant role in plant growth (Newcomb, 1980). These result agreed with El-Wakeil and El-Sebai (2009), who found a significant positive effect of rhizobium strains on fresh and dry weight of leaves and stems, root/ shoot ratio, pods/flowers ratio, as well as, the number and weight of nodules compared to NPK fertilizer plots. Ögutcu *et al*. (2008) found significant differences among rhizobial strains for various parameters such as nodule dry weight and shoot dry weight. Deshwal, *et al*. (2003) reported that rhizobia are known to increase nodulation and nodule dry weight in legumes along with increase in host plant growth and development. Rudresh *et al*. (2004) found that a combined inoculation of *Rhizobium* and a phosphate solubilizing *Bacillus* increased germination, nutrient uptake, plant height, number of branches, nodulation, yield and total biomass of check pea if compared to either individual or uninoculated control.

**Table 4. Mean of growth performance in inoculated plants.**

| Treatments | Root DW† (g/plant) | | Shoot DW† † (g/plant) | | L.A/P† (cm2) | | Plant height (cm) | |
|---|---|---|---|---|---|---|---|---|
| | I | II | I | II | I | II | I | II |
| Uninoculated plants | 0.6 | 2.7 | 6.5 | 9.4 | 844 | 748 | 63 | 59 |
| Full dose of N | 3.0 | 2.8 | 14.3 | 13.6 | 3070 | 3333 | 85 | 81 |
| Half dose of N | 2.4 | 1.8 | 12.3 | 11.7 | 1295 | 2553 | 79 | 75 |
| RL-12612 | 2.1 | 1.0 | 14.7 | 10.1 | 2619 | 2054 | 74 | 78 |
| RL-3841 | 1.9 | 1.2 | 15.0 | 11.6 | 2971 | 2627 | 71 | 77 |
| RL-207 | 2.0 | 1.4 | 11.6 | 11.2 | 2120 | 1690 | 75 | 70 |
| PF-23932 | 1.8 | 0.9 | 14.6 | 10.0 | 4215 | 2694 | 72 | 75 |
| RL-12612+ PF-23932 | 2.9 | 2.6 | 12.6 | 15.0 | 3909 | 4364 | 71 | 79 |
| RL-3841+ PF-23932 | 3.4 | 2.7 | 11.3 | 12.3 | 2182 | 1375 | 77 | 82 |
| RL-207+ PF-23932 | 3.3 | 2.5 | 15.0 | 13.7 | 3053 | 2782 | 85 | 91 |
| F-test | * | * | * | * | ** | * | ** | ** |
| LSD 0.05 | 0.37 | 0.53 | 4.8 | 2.13 | 3055.66 | 2209.53 | 12.89 | 10.35 |
| 0.01 | 0.51 | 0.73 | 6.58 | 2.92 | 4190.62 | 3030.22 | 17.67 | 14.19 |

***,** = Significant at 0.05 and 0.01 probability levels, respectively.**

**†= Leaf area per plant. I, II = Growing seasons of 2013/2014 and 2014/2015, respectively.**

## Yield parameters

Data presented in Table 5 showed the effect of inoculation on yield components (number of pods per plant and seeds dry weight) among two seasons. The results revealed a significant increase in the number of pods developed per plant above uninoculated plants in both seasons when plants inoculated with RL-12612 and co-inoculation (RL-12612+ PF-23932, RL-3841+ PF-23932 and RL-207+ PF-23932). Whereas, *Rhizobium* strains RL-3841 and RL-207 appeared significant increase in the number of pods per plant in 2013/2014 season and seeds dry weight among both seasons. This result agreed with Fatnassi *et al*. (2015), who found that *Vicia faba* inoculated with *Rhizobium leguminosarum* bv. *Viciae* appeared significant increases the number and the weight of nodules by 50%. Whereas, co-inoculation between *Rhizobium* and *Pseudomonas* positively influence the growth and seed yield per plant. Dashadi *et al*. (2011) found that co-inoculation of *Rhizobium* and *Azotobacter* increased total nitrogen content, nodulation, seed yield and biological yield under water deficit condition. However, El-Wakeil and El-Sebai (2009) found that the highest number of pods was achieved in treatment of rhizobia mixed with mycorrhiza or *pseudomonas*. Several workers have reported that seed inoculation with *Rhizobium* has significantly increased the growth and yield of legume crops (Pathak *et al*., 2001).

**Table 5. Means of yield parameters**

| Treatments | Pods/plant | | Seeds (g/plant) | |
|---|---|---|---|---|
| | I | II | I | II |
| Uninoculated plants | 12.33 | 11.33 | 18.20 | 25.95 |
| Full dose of N | 31.67 | 27.33 | 52.07 | 58.28 |
| Half dose of N | 22.67 | 21.00 | 36.60 | 27.68 |
| RL-12612 | 22.00 | 19.33 | 43.01 | 40.21 |
| RL-3841 | 20.00 | 18.00 | 41.23 | 42.20 |
| RL-207 | 13.33 | 20.67 | 37.74 | 39.71 |
| PF-23932 | 11.00 | 15.00 | 26.83 | 32.56 |
| RL-12612+ PF-23932 | 25.00 | 23.67 | 51.91 | 50.92 |
| RL-3841+ PF-23932 | 23.33 | 24.00 | 46.89 | 57.97 |
| RL-3841+ PF-23932 | 32.33 | 25.67 | 58.99 | 57.13 |
| F-test | * | ** | * | ** |
| LSD 0.05 | 8.29 | 6.36 | 8.88 | 8.59 |
| 0.01 | 11.37 | 8.72 | 12.18 | 11.78 |

***, ** = Significant at 0.05 and 0.01 probability levels, respectively.**
**I, II = Growing seasons of 2013/2014 and 2014/2015, respectively.**

## Biochemical traits

Data presented in Table 6 showed that RL-207 and co-inoculation (RL-12612+ PF-23932, RL-3841+ PF-23932 and RL-207+ PF-23932) play an important role to improve chlorophyll b at 60 days plant-old at the first season. These results agreed with Kosslak and Bohlool (1984), who found that photosynthetic capacity of the host plant may be affected by number of successful infections. Benefits to plants from PGPR have been shown to include increases in seed germination rate, root growth, yield, leaf area, chlorophyll content, nutrient uptake, protein content, hydraulic activity, tolerance to abiotic stress, shoot and root weights, biocontrol, and delayed senescence (Yang *et al*.2009). Dakora (2003) reported that the release phytohormones produced by rhizobia into cropping systems promoted plant growth through $N_2$ fixation.

**Table 6. Chlorophyll pigments in the leaves of faba bean plants.**

| Treatments | Chlorophyll content (mg/g) | | | | | | Carotene | |
|---|---|---|---|---|---|---|---|---|
| | Chl†.a | | Chl.b | | Total Chl. | | | |
| | I | II | I | II | I | II | I | II |
| Uninoculated plant | 1.45 | 1.58 | 0.23 | 0.43 | 1.68 | 2.01 | 1.42 | 1.28 |
| Full dose of N | 1.63 | 2.87 | 0.23 | 0.59 | 1.87 | 3.46 | 1.41 | 2.21 |
| Half dose of N | 2.12 | 2.40 | 0.23 | 0.43 | 2.35 | 2.83 | 1.90 | 1.57 |
| RL-12612 | 1.77 | 1.80 | 0.24 | 0.36 | 2.01 | 2.16 | 1.90 | 1.64 |
| RL-3841 | 2.98 | 1.80 | 0.45 | 0.42 | 3.43 | 2.23 | 2.32 | 1.41 |
| RL-207 | 2.84 | 1.80 | 0.61 | 0.40 | 3.45 | 2.21 | 2.24 | 1.30 |
| PF-23932 | 2.00 | 1.64 | 0.52 | 0.35 | 2.53 | 1.99 | 1.84 | 1.27 |
| RL-12612+ PF-23932 | 3.06 | 1.49 | 0.75 | 0.37 | 3.81 | 1.86 | 2.41 | 1.15 |
| RL-3841+ PF-23932 | 2.66 | 2.14 | 0.64 | 0.54 | 3.30 | 2.68 | 2.20 | 1.85 |
| RL-207+ PF-23932 | 1.98 | 2.11 | 0.57 | 0.46 | 2.55 | 2.57 | 1.87 | 1.81 |
| F-test | NS | * | ** | * | NS | * | NS | NS |
| LSD 0.05 | | 0.76 | 0.23 | 0.19 | | 0.87 | | |
| 0.01 | | 1.04 | 0.31 | 0.27 | | 1.20 | | |

**NS,*,** = Insignificant differences , significantce at 0.05 and 0.01 probability levels, respectively.**
**†= chlorophyll. I, II = Growing seasons of 2013/2014 and 2014/2015, respectively.**

Data summarized in Table 7 revealed that all treatments appeared significant increase in NPK content at 60 days plant-old above uninoculated plants among two seasons except for potassium content in the second season. This result agreed with Kucuk and Kivanc (2008), who found significant increase in seed yield, seed weight and protein range in three bean (*Phaseolus vulgaris* L.) varieties inoculated by *Rhizobium* spp. The increase in dry-matter and nitrogen content of co-inoculated plants may be attributed to increased nodulation, higher $N_2$-fixation and improvement of root development under controlled or field conditions (Dashti *et al*. 1998). PGPR bacteria can increase P availability to plants through solubilizing insoluble phosphates and this may improve biological nitrogen fixation and availability of other nutrients (Gyaneshwar *et al*., 2002).

**Table 7 . Mean of NPK content of faba bean grown under field conditions.**

| Treatments | N | | p | | k | |
|---|---|---|---|---|---|---|
| | I | II | I | II | I | II |
| Uninoculated plants | 1.3 | 1.3 | 0.10 | 0.12 | 1.15 | 1.16 |
| Full dose of N | 1.7 | 1.8 | 0.23 | 0.25 | 1.69 | 1.90 |
| Half dose of N | 1.8 | 1.8 | 0.24 | 0.21 | 1.85 | 1.76 |
| RL-12612 | 1.9 | 1.9 | 0.21 | 0.22 | 1.60 | 1.69 |
| RL-3841 | 2.2 | 1.9 | 0.24 | 0.23 | 1.65 | 1.77 |
| RL-207 | 1.9 | 2.1 | 0.30 | 0.27 | 1.89 | 1.73 |
| PF-23932 | 1.6 | 1.5 | 0.27 | 0.24 | 1.62 | 1.79 |
| RL-12612+ PF-23932 | 2.3 | 2.0 | 0.31 | 0.31 | 1.74 | 1.75 |
| RL-3841+ PF-23932 | 1.7 | 2.2 | 0.22 | 0.26 | 1.83 | 1.76 |
| RL-207+ PF-23932 | 2.9 | 2.6 | 0.34 | 0.29 | 1.73 | 1.85 |
| F-test | * | * | * | * | * | NS |
| LSD 0.05 | 0.36 | 0.19 | 0.06 | 0.04 | 0.26 | |

**NS,* = Insignificant differences and significantce at 0.05 probability levels, respectively.**
**I, II = Growing seasons of 2013/2014 and 2014/2015, respectively.**

Data in Table 8 showed that all treatments induced significant increase in total protein of seeds above uninoculated plants at the first season. The same effect was achieved by RL-207 and co-inoculation (RL-12612+ PF-23932, RL-3841+ PF-23932 and RL-207+ PF-23932) at the second season. Co-inoculation (RL-12612+ PF-23932 and RL-207+ PF-23932) appeared significant increase in total free amino acids in shoot above uninoculated plants among two seasons. However, bacterial strains RL-12612, RL-3841, RL-207, PF-23932 and co-inoculation (RL-3841+ PF-23932) appeared significant increase in total free amino acids in shoots above uninoculated plants among the second season. All treatments except for half recommended dose and RL-3841 appeared significant increase in shoot phenol content above uninoculated plants at the first season. These results agreed with Yadegari and Asadi Rahmani (2010), who found that co-inoculation strains with *P. fluorescens* gave the highest seed yield, number of pods per plant, weight of 100 seed, seed protein yield, number of seed per pod, and seed protein yield of bean (*Phaseolus vulgaris*). However, Solaiman and Rabbani (2005) found that the performance of *Rhizobium* inoculant was superior in relation to uninoculated control concerning protein content in green and mature seeds of pea.

**Table 8. Protein chemical traits in faba bean plants inoculated with rhizobia and *Pseudomonas*.**

| Treatments | Protein % in seeds | | Total free amino acids (µg/g) | | Total phenol (mg 100 $g^{-1}$ fresh weight) | |
|---|---|---|---|---|---|---|
| | I | II | II | I | I | II |
| Uninoculated plants | 16.9 | 17.0 | 11.41 | 8.40 | 122.3 | 151.5 |
| Full dose of N | 23.6 | 23.6 | 43.09 | 19.29 | 285.3 | 303.9 |
| Half dose of N | 20.2 | 20.1 | 10.63 | 11.10 | 176.4 | 185.51 |
| RL-12612 | 21.1 | 19.6 | 15.62 | 16.49 | 254.3 | 211.0 |
| RL-3841 | 21.0 | 21.2 | 14.28 | 14.39 | 208.2 | 253.0 |
| RL-207 | 20.0 | 19.9 | 23.94 | 18.17 | 288.2 | 231.8 |
| PF-23932 | 19.7 | 18.4 | 16.15 | 21.02 | 244.8 | 212.3 |
| RL-12612+ PF-23932 | 22.6 | 22.5 | 52.06 | 32.15 | 286.9 | 271.2 |
| RL-3841+ PF-23932 | 22.4 | 24 | 23.83 | 24.71 | 264.6 | 294.3 |
| RL-3841+ PF-23932 | 24.9 | 23.5 | 41.96 | 28.54 | 257.3 | 314.8 |
| F-test | * | * | * | ** | ** | * |
| LSD | | | | | | |
| 0.05 | 2.72 | 3.68 | 15.44 | 5.66 | 96.82 | 92.96 |
| 0.01 | 3.73 | 5.05 | 21.17 | 7.76 | 132.78 | 127.49 |

***,** = Significant at 0.05 and 0.01 probability levels, respectively.**
**I, II = Growing seasons of 2013/2014 and 2014/2015, respectively.**

In conclusion, the present study demonstrated a positive effect of co-inoculation between *Rhizobium* with *Pseudomonas* on symbiotic effectiveness and chlorophyll contents in faba bean plants, which improving the yield and yield components.

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**تعزيز نمو محصول الفول البلدى بواسطه التلقيح المشترك بين سلالات الرايزوبيم و سلالة السيدوموناس فلوروسنز PF-23932 كسماد حيوى محفز لنمو النباتات**

**أحمد إبراهيم السيد ، أحمد سامى عمر أحمد السنوسى و محمود عبد المقصود ناصف**
**قسم الميكروبيولوجيا الزراعية- معهد بحوث الأراضى و المياه و البيئة – مركز البحوث الزراعية.**

استخدمت فى هذه الدراسة اربعة سلالات بكتيرية . تم تقيم السلالات المستخدمة بالنسبه لإنتاجها من هرمون النمو اندول حمض الخليك فى بيئتى النمو الكاملة و الحديه المدعومة بالحامض الأمينى تربتوفان. أظهرت سلالة الرايزوبيم Rl-12612 زيادة معنوية فى إنتاج أندول حامض الخليك على مستوى بيئة النمو الكاملة و الحدية مقارنة بباقى السلالات البكتيرية. بينما أظهرت سلالة PF-23932 زيادة معنوية فى انتاج أندول حامض الخليك على مستوى بيئة النمو الحدية مقارنة بباقى السلالات البكتيرية . تم زراعة التجربة الحقلية فى المزرعة البحثية بمحطة تاج العز للبحوث الزراعية خلال موسمي 2014/2013 ،2015/2014.

أظهر التلقيح المشترك بين سلالات الرايزوبيم و سلالة السيدوموناس فلوروسنز PF-23932 زيادة معنوية فى الصفات الخاصة بتكوين العقد البكتيرية على جذر نبات الفول البلدى خلال موسميين النمو . كما أظهرت كل المعاملات المستخدمة زيادة معنوية فى الوزن الجاف للمجموع الجذرى مقارنة بالنباتات الغير ملقحة خلال موسمى الزراعة ، بينما كان نفس التاثير بالنسبه لصفة طول النبات خلال موسم النمو الأول.

اظهرت السلالة RL-12612 ، معاملات التلقيح المشترك بين سلالات الرايزوبيم و سلالة السيدوموناس فلوروسنز PF-23932 زيادة معنوية فى عدد القرون فاقت النباتات الغير ملقحة. لعبت سلالة الرايزوبيم RL-207 و معاملات التلقيح المشترك بين سلالات الرايزوبيم و سلالة السيدوموناس فلوروسنز PF-23932 دورا هاما فى تحسين نسبه كلوروفيل b فى الاوراق عند عمر 60 يوم خلال موسم النمو الأول بينما أظهرت المعاملات زيادة معنوية فى محتوى الأوراق من المعادن النيتروجين ، الفوسفور ،البوتاسيوم خلال موسمى النمو (ماعد عنصر البوتاسيوم خلال الموسم الثانى فقط ) مقارنة بالنباتات الغير ملقحة .

عموما اوضحت النتائج إمكانية الإعتماد على استخدم نصف الجرعات من التسميد الكيماوى للنيتروجين مع الجرعة الكاملة للفوسفور مع إستخدام التلقيح المشترك لبكتريا العقد الجذرية الرايزوبيم و السيدوموناس كمخصب حيوى فعال .