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Pseudomonas fluorescens Isolates Used as a Plant Growth Promoter of Faba Bean (*Vicia faba*) in Vitro as Well as in Vivo Study in Ethiopia

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Abstract: Production of the crop is affected by deficiency of fertilizers and low number of plant growth promoting rhizobacteria in soil. At the present study, Pseudomonas fluorescens isolates possess a variety of promising properties which make it a good plant growth promoting traits. Twelve *P. fluorescens* isolates from rhizospheric soil of faba bean were isolated and assessed in vitro for their plant growth promoting activity based on their ability to produce hydrogen cyanide (HCN), siderophores, indole acetic acid (IAA), and ammonia and phosphate solubilization. The results indicated that most of the isolates tested possess plant growth promoting traits. Bio-primed faba bean seed with *P. fluorescens* 9 and *P. fluorescens*10 for plant growth promoting activities in green house showed a positive result. In addition, these to two isolates increased faba bean leaves number, branches number, height, root length, lateral roots and number of nodule per plant. Therefore, from this study it is possible to conclude that the use of *P. fluorescens* 9 and 10 isolates could increase the faba bean growth and yield performance. These isolates can be used as potential biofertilizers and plant growth promoter.

Keywords: Faba Bean, Growth Promote, Phosphate Solubilization, *P. fluorescens*, Siderophore

1. Introduction

The diversity and beneficial activity of the plant-bacterial association and its understanding is important to sustain agro-ecosystems for sustainable crop production (Germida et al., 1998). The rhizosphere is representing the thin layer of soil surrounding plant roots. The rhizosphere, supports large active groups of bacteria (Villacieros et al., 2003) known as plant growth promoting rhizobacteria (PGPR) (Kloepper et al., 1980). Plant growth promoting rhizobacteria are known to rapidly colonize the rhizosphere, suppress soil borne pathogens at the root surface (Rangajaran et al., 2003) and to stimulate plant growth (Bloemberg and Lugtenberg, 2001; Moeinzadeh et al., 2010). Some *Pseudomonas* sp *P.*, especially fluorescent *pseudomonads*, are particularly suitable to be used as agricultural biocontrol agents because they can produce large amounts of secondary metabolites to protect plants from phytopathogens and stimulate plant growth.

Plant growth promoting rhizobacteria (PGPR) were defined as the soil bacteria that colonize the roots of plants by following inoculation on to seed and that enhance plant growth (Kloepper and Schroth, 1978). The plant growth-promoting ability of these bacteria is generated mainly by the production of indole-3-acetic acid (IAA), siderophores and some well-known antibiotics (Nagarajkumar et al., 2004), hydrogen cyanide (HCN) (Voisard et al., 1989), phosphate solubilize (Rodríguez and Fraga, 1999).

Pseudomonas fluorescens is adapted to survival in soil and colonization of plant roots (Kiely et al., 2006) and 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. While the biofertilizer organisms make the nutrients available to plants, biocontrol agents protect the plants against the pathogenic organisms and insect pests. Bio-priming, a seed treatment system that integrates the biological and physiological aspects of disease control, involves coating the seed with fungal or bacterial biocontrol agents (El-Mougy and Abdel-Kader, 2008). Bioprotectants applied to seeds, protect seeds (Sivan and Chet, 1986) colonise and protect roots (Chang et al., 1986)

and may increase plant growth. A successful antagonist should colonise the rhizosphere during seed germination (Weller, 1983).

Indole-3-acetic acid (IAA) is implicated in signaling between microorganism and plants (Spaepen et al., 2007) leading to stimulation of cell division, initiation of lateral and adventitious roots (Malamy and Benfry, 1997), cell enlargement (Salisbury, 1994) and results into elongation of stems and roots. The stimulation of growth of roots results in enhances uptakes of nutrients plants association (Lifshitz et al., 1987). Biofertilizers such as microbial inoculants promote plant growth, productivity and increase the nutrient status of the host plant. This produce has internationally been accepted as an alternative source of chemical fertilizers (Vessey, 2003).

Siderophores act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation (Indiragandhi et al., 2008). Antibiotics are chemically heterogeneous group of organic, low molecular weight compounds produced by microorganisms (Raaijmakers et al., 2002). This is first study of *P. fluorescens* role on faba bean crop to increase the growth and yield. As result these isolates have great contribution on growth promoting of faba bean in agriculture production. The present study was firstly designed to isolate certain rhizospheric of *Pseudomonas fluorescens* from faba bean root soil for assessment of their production of plant growth promoting substances (PGPS) in vitro study. Secondly to evaluate plant growth promoting substances effect on faba bean crop growth as well as yield in green house test through bio-priming method on faba bean seed.

2. Materials and Methods

2.1. Soil Sample Collection and bacteria Isolation

Rhizospheric soil samples were collected in an envelope from fields growing faba bean (*Vicia faba* L.) from five locality area of North Showa of Oromeia Regional state of Salele Zone, Ethiopia. The soils were brought to Mycology Laboratory, Addis Ababa University. Ten gram (10 g) of rhizosphere soil sample was suspended in 90 ml of sterile distilled water. Samples were serially diluted up to 10^{-2} and 0.1 ml of sample was spreaded on King's B medium plates (King et al., 1954) incubated at 28°C for 48 h. After incubation the plates were exposed to UV light at 365 nm for few seconds and the colonies exhibiting the fluorescence were picked up and purified on King's B medium plates. Twelve strains of *P. fluorescens* were isolated and they were designated as Pf 1 up to Pf 12 for further studies.

2.2. Source of Faba Bean Seed and Chocolate Spot Pathogen

One isolate of *Botrytis fabae* was obtained from Holeta Agricultural Research Centre, Ethiopia which was isolated from the leaf of infected faba bean which was grown around Holeta locality. The seeds of faba bean varieties used in the present work were obtained from Holeta Agriculture Research Centre, Ethiopia. The three varieties of faba bean seed (namely: NC 58 susceptible variety, Moti moderate variety and ILB 938 restively resistant variety) was obtained from Holeta Agriculture Research Center (figure 1).

Figure 1. *Faba bean sample.*

2.3. Biochemical Characterization of P. fluorescens Isolates for Plant Growth Promoting (PGP) Traits

2.3.1. Assay for Siderophore Production

Siderophore production was tested by growing *P. fluorescens* isolates on the king's B medium at 28°c for 48 hours. The plates were exposed to UV light for 30 seconds and the isolate with pigment were exhibiting the fluorescence (Ramyasmruthi et al., 2012).

2.3.2. Assay for Hydrogen Cyanide Production

Hydrogen cyanide production was assayed by the method suggested by (Castric, 1977; Lorck, 1948). For the production of HCN, *P. fluorescens* isolates were streaked into King's B agar plates supplemented with glycine (4.4 g/l). After wards, plates were inverted and a piece of filter paper impregnated with 0.5% picric acid and 2% of sodium carbonate was placed on the lid. Petri plates were sealed with parafilm and incubated at 28° C for 96 h. Production of cyanide was determined by a color shift from yellow to orange in the filter paper (Castric, 1975).

2.3.3. Assay for Indole Acetic Acid (IAA) Production

All the *Pseudomonas fluorescens* isolates were tested for IAA production (Loper and Schroth, 1986). *P. fluorescens* isolates were inoculated in nutrient broth with L-tryptophan 500 mg/l at 28 ^{0}C for 1 week. Fully grown cultures were centrifuged at 3000 rpm for 30 min, then 2 ml of supernatant was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M FeCl3 solution). Development of pink color indicates IAA production was determined (Bric et al., 1991).

2.3.4. Assay for Ammonia Production

P. fluorescens isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water and incubated for 72 h at 28^{0}C. Then Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was a positive for ammonia production (Cappuccino and Sherman, 2005).

2.3.5. Assay for Phosphate-Solubilization

Phosphate-solubilization test was conducted qualitatively by plating the *P. fluorescens* isolates in agar containing precipitated tricalcium phosphate. The medium was a modification of Pikovskaya medium (Subba Rao, 1999), consisted of 10 g glucose, 5 g tribasic phosphate ($Ca_3(PO_4)_2$), 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4.7H_2O$, 0.2 g NaCl, 0.2 g KCl, 0.002 g trace of $MnSO_4.H_2O$ and $FeSO_4$ $.7H_2O$, 0.5 g yeast extract, and 20 g agar, in 1,000 ml distilled water. *Pseudomonas fluorescens* isolates culture were streaked on the surface of agar plates and incubated at 28°C for 3 days. After 3 days of incubation, the colonies showing the clear zones around them were considered as positive for positive P-solubilization.

2.4. Preparation of Bacteria Inoculum and Bio-Priming of Faba Bean Seeds

Carboxymethyl cellulose (CMC) and pectin were used as adhesive polymers for the bio-priming process of three varieties of faba bean seeds with antagonistic bioagent. *P. fluorescens* isolates was grown for 48 h in King's B (KB) broth medium, and then cells were harvested by centrifugation. Two isolates of *P. fluorescens* (Pf 9 and Pf 10) were resuspended in sterile distilled water and the concentration adjusted to give 10^9-10^{10} cells/ml (El-Mougy and Abdel-Kader, 2008). Ten grams of either CMC or pectin was suspended in 1 L of *P. fluorescens* isolates suspensions, which were shaken for 10 min on a magnetic stirrer plate. Seeds of faba bean (at the ratio of 500 g/L) were imbibed in each of the prepared priming solutions for 16 h (Jensen et al., 2004).

The bio-primed seeds were then air-dried on filter paper for 1 h and stored in a refrigerator at 5^o C until required. Another group of surface-sterilized faba bean seeds (70% ethanol for 2min) were prepared as control treatments (El-Mougy and Abdel-Kader, 2008).

2.5. Growth and Yield Parameters Data Collection

Growth parameters (plant height (cm), number of leaves, branches, nodule number (Alemayehu, 2009) root length (cm) per plant) were collected at 70 days (El-Ghamry et al., 2009) and after 5 months yield parameters (shoot fresh weight (g), number of pods per plant, weight of pods/plant (g) and number of seeds/ plant) were collected.

3. Data Analysis

All the measurements were replicated three times for each assay and the results are presented as mean ± SD. IBM SPSS 20 Version statistical software package was used for statistical analysis of growth and yield parameter in each case.

4. Results

Isolation of Pseudomonas fluorescens

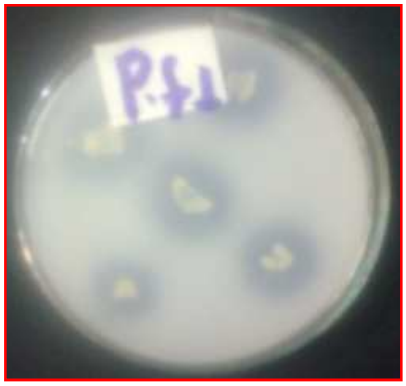
At the present study, twelve P. fluorescens were isolated from rhizospheric soil of healthy faba bean on King's B medium and observed under UV light at 365 nm for 30 seconds as indicated figure 2.

Figure 2. *Primarily screening of Pseudomonas fluorescens based on pigment production under UV light*

4.1. In Vitro Production of Plant Growth Promoting Substances

All of *P. fluorescens* isolates were positive for produced plant growth promoting substances. All the strains were positive for siderophore, hydrogen cyanide (HCN), indole acetic acid (IAA) and ammonia production and also for phosphate-solubilization as indicted figure 3.

A



B

C

D

E

Figure 3. *Production of plant (crop) promoting traits by Pseudomonas fluorescens isolates: A, Siderophore production; B, Phosphate-Solubilization; C, Hydrogen cyanide (HCN) production; D, Indole acetic acid (IAA) production and E, Ammonia production.*

4.2. Plant Growth Parameters

4.2.1. Effect of Pseudomonas fluorescens Isolates (Pf 9 and Pf 10) on Growth Parameters under Greenhouse Conditions

The use of bio-priming seeds of faba bean of NC 58, Moti and ILB 938 varieties with Pf 9 and Pf 10 treatments were showed significantly increase number of leaves/plant over untreated (negative control or infected untreated plant) and positive control (Uninfected untreated plants) as indicated in Table 1 after 70 days of planting of faba bean.

Two isolates of *P. fluorescens* (Pf 9 and Pf 10) enhanced the number of branches/plant significantly over negative and positive control. Bio-primed seed of faba bean with Pf 9 increased the number of branches per plants on NC 58, Moti and ILB 938 varieties, while bio-primed seed of faba bean with Pf 10 increased the number of branches per plants on NC 58, Moti and ILB 938 varieties as indicated in Table 1 after 70 days of planting of faba bean.

The result in Table 1 indicated bio-primed seed of faba bean with Pf 9 increased height of plants on of Moti, NC 58 and ILB 938 varieties and with Pf 10 increased the height of plants on NC 58, ILB 938 and Moti varieties as, compared negative and positive control after 70 days of planting of faba bean.

The result showed that bio-primed faba bean seeds with Pf 9 and Pf 10 showed an increase in the nodule number average per plant of the varieties in Table 2.

Bio-primed faba bean seeds with Pf 9 and Pf 10 showed increase of the root length per plant of NC 58, ILB 938 and Moti after 70 day as showed in Table 2.

Table 1. *Effect of two Psedomonas fluorescens isolates (Pf 9 and Pf 10) on growth parameter of faba bean plants.*

Treatments and controls	After 70 days		
	Nº leaves per plant Mean ± SD	Nº branches per plant Mean ± SD	Plant height(cm) Mean ± SD
Pf 9 NC 58	65.33 ± 8.927	1.75 ± 1.055	87.25 ± 3.720
Pf 9 Moti	75.25 ± 21.239	2.25 ± 0.965	86.92 ± 9.624
Pf 9 ILB 938	67.83 ± 13.114	2.25 ± 1.357	88.25 ± 6.254
Pf 10 NC 58	70.58 ± 9.811	1.67 ± 0.651	84.67 ± 2.807
Pf 10 Moti	83.08 ± 11.261	2.42 ± 0.793	91.92 ± 7.573
Pf 10 ILB 938	79.50 ± 34.259	2.58 ± 0.996	90.08 ± 16.149
Negative Control NC58	61.67 ± 7.608	1.17 ± 0.937	84.42 ± 6.626
Negative Control Moti	66.33 ± 11.372	1.58 ± 0.515	82.17 ± 8.840
Negative Control ILB 938	60.42 ± 13.787	2.00 ± 0.603	83.75 ± 8.081
Positive Control NC 58	62.00 ± 8.224	1.42 ± 1.165	84.25 ± 6.982
Positive Control Moti	72.08 ± 10.131	1.83 ± 0.835	81.00 ± 4.767
Positive Control ILB 938	61.50 ± 8.459	2.08 ± 1.165	81.01 ± 4.767

Key: Mean, SD= Standard deviation, NC 58, Moti and ILB 938 are faba bean varieties

Table 2. *The effect Pseudomonas fluorescens isolates (Pf 9 and Pf 10) on nodulation and Root length of faba bean.*

Treatments and controls	After 70 days	
	Nodule number/plant Mean ± SD	Root length (cm) root system Mean ± SD
Pf 9 NC 58	33.25 ±3.304	32.50 ± 2.082
Pf 9 Moti	61.50 ±1.915	49.75 ±15.966
Pf 9ILB 938	50.00 ±17.049	44.75 ±5.500
Pf 10 NC 58	35.00 ±10.893	34.75 ±13.598
Pf 10 Moti	68.25 ±9.215	53.00 ±11.518
Pf 10 ILB 938	81.25 ±12.066	45.00 ±3.916

Treatments and controls	After 70 days	
	Nodule number/plant Mean ± SD	Root length (cm) root system Mean ± SD
NC 58 Negative control	28.50 ± 13.669	21.00 ±7.257
Moti Negative Control	48.25 ± 8.884	22.75 ±13.048
ILB 938 Negative control	33.50 ± 10.661	30.25 ± 7.762
NC 58 Positive control	29.00 ± 11.576	24.25 ± 3.775
Moti Positive control	56.00 ±18.312	35.13 ±18.993
ILB 938 Positive Control	40.75 ±15.649	35.25 ±12.553

Key: Negative control= seed of faba bean (NC 58, Moti and ILB 938) with *Botrytis fabae* without using *P. fluorescens* as Bio-priming sowing
Positive control= only sowing faba bean seed (NC 58, Moti and ILB 938) without of inoculating both bio-control (*P. fluorescens*) and pathogen (*Botrytis fabae*)

4.2.2. Effect of Pseudomonas fluorescens Isolates (P f9 and P f10) on Yield Parameters under Greenhouse Conditions

The use of bio-priming seeds of faba bean (NC 58, Moti and ILB 938 varieties) with Pf 9 and Pf 10 showed increase in shoot fresh weight of faba bean over untreated (negative control or infected untreated plant) and positive control (Uninfected untreated plants) as indicated in Table 3.

The two isolates of *P. fluorescens* enhanced the number of pods per plant significantly over negative and positive control. Bio-primed seed of faba bean with Pf 9 increased the number of branches per plants on NC 58, Moti and ILB 938 varieties, while bio-primed seed of faba bean with Pf 10 increased the number of pods per plants on NC 58, Moti and ILB 938 varieties as indicated in Table 3.

As result indicated bio-primed seed of faba bean with Pf 9 increased height of plants on of NC 58, Moti and ILB 938 varieties and with Pf 10 increased the weight of pods/plant on NC 58, Moti and ILB 938 varieties as, compared negative and positive control in Table 3.

The result showed that bio-primed of faba bean seeds with Pf 9 were increased the number of seeds/plant of NC 58, Moti and ILB 938, while bio-primed with Pf 10 also increased the number of seeds/ plant of NC 58, Moti and ILB 938 as indicated in Table 3 after 5 months.

Table 3. *Effect of P. fluorescens 9 and 10 strains treatments on yield parameters of faba bean plants under greenhouse condition after 5 months.*

Treatments and Controls	Yield Parameters			
	Shoot fresh weight (g)	Number pods/plant	Weight pods/plant (g)	Number of seeds/ plant
Pf 9 NC 58	83.20	4.13	4.50	6.35
Pf 9 Moti	232.10	5.50	8.50	10.25
Pf 9 ILB 938	360.30	6.34	9.24	17.32
Pf10 NC 58	235.12	5.37	5.63	7.75
Pf 10 Moti	225.90	5.16	7.12	9.89
Pf 10 ILB 938	517.70	9.89	17.40	44.12
Negative Control NC58	131.30	1.20	1.50	2.10
Negative Control Moti	200.40	1.89	1.90	2.13
Negative Control ILB 938	257.09	0.00	0.00	0.00
Positive Control NC 58	165.47	3.57	3.96	4.81
Positive Control Moti	215.01	4.12	4.53	5.21
Positive Control ILB 938	350.00	8.76	12.17	18.28

5. Discussion

5.1. In Vitro Production of Plant Growth Promoting Substances (PGPS)

In present study, *P. fluorescens* isolates showed PGP traits like phosphate-solubilization, siderophore production, hydrogen cyanide production, ammonia production, and indole acetic acid (IAA) production. Hence, these isolates have been used as biocontrol agents and plant growth promoting rhizobacteria (PGPR). The exact mechanism by which PGPR stimulate plant growth is not clearly known, although several mechanisms such as production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilization and promotion of the mineral nutrient uptake are usually believed to be involved in plant growth promotion (Glick, 1995) and inhibit the growth of plant pathogens by diverse mechanisms such as antibiotic production (Nagarajkumar et al., 2004), siderophore production (Loper, 1988), HCN release (Voisard et al., 1989) and competitive colonization of plant roots (Weller, 1985).

In this study, the qualitative estimation of siderophores by *Pseudomonas fluorescens* isolates showed that they were compare with producer of siderophores under limited iron on King's B medium. The production of siderophores by *Pseudomonas fluorescens* isolates indicated that these bacteria isolates can be used as a bio-control against soil borne phytopathogens. Similarly, (Ramyasmruthi et al., 2012) reported that *P. fluorescens* as siderophore producer on King's B medium. Siderophores provide a competitive advantage to producer organism over fungal pathogens for the absorption of available iron (Jeffrey et al., 2003).

In the present study, all *P. fluorescens* isolates were positive for HCN production, which acts as an inducer of plant resistance. On other study, HCN is a secondary metabolite produced by gram negative *P. fluorescens*, *P. aeruginosa* and *Chromobacterium violaceum* (Askeland and Morrison, 1983) and played a role in biological control of pathogens. This compound, although reported as a potential inhibitor of enzymes involved in major plant metabolic processes (Castric, 1975) is currently attracting remarkable attention and wide applications in areas of biocontrol methods. HCN production by rhizobacteria has been postulated to play an important role in the biological control of pathogens (Lifshitz et al., 1987).

Applications of growth promoter of *Pseudomonas fluorescens* isolates on faba bean seed were brought significant increases on growth and yield of cro*P.* The increment in growth parameter may be due to that Pf 9 and Pf 10 are extremely important component because they constitute a stable fraction of carbon, thus regulating the carbon cycle and release of nutrients, including nitrogen and phosphorus which decreasing the need for inorganic fertilizer for plant growth. Similarly, the use of *Tichoderma viride* tag4 in combined with *Rhizobium leguminosarum* is an effective strategy for an integrated management of chocolate spot disease as well as increasing growth and productivity of faba bean (Saber et al., 2009).

5.2. Growth Promotes

Bio-primed seed of faba bean with Pf 9 and Pf 10 increased the number of leaves per plants on NC 58, Moti and ILB 938 varieties. The highest number of leaves per plants (75.25 leaves/plant) observed on ILB 938 variety. On other study, *Trichoderma harzianum* significantly increased number of leaves in treated bean plants were 15.2 leaves/plant, while in untreated plants were 9.5 leaves/plant (Abd-El-Khair et al., 2010). Application of humic acids (HA) at 2000+ amino acids (AA) at 2000 ppm came in the top of other treatments in increasing 46.00 leaves number per plant (El-Ghamry et al., 2009). It has been reported that seed treatment with Bion and Salicylic acid achieved highest faba bean shoot length at 0.36 and 0.34 double field dose (D.F.D.) 26.0 leaves per plants respectively (Mahmoud et al., 2011).

Pf 9 showed the maximum number of branches in Moti and ILB 938 varieties 2.25 branches/plant and bio-primed with Pf 10 was 2.00 branches/plant of ILB 938 varieties. It has reported that, *Trichoderma harzianum* significantly increased the branches number average 6.3 branch/plant, compared to 3.7 branch /plant in control treatment (Abd-El-Khair et al., 2010). Application of HA at 2000+ AA at 2000 ppm came in the top of other treatments in increasing 4.67 branches number per plant (El-Ghamry et al., 2009).

The highest shoot length 88.25 cm was recorded on faba bean plants of ILB 938 variety inoculated with Pf 9 and 91.92 cm was recorded on Moti variety inoculated with Pf 10. On other study, *Trichoderma hamatum* gave the highest increase of plant height 49.8 cm compared to 37.3 cm in the control plants (Abd-El-Khair et al., 2010). Application of AA at 3000 ppm increased plant height to 74.33 cm (Loper, 1988). As Mahmud reported that seed treatment with Bion achieved highest faba bean shoot length at 0.36 D.F.D 54.3cm (Mahmud et al., 2011).

The highest numbers of nodule per plant 81.25 and 61.50 nodule/plant were recorded in faba bean ILB 938 and Moti bio-primed with Pf 10 and Pf 9 respectively. It has been reported that highest average nodule number was observed 96 nodules/ plant (Alemayehu, 2009) in faba bean.

The highest root length per plant 49.75 and 53.00 cm root length/faba bean were recorded in faba bean Moti bio-primed with Pf 9 and Pf 10 respectively. It has been reported that *Pseudomonas* species produced significant levels of IAA and caused shoot and root elongation in soybean (Xie et al., 1996).

5.3. Yield Parameters

Bio-primed seed of faba bean with Pf 9 and Pf 10 increased the shoot fresh weight on 3 varieties. The highest shoot fresh weight (360.30 g) was observed on ILB 938 variety, the while bio-primed seed of faba bean with Pf 10 increased the shoot fresh weight on Moti, NC 58 and ILB 938 varieties and gave maximum shoot fresh weight (517.70 g) on ILB 938 variety. The study conducted by (Mahmoud et al., 2011) reported that shoot treatment with bion was the most effective for shoot fresh weight parameters at field dose (0.18) and double filed dose (0.36) dose. It has reported that application of *Trichoderma spp.* increased the pods number average per plant from 4.2 fresh weight/plant with the control plant to 43.1-77.4 fresh weight/plant in treated plant (Abd-El-Khair et al., 2010).

Treatement Pf 9 showed the maximum number of pods per plant of ILB 938 variety 6.34 numbers of pods /plant and bio-primed with Pf 10 were 9.89 numbers of pods /plant of ILB 938 variety. It has reported that in the case of shoot treatment, salysilic acid was the most effective for number pods/ plant parameters at both field dose (0.32) and double filed dose (0.34), 2.0 and 2.5 (Mahmoud et al., 2011), while in the case of seed treatment with bion at both filed dose (0.18) and double filed dose (0.36) with 2.5 and 4.0 pod/plant respectively. The study conducted by (Abd-El-Khair et al., 20210) has reported that application of *Trichoderma spp.* significantly increased the pods number average per plant to 15.2-20.0 pods/plant, compared with10.8 pods/plant in the control plants. It has reported that spraying faba bean plants with humic acid (HA) (2000 ppm) + amino acid (AA) (2000 ppm) significantly improved number of pods/ plant at highest level at 67.33 (El-Ghamry et al., 2009). It has reported that chemical inducer of KH_2PO_4 with seed treated increase the number of pods/plant 24.7, as compared with control 16.5 (El-Hendawy et al., 2010).

The highest weight of pods/plant 17.40 and 9.24 g were recorded in faba bean ILB 938 bio-primed with Pf 10 and Pf 9 respectively. The study conducted has reported that application of *Trichoderma spp.* significantly increased the pods number average per plant where the number were in the range of 2.7-4.0 pod weight/plant ,compared to 2.7 pod

weight/plant in the control plants (Abd-El-Khair et al., 2010). On other study has reported that chemical inducer of KH_2PO_4 with seed treated increase the weight of pods/plant 75.7 g, as compared with control 50.7 g (El-Hendawy et al., 2010).

The highest number of seeds/plant was recorded on ILB 938 variety inoculated with and Pf 10 (44.12) and Pf 9 (17.32). On other study, (El-Hendawy et al., 2010) had reported that chemical inducer of KH_2PO_4 with seed treated increase the number of seeds/plant 58.3, when compared with control 42.8.

In the present study, IAA productions in all *P. fluorescens* isolates were positive and also indicate their finding. Similarly, Plant growth promoting rhizobacteria (PGPR) traits showed positive for the traits of IAA (Ramyasmruthi et al., 2012). The ability of bacteria to produce IAA in culture depends on the availability of precursors. Similarly, it has been reported that IAA production by PGPR can be influenced by substrate availability (Mirza et al., 2001).

It is expected that inoculation with *P. fluorescens* isolates containing PGP characteristics consequently promote root, shoot growth and lateral roots as well as nodulation. The ability of bacteria to produce IAA in the rhizosphere depends on the availability of precursors and uptake of microbial IAA by plant. Microbial biosynthesis of IAA in the soil is enhanced by tryptophan from root exudates or decaying cells (Benizri et al., 1998) and also improves plant growth by increasing the number of root hairs and lateral roots (Okon and Kapulnik, 1986).

In this study, mostly all isolates of *P. fluorescens* were able to produce ammonia. Similarly, *P. fluorescens* isolate was produced ammonia as reported according to (Ramyasmruthi et al., 2012). Production of inhibitory volatiles may increase the survival rate of bacteria in soil, by eliminating potential competitors for nutrients (Mackie and Wheatley, 1999).

In the present study, all isolates of *P.fluorescens* bacteria showed zone of phosphate solubilization. Isolates 1, 3 and 9 of *P.fluorescens* showed the clearer zone in PVK medium. Similarly, the highest phosphate solubilization zone was also recorded by *Pseudomonas* sp*p.* on PVK medium (Kumar et al., 2012).

To conclude that, application of fungicides for plant disease control are largely affecting human health, normal flora of soil and environment and also pathogenic fungi became very fast resistant to them. For this reason, seed inoculation with *Pseudomonas fluorescens* isolates as a bio-primed seed showed as growth promoter and bio fertilizers are an acceptable alternative to chemical application. Based on present studies, *Pseudomonas fluorescens* isolates under investigation possess a variety of promising properties which make them better growth promoter that are capable of producing plant growth promoting substances, growth and subsequent enhancement of yield of the crop. The use of environmental sociable *Pseudomonas fluorescens* isolates also use for increasing soil fertility and production of faba bean crop through management of chocolates spot disease (*Botrytis fabae*).

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