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*Full Length Research Paper*

# *Pseudomonas fluorescens* isolates as an inducer of physiological activities of faba bean (*Vicia faba*)

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**Plant protection is an important area which needs attention since most of the hazardous inputs added into the agricultural system are in the form of plant protection chemicals. Production of the crop is, however, constrained by fungal plant pathogens infections. The present study was also carried out for estimation of total phenols and flavonoids present in fresh faba bean leaf extract with ethanol. Applying the *Pseudomonas fluorescens* 9 and 10 by bio-primed seed of faba bean treatment enhanced the accumulation of total phenols and flavonoids compared to untreated infected and uninfected untreated faba bean. Bio-priming faba bean seeds with P f9 and P f10 were increased the faba bean total flavonoids in NC 58, Moti and ILB 938 varieties with range 450.58 to 770.19 mg/g compared negative and positive control. The maximum amount of total phenol was recorded 152.25 mg/g in Moti variety with P f9 isolate and 123.70 mg/g was recorded in ILB 938 variety with P f10 isolates. On another hand, all isolates of *P. fluorescens* were tested for certain biochemical production. Consequently all isolate are positive for all tests. Therefore, from this study it is possible to conclude that the use of *P. fluorescens* 9 and 10 isolates could reduce the disease incidence and severity of caused by chocolate spot (*Botrytis fabae*) and simultaneously increased the plant growth promoting and yield performance of faba bean. These isolates can be used as potential biofertilizers, inducer of ant-fungal compounds and also as biocontrol agent against *Botrytis fabae*. So all isolates of *P. fluorescens* can be used as plant growth promoting producing bacteria for increasing the yield production and quality of faba bean.**

**Key words:** *Botrytis fabae*, faba bean, flavonoids, phenols, *Pseudomonas fluorescens*.

## INTRODUCTION

*Pseudomonas fluorescens* is adapted to survival in soil and colonization of plant roots (Kiely et al., 2006) and also applies to the particular fungal and bacterial as biocontrol agents. Biocontrol strains have noticeably been observed at the root surface, the rhizoplane often forming micro colonies or discontinued biofilms in the grooves between epidermal cells (Couillerot et al., 2009). Induced systemic resistance (ISR) is broadly defined as activation of latent defense mechanisms in plants prior to pathogenic attack. The mechanism has been hypothesized in recent years to be an operable mechanism in several rhizobacterial systems. Induced systemic resistance is a state of enhanced defensive capacity developed in plants when appropriately stimulated (Kuc, 2001). The bacterial factors involved in ISR induction comprise antibiotics, flagella, lipopolysaccharides, siderophores and salicylic acid (Bakker, 2007; van Loon et al., 1998).

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Induced systemic resistance is associated with increased synthesis of certain enzymes in plant root such as peroxidase, chitinases and phenylalanine ammonia lyase (Lagriminis and Ruthstein, 1987; Hammerschmid, 1999; Hargreaves et al., 1977), increased levels of certain acid soluble proteins (Zdor and Anderson,1992), enhanced phytoalexin production (Marley and Hillocks, 1993) and the accumulation of phytoalexins in the induced plant tissue (Van peer et al., 1991), strengthening of epidermal and cortical cell walls and deposition of newly formed barriers beyond infection sites including callose, lignin and phenolics (Yedida et al., 1999) enhanced expression of stress-related genes (Zhang et al., 2002).

The seed bacterization of common bean with *P. fluorescens S97* suppressed the halo blight caused by *P. syringe pv phaseolicola* through induced systemic resistance mechanism (Alstrom, 1991). *P. fluorescens* induced systemic resistance against *Rhizoctonia solani* causing sheath blight in rice with a twofold increased activity of pathogenesis related peroxidase and chitinase proteins (Nandakumar et al., 1998).

ISR due to *P. fluorescens* has been demonstrated in several species including carnation (Van peer et al., 1991), radish (Leeman et al., 1995), arabidopsis (Pieterse et al., 1996), cucumber (Wei et al., 1991), and tobacco (Maurhofer et al., 1995). One of the most extensively studied faba bean (*Vicia faba* L.) tissues produce defence responses to pathogen infection is the induced accumulation of secondary metabolites such as furanoacetylenic phytoalexins and chemicalbarriers to microbial attack (phytoanticipins) (Hammerschmidt, 1999; Hargreaves et al., 1977; Fawcett et al., 1971; Ingram and Hebblethwaite, 1976).

Phytoalexins are a diverse group of low molecular weight anti-microbial compounds that are synthesized and accumulated in appreciable amounts in plants after stimulation by various types of pathogens, and are toxic to pathogens (Mansfield and Deverall, 1974; Smith, 1996). Two of the most important of furanoacetylenic phytoalexins are wyerone acid and its methyl ester wyerone, and their induced accumulation in infected tissues causes inhibition of fungal growth (Hammerschmidt, 1999; Mansfield and Deverall, 1974; Letcher et al., 1970; Rossall et al., 1980). Wyerone acid is one of the major furanoacetylenic phytoalexins from broad bean produced in response to infection by *Botrytis fabae Sard.,* the causal organism of chocolate spot disease (Letcher et al., 1970; Soylu et al., 2002). Wyerone is subsequently converted by *B. fabae* to wyerol in broad bean cotyledons (Hargreaves et al., 1976) and to a more anti-fungal wyreone acid in broad bean leaves and pods (Fawcett et al., 1971).

Phytoanticipin are low molecular weight antimicrobial compounds that are present in plants before challenge by microorganisms or are produced after infection solely from preexisting constituents. Some phytoanticipins are found at the plant surface. Others are sequestered as preformed compounds in vacuoles or organelles and released through a hydrolyzing enzyme after pathogen challenge. Because the enzyme involved in the final liberation of the molecule is not formed de novo these compounds are not considered as phytoalexins (Lamothe et al., 2009).

The greater ability of *B. fabae* to colonize faba bean tissues seems to be related to its capacity to detoxify faba bean phytoalexins and to reduce their toxic effects (Hammerschmidt, 1999; Mansfield and Deverall, 1974; Rossall et al., 1980; Hargreaves and Mansfield, 1975; Madeira et al., 1993; Rossall and Mansfield, 1984; Nawar and Kuti, 2003). Phenolic compounds are a class of antioxidant agents which act as free radical terminators (Om Prakash and Yamini, 2007). It is reported that the phenolics are responsible for the variation in the antioxidant activity of the plant (Luo et al., 2004). The mechanisms for phenolic toxicity to microorganisms include substrate deprivation, membrane disruption, and enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Cowan, 1999).

Flavonoids are hydroxylated phenolic substances. Since they are synthesized by plants in response to microbial infection, they have not been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes. Flavonoid compounds exhibit inhibitory effects against multiple viruses (Cowan, 1999). Based on the skeleton of flavonoids some subgroups are: flavones, flavonols, isoflavones, chalcones, aurones (Dewick, 2001).

This study has a great role to create awareness about beneficial aspects of *P. fluorescens* that will be help for proper management and control some fungal disease. Additional this will be serving as primary information for further research. Therefor, this finding is introduce to the *P. fluorescens* isolates have a potential to induce certain secondary metabolites in faba bean crop that responsible against fungal pathogens as well as producing plant growth promoting substance. The present study was designed to isolate certain rhizospheric bacteria of *P. fluorescens* isolates for their stimulate biochemical in faba bean leaves and for biochemical production test.

## MATERIALS AND METHODS

### Soil samples collection

The rhizospheric soil samples were collected in polyethylene from faba bean (*Vicia faba* L.) fields growing of five Localities: Mechale Wartsu at altitude of 2560 m above sea level, Wachale at altitude of 2540 m above sea level, Gore Kateme at altitude of 2590 m above sea level, Eveno at altitude of 2510 m above sea level and Gago at altitude of 2520 m above sea level of North Showa of Oromeia

Region of Salele Zone, Ethiopia. The soils were brought to Mycology Laboratory, Department of Microbial, Cellular and Molecular Biology, College of Natural and Computational Sciences, Addis Ababa University.

### Isolation of *P. fluorescens*

Isolation of *P. fluorescens* isolates were carried out on King's B medium (King et al., 1954). Ten gram of rhizosphere soil sample was suspended in 90 ml of sterile distilled water. Samples were serially diluted and 0.1 ml of sample was spread on King's B medium plates and was incubated at 28°C for 48 h. After incubation, the plates were exposed to UV light at 365 nm for 30 s and the colonies exhibiting the fluorescence were picked up and streaked on the slants for maintenance, purified on King's B medium plates and also designated as Pf 1 to 12 which stand for *P. fluorescens* isolates used for further studies.

### Test pathogen

Standard one isolate of *Botrytis fabae* was obtained from Holeta Agricultural Research Centre, Ethiopian Agricultural Research Institute (EARI), Ethiopia which was isolated from the leaf of infected faba bean which was grown around Holeta locality.

### Plant material

Faba bean seeds used in the present study were obtained from Holeta Agriculture Research Centre, EARI. From this Research Center obtained three varieties of faba bean seeds namely, NC 58 (susceptible), Moti (moderate), and ILB 938 (resistant).

### Biochemical parameters of tested faba bean leaves

#### *Collection of plant material*

The leaves of the plant faba bean collected from Ecology and Ecophysiology Greenhouse, College of Natural and Computational Sciences, Addis Ababa University after 70 days which were sown in the month of May 2012. The faba bean leaves were brought to Mycology Laboratory, Department of Microbial, Cellular and Molecular Biology, College Natural and Computational Sciences, Addis Ababa University for the study of biochemical parameters and testing.

#### *Estimation of total phenol*

The measurement of total phenol was done according to (Mallick and Singh, 1980). For preparation of the calibration curve, 1 ml aliquots of 0.2, 0.4, 0.6, 0.8 and 1.00 mg/ml ethanolic catechol solutions were combined with 0.5 ml Folin-ciocalteaus reagent. After 3 min, 2 ml of 20% sodium carbonate was added and the contents were mixed thoroughly. The final color change was measured calorimetrically at 650 nm and the calibration curve was drawn. Half (0.5 g) of fresh plant tissue was ground using a pestle and mortar with 10 ml of 80% ethanol and centrifuged at 10,000 rpm for 20 min. The supernatant was evaporated to dryness and preserved. The residue was dissolved in 5 ml of distilled water and used as the extract. To 2 ml of the extract, 0.5 ml of Folin Ciocalteau reagent was added. After 3 min, 2 ml of 20% $Na_2CO_3$ solution was mixed in throughly. The mixture was kept at 40°C in water bath for exactly 1 min and after cooling the absorbance was read at 650 nm in a spectrophotometer. The total phenol was determined using a standard curve prepared with different concentrations of gallic acid ($C_7H_6O_5$ $H_2O$). The content of phenolic compounds in plants ethanolic extracts was calculated in the following equation:

$$T = \frac{C+V \times 100}{M}$$

where, T is total content of phenolic compounds, in mg of catechol/100 g of fresh weight material, C is concentration of established from the calibration curve, in mg $ml^{-1}$, V is the volume of extract in ml and M is the weight of pure plant ethanolic extract in g.

### Estimation total flavonoids

Half (0.5 g) of fresh plant tissue was ground using a pestle and mortar with 10 ml of 80% ethanol and centrifuged at 10,000 rpm for 20 min. The supernatant was evaporated to dryness and preserved. The residue was dissolved in 5 ml of distilled water and used as the extract. To 2 ml of the extract, 0.3 ml of 5% sodium nitrate was added to the tubes. After 5 min, 0.3 ml of aluminum chloride ($AlCl_3$) (10%) was added to all the tubes. At the 6 min, 2 ml of sodium hydroxide (1 M) was added to the mixture. Immediately, the contents of the reaction mixture were diluted with 2.4 ml of distilled water and mixed thoroughly. Absorbance of the mixture was determined at 510 nm versus a prepared blank immediately. Gallic acid was used as the standard compound for quantification of total flavonoids (Zhisen et al., 1999).

### Biochemical characterization of *P. fluorescens*

All isolates of *P. fluorescens* were tested for Siderophore (Ramyasmruthi et al., 2012), hydrogen cyanide (Castric, 1975) indole acetic acid (Bric et al., 1991) and ammonia production (Cappuccino and Sherman, 2005) and phosphate-solubilization (Subba Rao, 1999).

### Determination of minimum inhibitory concentration (MIC) for fungicides against *Botrytis fabae*

Different amount of fungicide (10 to 80 mg) were dissolved with 1 ml of sterilized distilled water in test tubes to obtain 10 to 80 mg/ml concentration. Different concentration of fungicide was transferred to King's B medium and mixed uniformly. After complete solidification, 4 mm mycelial disc of *B. fabae* inoculated at the center. They were incubated at 28°C for seven days. The plate containing the least concentration of fungicides showing no visible sign of growth was considered as MIC (Andreuos, 2001).

### Data analysis

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

## RESULTS AND DISCUSSION

### Isolation of *P. fluorescens*

During this research investigation, totally 12 isolates of *P.*

**Figure 1.** *Pseudomonas fluorescens* showing pigment under UV light at 365 nm.


**Figure 2.** *Pseudomonas fluorescens* isolates confirmed again under UV light at 365 nm.

*fluorescens* were isolated from rhizospheric soil of healthy faba bean from five localities of Salale zone of Oromeyia region on King's B medium and observed under UV light at 365 nm for few seconds as indicated Figure 1. Subsequently the culture was purified again on same medium and observed under UV light at 365 Figure 2. All the rhizospheric isolates were tentatively named as indicated in Table 1 and maintained on nutrient agar slants for further study and testing of biochemical production.

**Assessment of secondary metabolites induced by two isolates of *P. fluorescens* (P f9 and P f10) on faba bean varieties**

***Total phenol of faba bean***

Bio-priming faba bean seeds with P f9 and P f10 were increased in the faba bean NC 58, Moti and ILB 938 varieties in total phenol as compared with negative and positive control. The lowest amount of total phenol content (106.42 mg/g) was recorded in NC 58 variety with P f9 isolate whereas 100.50 mg/g was recorded in NC 58 variety with P f10 as indicated in Table 1 after 70 day of sowing of faba bean varieties in the green house.

***Total flavonoids of faba bean***

Bio-priming faba bean seeds with P f9 and P f10 were increased the faba bean total flavonoids in NC 58, Moti and ILB 938 varieties with range 450.58 to 770.19 mg/g compared negative and positive control (383.66 to 532.27 and 358.62 to 447.12) respectively. The lowest amount of flavonoids content 450.58 mg/g was recorded in NC 58 variety with P f9 and 489.81 mg/g was recorded in NC 58 variety with P f10 as indicated in Table 1 after 70 day of planting.

**Biochemical characterization of *P. fluorescens***

All isolates of *P. fluorescens* are positive for all biochemical characterization as indicated in Table 2.

**Determination of minimum inhibitory concentration (MIC) for fungicide**

B. *fabae* was tested in this study to see the in vitro antifungal activity of five types of fungicide Bayleton ®, Curzate ® WP (Cymoxanil 4.2%, cupper oxychloride 39.75 and rest inert), Ridomil Gold MZ (metenoxam 4%, mancozeb 64% and inert 32%) and Sancozeb 80% WP (mancozeb 80% and inert 20%) at different concentration ranges from 10 to 80mg/ml as indicated in Figure 3. *B. fabae* plant pathogen showed differences in their colony diameter growth patterns (mm) at different concentration of Bayleton ®. The result of *in vitro* susceptibility of the tested pathogen. The colony diameter of *B. fabae* was ranges from 54.67 to 27.67 mm and the lowest mycelia growth showed at 80 mg/ml concentration. Whereas the

**Table 1.** The total phenolic and flavonoids content of leaf extract of faba bean (*Vicia fabae*).

| No. | Bio-priming of Faba bean seed | Varieties of Faba bean | Parameter analyzed | |
|---|---|---|---|---|
| | | | Total phenols (mg cat/100 g fresh wt) | Total flavonoids (mg /100 g) |
| 1 | *P. fluorescens* 9 | NC 58 | 106.42 | 450.58 |
| | | Moti | 152.25 | 770.19 |
| | | ILB 938 | 122.32 | 666.35 |
| 2 | *P. fluorescens* 10 | NC 58 | 100.50 | 489.81 |
| | | Moti | 123.03 | 617.65 |
| | | ILB 938 | 123.70 | 595.15 |
| 3 | Negative control | NC 58 | 84.80 | 383.66 |
| | | Moti | 90.60 | 457.85 |
| | | ILB 938 | 102.93 | 532.27 |
| 4 | Positive control | NC 58 | 94.52 | 358.62 |
| | | Moti | 99.29 | 359.42 |
| | | ILB 938 | 109.55 | 447.12 |

Negative control, seed of faba bean treated only with pathogen (*Botrytis fabae) P. fluorescens*, *P. fluorescens,* positive control, seed only not treat with both *Botrytis fabae* and *P. fluorescens*, NC 58, varieties of faba bean seeds has susceptible traits, Moti, varieties of faba bean seeds has moderate traits, ILB 938, varieties of faba bean seeds has resistant traits.

**Table 2.** *P. fluorescens* isolates showing different plant growth promoting activities qualitatively.

| ***Pseudomonas fluorescens* isolates** | **Phosphate solubilization** | **IAA production** | **Ammonia production** | **HCN production** | **Catalase Activity** | **Siderophore Production** |
|---|---|---|---|---|---|---|
| P f1 | + | + | + | + | + | + |
| P f2 | + | + | + | + | + | + |
| P f3 | + | + | + | + | + | + |
| P f4 | + | + | + | + | + | + |
| P f5 | + | + | + | + | + | + |
| P f6 | + | + | + | + | + | + |
| P f7 | + | + | + | + | + | + |
| P f8 | + | + | + | + | + | + |
| P f9 | + | + | + | + | + | + |
| P f10 | + | + | + | + | + | + |
| P f11 | + | + | + | + | + | + |
| P f 12 | + | + | + | + | + | + |
| Control | No | - | - | - | No | No |

+ = Positive result, **-** =Negative result, No= not need control, *P. fluorescens* isolate 1=P f1, *P. fluorescens* isolate2 =P f2, *P. fluorescens* isolate 3=P f3, *P. fluorescens* isolate 4=P f4, *P. fluorescens* isolate 5=P f5, *P.fluorescens* isolate 6=P f 6, *P. fluorescens* isolate 7=P f7, *P. fluorescens* isolate 8=P f 8, *P. fluorescens* isolate9=P f9, *P. fluorescens* isolate 10=P f9, *P. fluorescens* isolate 11=P f11, *P. fluorescens* isolate 12=P f 12, P f = *P. fluorescens* 1,2,3,4,5,6,7,8,9,10,11,12 = isolate code.

colony diameter of B. *fabae* was range from 48.33 to 24.33 mm and the complete mycelia growth inhibited at 80mg/ml concentration of Curzate ® WP. On the other hand, Ridomil Gold MZ showed difference mycelial growth ranges from 28.33 to 14.00 mm at difference concentration of *B*. *fabae* and the lowest growth at observed at 80 mg/ml concentration. While using different concentration of Sancozeb 80% WP on mycelia growth

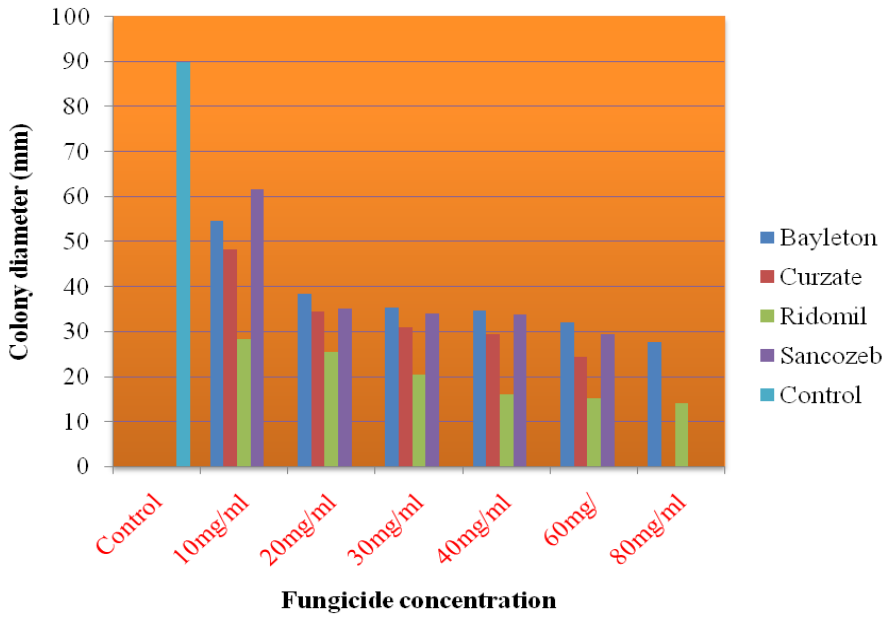


**Figure 3.** MIC for Bayleton ®, Curzate ® WP, Ridomil Gold MZ and Sancozeb 80% WP.

of *B*. *fabae* was ranges 61.67 to 29.33 mm and at 80 mg/ml prevented the mycelial growth of *B*. *fabae*.

The application of bio-primed faba bean seed with Pf 9 isolate increased total phenols in NC 58, ILB 938 and Moti varieties as well as bio-primed with P f10 isolate was increased total phenols in NC 58, Moti, ILB 938 varieties. The maximum amount of total phenol was recorded 152.25 mg/g in Moti variety with P f9 isolate and 123.70 mg/g was recorded in ILB 938 variety with P f10 isolate. Phenolic compounds are secondary metabolites which synthesize in plants for the reduction of plant pathogens. It has been indicated that plants posses some biological properties such as: antioxidant, anti-apoptosis, anti-aging, and anti-inflammation. These results indicate that there is a correlation between the disease resistances due to the concentration of total phenols in faba bean tissue. In a similar way, Mahmoud et al. 2011 has reported that total phenol increased in treated faba bean followed by uninfected untreated plants and infected untreated plants. The total phenols content of infected faba bean was significantly increased due to the effects of the pathogens. It resulted in 238.80 mg catechonl/100g fresh weight compared with the control treatment, which had 149.21 mg catechol/100 g fresh weight (Elwakil et al., 2009). Indole acetic acid (IAA) leads to increase in total phenol content, this material protect plants against pathogen stress (Chowdhury, 2003). Total phenols and phenolics have long been considered as important defence-related compounds whose levels are naturally high in the resistant varieties of many crops (Onyeneho and Hettiarachchy, 1992; Saini et al., 1988).

The value of phenolic content indicates that the plant has antioxidant activity (Rani et al., 2011). The level of polyphenols in the ethanol extract was 5.1 mg/g which was higher when compared to methanol, chloroform, hexane, and aqueous extracts of *Tinospora cordifolia* leaves (Premanath and Lakshmidevi, 2010). The ethanol extracts of *Leptadenia pyrotechnica, Haloxylon salicornicum and Ochradenus baccatus* have high total phenolic content 158.3, 164.4 and 145.3 mg/g extract (Alqasoumi et al., 2012) respectively. The total Phenol content in *Acacia concinna* has been found to be 650 mg/100 ml (Raja and Sama, 2012).

Bio-priming faba bean seeds with P f9 were increased the faba bean of total flavonoids in NC 58, ILB 938 and Moti varieties, where as P f10 increased total flavonoids in the faba bean varieties NC 58, ILB 938 and Moti. The highest amount of flavonoids content 770.19 mg/g was recorded in Moti variety with P f9 and 617.65 mg/g was recorded in Moti variety with P f10. The antioxidant activity of flavonoids is due to their ability to reduce free radical formation and to scavenge free radicals. On other hand, it has been reported as indicate flavonoids are phenolic acids which serve as an important source of anti-oxidants found in different medicinal plants and related phytomedicies (Pietta, 1998). Ethanol extract of the leaves had a flavonoids content of 0.52 mg/g which was higher when compared to methanol, chloroform, hexane, and aqueous extracts of *Tinospora cordifolia* (Premanath and Lakshmidevi, 2010). *Haloxylon salicornicum* and *Leptadenia pyrotechnica* were found to have the highest total flavonoid content 92.0 and 89.0 mg/g extract respectively, while *Ochradenus baccatus* displayed lower total flavonoid content 85.0 mg/g extract (Alqasoumi et al., 2012). The anti-oxidant activity of flavonoids is due to their ability to reduce free radical

formation and to scavenge free radicals or chelating process (Kessler et al., 2003). It acts as scavengers of various oxidizing species that is, super oxide anion ($O_2^-$), hydroxyl radical or per oxy radicals, they also act as quenchers of singlet oxygen (Das and Ratty, 1986). It is evident that the application of fungicides for 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 *P. fluorescens* isolates as a bio-primed seed showed that an inducer of phenol and flavonoids compound in faba bean having inhibitor effect against *B. fabae* is an acceptable alternative to chemical fungicides application. Based on present studies, *P. fluorescens* isolates under investigation possess a variety of promising properties which are capable of producing, enhance the accumulation of antifungal (antioxidant) compound in faba bean leaves and subsequent increased the plant growth performance and yield of the crop.

In this study, the *P. fluorescens* isolates exhibited the plant growth promoting traits like Phosphate-Solubilization, Siderophore production, Hydrogen cyanide production, ammonia production, and indole acetic acid (IAA) production. Hence, they have been advocated as biocontrol agents and plant growth promoting rhizobacteria. Similarly, plant growth promoting rhizobacteria are known to inhibit the growth of plant pathogens by diverse mechanisms such as siderophore production (Loper, 1988), HCN release (Voisard et al., 1989), ammonia and IAA production (Ramyasmruthi et al., 2012) and phosphate solubilization (Kumar et al., 2012).

Bayleton ® 50% WP fungicid was slightly suppressed the radial growth of *B. fabae*. On other hand, Ridomil Gold MZ fungicide was gradually decreased the radial growth of *B. fabae*. While Curzate ® WP and Sancozeb 80% WP were reduced mycelial growth and finally inhibited the mycelia growth with high concentration. Kimber et al. (2007) reported Procymidone, Copper oxychloride and Captan were more effective than other fungicides to control the disease in field trials during 2005 and 2006.

## Conclusion

All isolates of *P. fluorescens* have a tendency to produce certain plant growth promoting substances and also trigger the production secondary metabolites in faba bean resulting in resistant of the crops.

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