J. Trop. Plant Pests Dis.
Vol. 22, No. 2, September 2022
Pages: 100–108

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.222100-108

RESEARCH PAPER

Involvement of secondary metabolites and extracellular lytic enzymes produced by plant growth promoting rhizobacteria in inhibiting the soilborne pathogens in Faba Bean Plants

Manal Sayed Mohammed Khalil[1], Mohamed Hassan Abdel-Rahem Hassan[2], Amer Fayz Mahmoud[2], & Kadry Mostafa Mohamed Morsy[1]

Manuscript received: 6 January 2022. Revision accepted: 9 March 2022. Available online: 2 July 2022.

ABSTRACT

Plant growth promoting rhizobacteria (PGPR) viz. *Pseudomonas fluorescens*, *Bacillus megaterium*, *B. subtilis*, and *B. cereus* and their metabolic products may play a pivotal role in controlling root rot and wilt diseases in faba bean plants caused by *Rhizoctonia solani*, *Fusarium solani*, *F. oxysporum*, and *Macrophomina phaseolina* and promote plant growth under greenhouse and field conditions. Cell cultures, extracellular metabolites, volatile metabolites of all tested PGPR strains were suppressed the linear growth of all tested pathogenic fungi *in vitro*. *P. fluorescens* followed by *B. megaterium* were more active than *B. subtilis* and *B. cereus* in reduction of the tested fungi radial growth. All PGPR strains were able to produce IAA, HCN, siderophore, Ammonia in media growth. *P. fluorescens* produced the highest levels of cyanide hydrogen and ammonia followed by *B. subtilis*, while the higher level of IAA was produced by *B. subtilis* followed by *P. fluorescens*. Also, *B. megaterium* was the most PGPR strain produced siderophore followed by *P. fluorescens*. All the tested PGPR strains successfully solubilized inorganic phosphate on Pikovskya's agar medium. Also, all plant growth promoting rhizobacteria strains (PGPR) were able to produce mycolytic enzymes viz. cellulase, chitinase β-1,3-glucanase, amylase and protease except *B. cereus* and *B. megaterium* not able to produce protease and amylase. *B. megaterium* recorded the highest activities of chitinase, β-1,3-glucanase, while, *B. cereus* produced the lowest levels of all tested enzymes.

Key words: faba bean, PGPR, root rot and wilt, secondary metabolites and extracellular lytic enzymes

INTRODUCTION

Faba bean (*Vicia faba* L.), family Fabaceae considered the most important nutritive popular food crop in the world and Egypt. It plays a major role in the Egyptian diet as a source of protein. Faba bean crop rich in protein (protein content ranges from 26 to 41%) and the supply of essential amino acids (Fernández et al., 1996). Several investigators recorded that diseases found on faba beans were considered the most destructive and caused considerable losses in yield (estimated at over 50%). Several root rot and wilt pathogens such as *R. solani*, *F. oxysporum*, *F. solani* and *M. phaseolina* are reported to attack faba bean roots and stem base causing serious losses in seed germination and plant stand as well (Abdel-Monaim, 2013).

Plant growth promoting rhizobacteria (PGPR) such as Bacillus strains and *P. fluorescens* are the major root colonizers (Zaim et al., 2013), and can elicit plant defense resistance. Different mechanisms have been reported for their performance such as production of antibiotics, siderophore cyanide hydrogen, competition for nutrition and space, induce resistance, inactivation of pathogen's enzymes and enhancement of root and plant development (Karimi et al., 2012). PGPR can prevent the proliferation of fungal and other pathogens by producing siderophores that bind most of the FeIII in the vicinity of the plant root, preventing pathogens from growing close to the plant roots. This is achieved by the PGPR out-competing the pathogens for available iron. Plants are not affected by the localized depletion of soil iron as most plants can tolerate much lower iron concentrations (~1000 fold less) than microorganisms. Similarly, cyanide is a secondary metabolite produced by Gram-negative bacteria.

HCN and CO_2 are formed from glycine and catalysed by HCN synthesis. HCN production by strains of PGPR suppresses disease, whereas mutant strains

Corresponding author:
Manal Sayed Mohammed Khalil (fowzy_2008@yahoo.com)

[1]Leguminous Crops and Fodder Disease Research Department, Plant Pathology Research Institute, ARC, Giza 12619,Egypt
[2]Plant Pathology Department Faculty of Agriculture, Assiut University, 71526 Assiut, Egypt

unable to synthesise HCN lose their ability to protect plants from disease. Also, many antifungal metabolites have been produced and shown to be effective in vitro. These antifungal metabolites are also suspected to have antifungal activity *in vivo*. These metabolites include ammonia, butyrolactones, 2-4-diacetylphloroglucinol, kanosamine, Oligomycin A, Oomycin A, phenazine-1-carboxylic acid (PCA), pyoluterin (Plt), pyrrolnitrin (pln), viscosinamide, xanthobaccin and zwittermycin A (Whipps, 2001). In addition, certain fungi have been shown to be sensitive to particular combinations of metabolites.

The microorganisms isolated from the rhizosphere of various crops have the ability to produce indole acetic acid as secondary metabolites due to rich supply of substrates. Indole acetic acid (IAA) helps in the production of longer roots with increased number of root hairs and lateral roots which are involved in nutrient uptake (Datta & Basu, 2000). IAA stimulates cell elongation by modifying certain conditions such as the increase in osmotic contents of the cell, increase in permeability of water into cell, decrease in wall pressure, an increase in cell wall synthesis and inducing specific RNA and protein synthesis. It promotes embial activity, inhibits it promotes embial activity, inhibits or delays the abscission of leaves, induces flowering and fruiting (Narasimhan et al., 2013).

Also, Chitinase, glucanase and other hydrolytic enzymes have many roles in a wide range of different biological systems. These enzymes are usually extracellular, of low molecular weight and high stability. In addition, they may be produced in multiple forms or isozymes that differ in charge, size, regulation, stability and ability to degrade cell walls (Ashwini & Srividya, 2014). The objectives of the present study were to test the possible role of in vitro production of mycolytic enzymes viz. chitinase, β-1, 3-glucanase, protease siderophores, HCN and IAA by PGPR strains *viz. B. subtilis* and *B. megaterium* in suppression of *F. oxysporum* f. sp. *ciceris*. In addition, to study the control wilt disease under artificial inoculation in greenhouse and under natural infection under field conditions as well as its effect on growth and yield parameters under field conditions.

MATERIAL AND METHODS

Research Site. All experiments were carried out at the Agricultural Research Station in the New Valley, New Valley Governorate, Egypt.

Source of Fungal Pathogen. The highly pathogenic isolate of *R. solani, F. solani*, *F. oxysporum* and *M. phaseolina* isolated from diseased chickpea plants collected from New Valley Governorate was used in this study (Khalil, 2019).

Preparation of Fungal Inoculum. The inoculum of pathogenic fungi was prepared from one week old culture grown on 50 mL potato dextrose broth (PDB) medium in conical flask (250 mL) and incubated at 25 ± 1 °C. The content of flask was homogenized in a blender for one min. Plastic pots were filled with sterilized soil and mixing with fungal inocula at rate of 100 mL homogenized culture per pot, seven days before planting.

Source of the PGPR. Plant growth promoting rhizobacteria (PGPR) namely, *B. megaterium* ((isolate BMM5), *B. subtilis* (isolate BSM1) and *B. cereus* (BCM8) and *P. fluorescens* (PFM1) were obtained from the Laboratory of Plant Pathology Department, New Valley Agric. Res. Station.

In Vitro Screening of Inhibitory Effect of PGPR. The tested isolates of antagonistic PGPR were streaked at one side on a PDA medium in plates and incubated for 24 hours at 25 °C ± 1, then one disc (7 mm in diameter) of tested pathogenic fungi *viz. R. solani, F. solani, M. phaseolina* and *F. oxysporum* were placed on the opposite side (Kaur et al., 2007). The inoculated plates with pathogenic fungus only were used as control. After 3-7 days incubation, linear growth of pathogenic fungi in all treatments was recorded. The decrease of percentage that occurred in linear growth of the pathogenic fungi was determined at the end of the experiment using formula as follows:

$$\text{Reduction in linier growth} = \left[\frac{(R1 - R2)}{R1}\right] \times 100$$

R_1= the radius of normal growth in control plates;
R_2= the radius of inhibited growth

Enzymatic Activities and Secondary Metabolites of the PGPR Strains

Production of Indole Acetic Acid (IAA). The production of IAA was determined by colorimetric measurement at 530 nm using Salkowski's reagent. Bacteria were grown under shaking (120 rpm) for 2 days at 30 ºC in Luria Bertani broth medium (Tryptone 10.0 g, Yeast Extract 5.0 g Sodium Chloride 10.0 g) supplemented with tryptophan (1 mg mL^{-1}) as IAA precursor. After incubation, the cells were centrifuged (3000 rpm for 10 min at 4 °C) and 1 mL of supernatant was combined with 2 mL of Salkowski's reagent (150 mL of 95-98% H_2SO_4,

7.5 mL of 0.5 M $FeCl_3 \cdot 6H_2O$, and 250 mL distilled water) and incubated for 30 min at room temperature. The quantification of IAA was carried out using a standard curve of pure IAA (Sigma−Aldrich, Co.).

Production of Hydrogen Cyanide (HCN). Bacterial strains were grown at 25 ± 2 °C on a rotary shaker in Tryptic Soy Broth (TSB). Filter paper (Whatman No. 1) was cut into uniform strips of 10 cm long and 0.5 cm wide saturated with alkaline picrate solution and placed inside the conical flasks in a hanging position. After incubation at 25 ± 2 °C for 48 hours, the sodium picrate present in the filter paper was reduced to reddish compound in proportion to the amount of hydrocyanic acid evolved. The developed colour was eluted in 10 mL of distilled water and the absorbance was measured at 625 nm.

Production of Siderophore. Bacterial strains were grown in KB broth for 3 days at 25 ± 2 °C and centrifuged at 3000 rpm for 10 min and the supernatants were collected. The pH of the supernatant was adjusted to 2.0 with diluted HCl and equal quantity of ethyl acetate was added in a separating funnel, mixed well and ethyl acetate fraction was collected. Five milliliters of ethyl acetate fraction were mixed with 5 mL of Hathway's reagent (1.0 mL of 0.1M $FeCl_3$ in 0.1 N HCl to 100 mL distilled water1.0 mL of potassium ferricyanide). The absorbance for dihydroxy phenols was read at 700 nm. A standard curve was prepared using dihydroxy benzoic acid. The quantity of siderophore synthesized was expressed as µmol benzoic acid/mL of culture filtrate.

Ammonia Production. Freshly grown culture was inoculated into peptone water and incubated for 7, 10, and 13 days at 27±2 °C. Broth was collected, centrifuged and the amount of ammonia in the supernatant was estimated by means of Nesslerization reaction 1 mL Nessler's reagent was added to 1 mL of supernatant and volume of this mixture was made up to 10 mL by addition of ammonia-free distilled water. Development of brown to yellow color was a positive test for ammonia production and optical density was measured by spectrophotometer at 450 nm (Demutskaya & Kalinichenko, 2010). The concentration of ammonia was estimated based on a standard curve of ammonium sulfate ranging from 0.1 to 1 µmol mL^{-1}.

Phosphate Solubilization. To detect the ability of organisms to solubilize phosphate, isolates were spot inoculated using sterile tooth picks onto Pikovskaya's Agar medium (Nautiyal, 1999) containing (per liter) tricalcium phosphate 2.5 g, glucose 13 g, (NH_4) SO_4 0.5 g, NaCl 0.2 g, MgSO4.$7H_2O$ 0.1 g, KCl 0.2 g, yeast extract 0.5 g, $MnSO_4$ trace, $FeSO_4.7H_2O$ trace, agar 15 g, pH adjusted to 7.2. Plates were incubated for three days at 28 ± 2°C. The strains showing halo zone of tricalcium phosphate solubilization were considered positive. Phosphate solubilization index (SI) was calculated by measuring the diameter of halo zone and bacterial colony using the formula (Edi-Premoto et al., 1996).

$$SI = \frac{\text{colony diameter} + \text{halo zone diameter}}{\text{colony diameter}}$$

Assay of Chitinase. PGPR strains were cultured at 25 ± 2 °C for 96 hours on a rotary shaker in 250 mL conical flasks containing 50 mL of chitin–peptone medium (glucose 0.5%, peptone 0.2%, colloidal chitin 0.2%, K_2HPO_4 0.1%, $MgSO_4.7H_2O$ 0.05% and NaCl 0.05%, pH 6.8). The cultures were centrifuged at 12,000 g for 20 min at 4 °C and the supernatant was used as an enzyme source. Colloidal chitin was prepared from crab shell chitin according to Berger & Reynolds (1958). The reaction mixture contained 0.25 mL of enzyme solution, 0.3 mL of 1 M sodium acetate buffer (pH 5.3) and 0.5 mL of colloidal chitin (0.1%). The reaction mixture was incubated at 50 °C for 4 hours in a water bath. Chitinase activity was determined by measuring the release of reducing sugars by the method of Nielsen & Sorensen (1999). One unit of chitinase was determined as 1 nmol of N-acetyl-β-D-glucosamine (GlcNAc) released per minute per mg of protein.

Assay of β-1, 3-glucanase. Bacterial strains were grown at 25 ± 2 °C for 96 hours on a rotary shaker in 250 mL conical flasks containing 50 mL of peptone medium containing laminarin (0.2%) (Laminaria digitata; Sigma). The cultures were then centrifuged at 12,000 g for 20 min at 4 °C and the supernatant was used as enzyme source. The reaction mixture contained 0.25 mL of enzyme solution, 0.3 mL of 0.1M phosphate buffer (pH 5.5) and 0.5 mL of laminarin (0.2%). The reaction mixture was incubated at 40 °C for 2 hours in water bath. β-1,3-glucanase activity was determined as 1 nmol of glucose released/min/mg of protein.

Protease Production. Bacterial strains were streaked on casein agar and/or incubated at 28 ± 2 °C for four days. At the end of the incubation, the plates were observed for halo zone around the colonies, which indicates the presence of protease. The halo zone diameters were recorded.

Cellulase Production. Carboxy methyl cellulose (CMC)

agar plates were prepared by screening for cellulose enzyme production according to the method by Kasana et al. (2008). A sterile paper disc was dipped into microbial culture and transferred onto the CMC agar plates. The plates were incubated overnight at 33 °C. After incubation, the plates were flooded with Congo red solution for 15 min, followed by de-staining with the salt solution for 15 min. Unstained areas indicate where the CMC has been degraded due to production of cellulose by the bacterial strain. The halo zone diameters were recorded.

Amylase Production. Amylase production was determined by the method described by Alariya et al. (2013). Fresh bacterial cultures were inoculated on the Starch Agar plate and incubated at room temperature for 24–48 hours. Plate was flooded by Iodine solution after incubation. The hydrolysis of starch is observed as a colourless zone around growth with violet background and is reported as trace to 4.

Determination of Extracellular Compounds. A 0.2 µm cellophane membrane was placed on PDA plates and 200 µL of antagonistic bacterial suspension (1×10^7 cfu/mL) were inoculated in the center of plates. The plates were incubated at 25 ± 2 °C for 48 hours, then the membrane with the grown bacterial strains was removed and the plate was inoculated in the middle with a five mm disk of a pure culture of *R. solani*, *F. solani*, *M. phaseolina* and *F. oxysporum*, individually. Plates were incubated at 25 ± 2 °C for 7 days and the radial growth of the pathogen was measured. Sterile double-distilled water replaced the bacterial suspension in control plates. There were four replicates for each treatment (Naureen et al., 2009; Kraus & Loper, 1990).

Production of Volatile Antibiotics. Firstly, 200 µL of bacterial suspension (1×10^7 cfu/mL) from each strain were spread on the surface of a Petri plate containing nutrient agar medium and incubated at 25 ± 2 °C for two days. In another Petri plate containing PDA medium, a 5 mm disk of a 7-days-old culture of tested pathogenic fungi *viz. R. solani*, *F. solani*, *M. phaseolina* and *F. oxysporum* were placed at the center. Then both half plates were placed face to face preventing any physical contact between the pathogen and the bacterial suspension (Abdel-Monaim, 2016). The plates were sealed with parafilm. In the control plates, pathogenic fungi were placed at the center. Plates were incubated at 25 ± 2 °C for 48 hours and the percentage of inhibition was calculated for each isolate in four replicates for each treatment.

Statistical Analysis. Analyses of variance were carried out using MSTATC, 1991 program (Ver. 2.10). Least significant difference was employed to test for significant difference between treatments at $p \leq 0.05$ (Gomez & Gomez, 1984).

RESULTS AND DISCUSSION

In vitro Screening Inhibitory Effect of PGPR. Plant growth promoting rhizobacteria *viz., B. megaterium*, *B. subtilis*, *B. cereus* and *P. fluorescens* strains were evaluated for antagonistic effect against *R. solani*, *F. solani*, *M. phaseolina* and *F. oxysporum* on Petri dishes containing PDA medium. Data in Figure 1 show that all PGPR strains succeeded in reducing the radial growth of all tested pathogenic fungi. *P. fluorescens* followed by *B. megaterium* were more active than *B. subtilis* and *B. cereus* for suppressing radial growth of tested fungi. On the other hand, *F. solani* followed by *F. oxysporum* was more affected with PGPR, while *R. solani* and *M. phaseolina* was the lowest one. Such results confirm previous reports indicating that PGPR has the potential to inhibit growth of soil borne pathogens (Abdel-Monaim, 2010; Susilowati et al., 2011; Sarhan & Shehata, 2014). Glick (1995) reported that the most effective mechanism that a PGPR can employ is to prevent proliferation of phytopathogens in the synthesis of antibiotics.

Enzymatic Activities and Secondary Metabolites of the PGPR Strains. Results of testing 4 PGPR strains (*B. megaterium*, *B. subtilis*, *B. cereus*, *P. fluorescens*) in vitro against tested pathogenic fungi showed that different strains exhibited different combinations of antimicrobial metabolites such as HCN, Siderophore, IAA, Ammonia production and Phosphate solubilisation. *Pseudomonas fluorescens* produced the highest levels of cyanide hydrogen and ammonia followed by *B. subtilis*. The higher level of IAA produced *B. subtilis* followed by *P. fluorescens*. On the other hand, *B. megaterium* is the most PGPR strain produced of siderophore followed by *P. fluorescens*. On the other hand, all the tested PGPR strains successfully solubilized inorganic phosphate on Pikovskya's agar medium (Table 1), indicated by forming a clear halo around their PGPR colony. The maximum phosphate solubilization after 72 hours for plates inoculated with *B. megaterium* (21.3 mm) followed by *P. fluorescens* (18.7 mm), while plates inoculated with *B. subtilis* and *B. cereus* where recorded phosphate solubilization 13.8 and 14.8 mm, respectively.

On the other hand, all plant growth promoting rhizobacteria strains (PGPR) were able to produce mycolytic enzymes *viz*. cellulase, chitinase β-1,3-

glucanase, amylase and protease in growth media except *B. cereus* and *B. megaterium* not able to produce protease and amylase in media growth, respectively (Table 1). *B. megaterium* recorded the highest activities of chitinase, β-1,3-glucanase in media growth compared with the other PGPR strains, whereas it produced 23.12 and 80.12 nmol/min/mg protein, respectively. While, *Ps. fluoresces* produced the highest protease and amylase in media growth (20.08 and 14.75 halo zone diameter). *B. subtilis* produced the highest cellulase level in media growth (16.35 halo zone diameter). On the other hand, *B. cereus* produced the lowest levels of all tested enzymes (chitinase, β-1,3-glucanase, protease, cellulase and amylase) in media growth).

The results agree with Kumar et al. (2009), Sarhan & Shehata (2014) and Choudhary & Sindhu (2015). Arora et al. (2007) found that the lytic enzymes produced by biocontrol agents interfere with the growth and activities of pathogen by hydrolyzing the polymeric compounds, including chitin, proteins, celluloses, hemicelluloses, etc., and consequently killing or suppressing the growth of pathogens. Hydrolytic enzymes showed their ability to control plant pathogens. A hydrolytic enzyme like chitinase, glucanase, protease and cellulase can degrade the fungal cell-wall and cause the cell lysis of fungal pathogens. Also, Abdel-Monaim (2016) found that PGPR strains *viz. B. subtilis* and *B. megaterium* were able to produce indole acetic acid (IAA), siderophore, hydrogen cyanide (HCN), extracellular compound and volatile antibiotics in vitro. In addition, both PGPR strains produced mycolytic enzymes *viz.* chitinase, β-1, 3-glucanase and protease in growth media. *B. megaterium* produced greater amounts of secondary metabolites than *B. subtilis*.

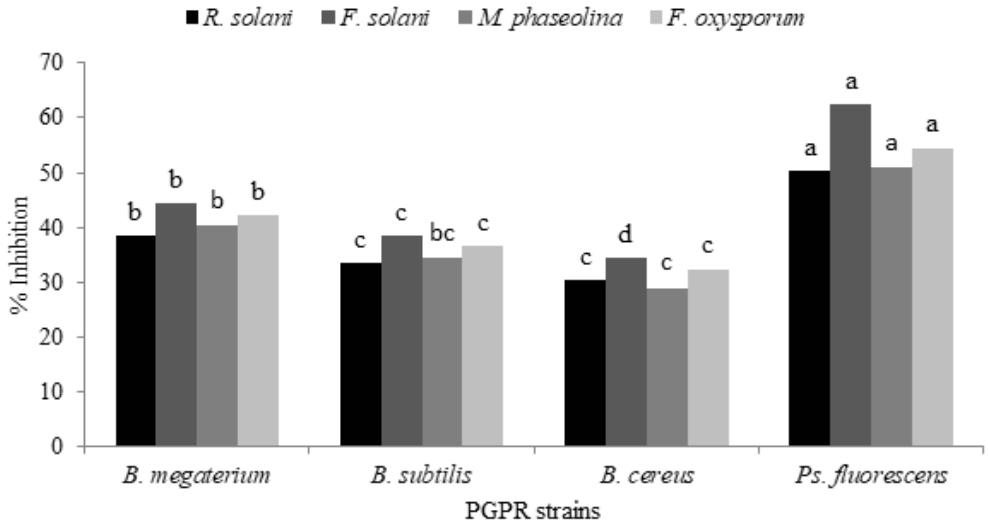

Figure 1. Effect of PGPR on mycelia growth of *R. solani, F. solani, M. phaseolina,* and *F oxysporum* in dual culture. Different letters indicate significant differences among treatments according to least significant difference test (LSD) (p= 0.05).

Table 1. *In vitro* production of various metabolites, enzymes by PGPR strains

Antifungal metabolites	*B. megaterium*	*B. subtilis*	*B. cereus*	*P. fluorescens*
Production of IAA (µg/mL)	0.805	1.124	0.736	1.086
Production of HCN (OD at 625 nm)	0.098	0.075	0.157	0.198
Production of siderophore (µmol benzoic acid/mL)	6.25	4.52	3.56	5.42
Ammonia production (µg/mL)	25.60	32.50	24.10	36.50
Phosphate solubilization index (mm)	21.30	13.80	14.80	18.70
Chitinase activity (nmol/min/mg protein)	23.12	17.45	15.43	19.69
β-1,3-glucanase activity (nmol/min/mg protein)	80.12	72.35	62.14	77.43
Protease production (halo zone diameter with mm)	14.35	8.36	0.00	20.08
Cellulase production (halo zone diameter with mm)	12.52	16.35	10.41	14.85
Amylase production (halo zone diameter with mm)	0.00	12.42	10.95	14.75

Effect of Extracellular Metabolites. Data present in Figure 2 show that all plant growth promoting rhizobacteria (*B. megaterium*, *B. subtilis*, *B. cereus* and *P. fluorescens* produced extracellular metabolites. *P. fluorescens* and *B. megaterium* were significantly the highest produced extracellular metabolites against all tested pathogenic fungi. While *B. cereus* and *B. subtilis* recorded the lowest produced extracellular metabolites in case of all pathogenic fungi. On the other hand, *F. solani* was the most affected with extracellular metabolites produced with PGPR, while *M. phaseolina* was the least affecting ones in this respect.

These results revealed that certain extracellular metabolites generated by PGPR affected mycelial growth and germ tube elongation. Some Bacillus species are known to produce numerous antimicrobial compounds which have been well characterized genetically and biochemically in vitro (Arguelles-Arias et al., 2009). Among these antagonistic compounds, the predominant lipopeptides of the surfactin, iturin and fengycin families have been well reported for their potential against a wide range of plant pathogens (Pathak et al., 2013).

Effect of Volatile Antibiotics. ANOVA results showed that the colony diameter of tested pathogenic fungi (*R. solani*, *F. solani*, *M. phaseolina* and *F. oxysporum* varied significantly ($p \leq 0.05$) depending on antagonistic treatments tested. In fact, data given in Figure 3, revealed that all tested pathogenic growth decrease, due to the inhibitory effects of volatile metabolites from the rhizobacteria strains tested, was varied by different PGPR strains and pathogenic fungi. *P. fluorescens* was recorded the highest inhibitory effect against all tested pathogenic fungi followed by *B. megaterium*, while *B. subtilis* and *B. cereus* gave the lowest ones against all pathogenic fungi in this respect. Volatile inhibitors that have been identified include ethylene, ammonia, allyl alcohol, acrylic acid, trimethylamine, benzaldehyde,

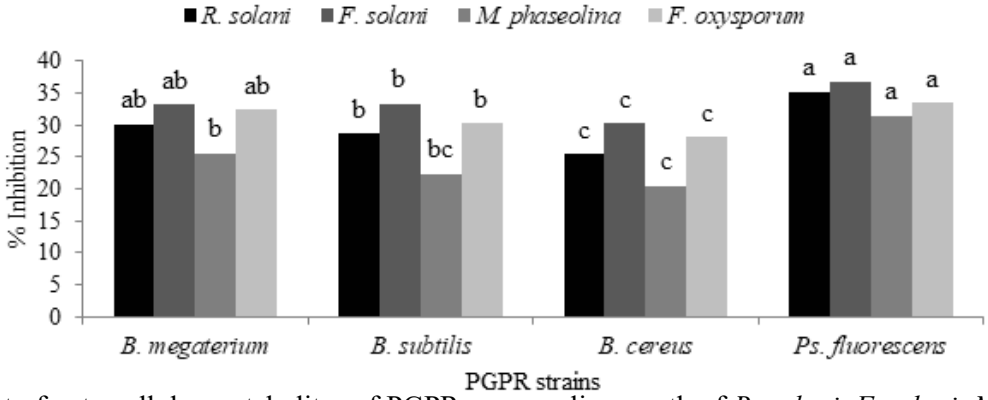

Figure 2. Effect of extracellular metabolites of PGPR on mycelia growth of *R. solani, F. solani, M. phaseolina,* and *F. oxysporum* in dual culture. Different letters indicate significant differences among treatments according to least significant difference test (LSD) ($p \leq 0.05$).

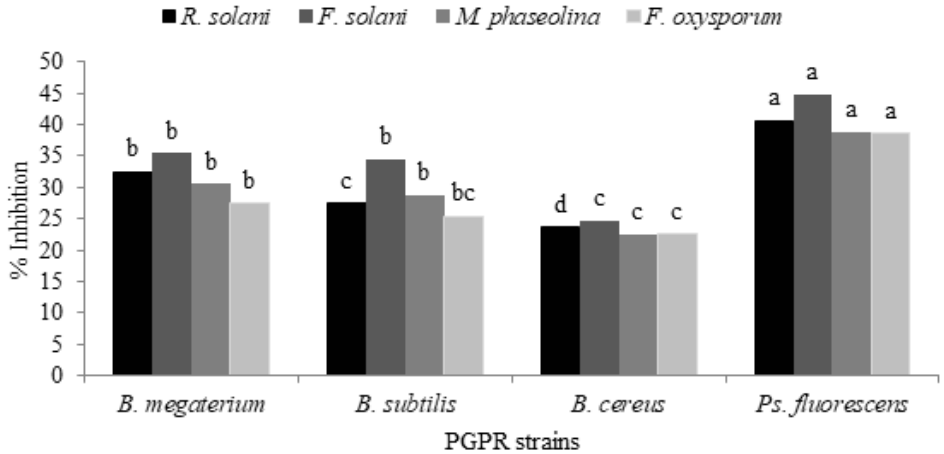

Figure 3. Effect of volatile antibiotics secreted by PGPR on mycelia growth of *R. solani, F. solani, M. phaseolina,* and *F. oxysporum*. Different letters indicate significant differences among treatments according to least significant difference test (LSD) ($p \leq 0.05$).

and N,N-dimethyloctylamine (Chuankun et al. 2004; El-Khoury & Makkouk, 2010). The spectrum of volatile compounds produced appears to depend not only on environmental conditions, such as nutrients, temperature, pH and moisture, but also on the organism itself. Ryu et al. (2004) demonstrated significant growth promotion of Arabidopsis by *B. subtilis* strain GB03 and *B. amyloliquefaciens* strain IN937a. Among VOCs released from strain GB03, 2,3-butanediol was found as a major component that promoted plant growth and elicited ISR against *Erwinia carotovora* subsp. *carotovora*. Several mutant lines of Arabidopsis, including brassinosteroid- and gibberellic acid-insensitive mutants, auxin-transport-deficient mutants, and cytokinin receptor-deficient mutants, were used to elucidate the signaling pathways that promote growth.

CONCLUSION

The application of PGPR and its secondary metabolites could control Fusarium wilt disease in faba bean. Their low cost, low toxicity to man and environmental pollution make them ideal for seed soaking for disease control under field conditions and also for increased seed yield and seed content of protein.

ACKNOWLEDGMENTS

The researchers extend their sincere thanks and appreciation to Prof. Dr. Montaser Fawzy Abdel-Moneim, Head of the Plant Diseases Department at the Regional Station for Agricultural Research in the New Valley, for his assistance in carrying out laboratory experiments at the station.

FUNDING

The research was funded by the Agricultural Research Center of the Egyptian Ministry of Agriculture within the ministry's plan to raise the efficiency of scientific research in the Arab Republic of Egypt.

AUTHORS' CONTRIBUTIONS

MSM implemented the research plan, applied it on the ground, and carried out experiments in the laboratory, MHARH developed a research plan and provided the necessary capabilities for the application and AFM & KMMM contributed to data analysis, statistical analysis and research writing.

COMPETING INTEREST

The research is an introduction to its publication to help the researcher to be promoted to a higher degree.

REFERENCES

Abdel-Monaim MF. 2010. Integrated management of damping-off, root and/or stem rot diseases of chickpea with sowing date, host resistance and bioagents. *Egypt J. Phytopathol*. 38(1): 45–61.

Abdel-Monaim MF. 2013. Improvement of biocontrol of damping-off and root rot/wilt of faba bean by salicylic acid and hydrogen peroxide. *Mycobiology*. 41(1): 47–55. https://doi.org/10.5941/MYCO.2013.41.1.47

Abdel-Monaim MF. 2016. Efficacy of secondary metabolites and extracellular lytic enzymes of plant growth promoting rhizobacteria (PGPR) in controlling Fusarium wilt of chickpea. *Egypt. J. Agric. Res*. 94(3): 573–589. https://doi.org/10.21608/ejar.2016.152587

Alariya SS, Sethi S, Gupta S, & Gupta BL. 2013. Amylase activity of a starch degrading bacteria isolated from soil. *Arch. Appl. Sci. Res*. 5(1): 15–24.

Arguelles-Arias A, Ongena M, Halimi B, Lara Y, Brans A, Joris B, & Fickers P. 2009. Bacillus amyloliquefaciens GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens. *Microb. Cell Fact.* 8: 63. https://doi.org/10.1186/1475-2859-8-63

Arora NK, Kim MJ, Kang SC, & Maheshwari DK. 2007. Role of chitinase and beta-1,3-glucanase activities produced by a fluorescent pseudomonad and in vitro inhibition of *Phytophthora capsici* and *Rhizoctonia solani*. *Can. J. Microbiol*. 53(2): 207–212. https://doi.org/10.1139/w06-119

Ashwini N & Srividya S. 2014. Potentiality of *Bacillus subtilis* as biocontrol agent for management of anthracnose disease of chilli caused by *Colletotrichum gloeosporioides* OGC1. *3 Biotech*. 4(2): 127–136. https://doi.org/10.1007/s13205-013-0134-4

Berger LR & Reynold DM. 1958. The chitinase system of a strain of *Streptomyces griseus*. *Biochim. Biophys. Acta*. 29(3): 522–534. https://doi.org/10.1016/0006-3002(58)90008-8

Choudhary SR & Sindhu SS. 2015. Suppression of *Rhizoctonia solani* root rot disease of clusterbean (*Cyamopsis tetragonoloba*) and plant growth promotion by rhizosphere bacteria. *Plant Pathol. J.* 14(2): 48–57. https://doi.org/10.3923/ppj.2015.48.57

Chuankun X, Minghe M, Leming Z, & Keqin Z. 2004. Soil volatile fungistasis and volatile fungistatic compounds. *Soil Biol. Biochem*. 36(12): 1997–2004. https://doi.org/10.1016/j.soilbio.2004.07.020

Datta C & Basu PS. 2000. lndole acetic acid production by a *Rhizobium* species from root nodules of a leguminous shrub *Cajanus cojan*. *Microbiol. Res*. 155(2): 123–127. https://doi.org/10.1016/S0944-5013(00)80047-6

Demutskaya LN & Kalinichenko IE. 2010. Photometric determination of ammonium nitrogen with the nessler reagent in drinking water after its chlorination. *J. Water Chem. Technol*. 32(2): 90–94. https://doi.org/10.3103/S1063455X10020049

Edi-Premono M, Moawad AM, & Vlek PLG. 1996. Effect of phosphate solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. *Indonesian J. Crop Sci*. 11: 13–23.

El-Khoury W & Makkouk K. 2010. Integrated plant disease management in developing countries. *J. Plant Pathol*. 92(4): S35–S42.

Fernández M, López-Jurado M, Aranda P, & Urbano G. 1996. Nutritional assessment of raw and processed faba bean (*Vicia faba* L.) cultivar major in growing rats. *J. Agric. Food Chem*. 44(9): 2766–2772. https://doi.org/10.1021/jf9505483

Glick BR. 1995. The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol* 41(2): 109–117. https://doi.org/10.1139/m95-015

Gomez KA & Gomez AA. 1984. *Statistical Procedures for Agricultural Research*. A Wiley-Interscience Publication, John Wiley & Sons. New York.

Karimi K, Amini J, Harighi B, & Bahramnejad B. 2012. Evaluation of biocontrol potential of *Pseudomonas* and *Bacillus* spp. against Fusarium wilt of chickpea. *Aust. J. Crop Sci*. 6(4): 695–703.

Kasana RC, Salwan R, Dhar H, Dutt S, & Gulati A. 2008. A rapid and easy method for the detection of microbial cellulases on agar plates using gram's iodine. *Curr. Microbiol*. 57(5): 503–507. https://doi.org/10.1007/s00284-008-9276-8

Kaur R, Kaur J, Singh RS, & Alabouvette C. 2007. Biological control of *Fusarium oxysporum* f.sp. *ciceri* by nonpathogenic Fusarium and fluorescent *Pseudomonas*. *Int. J. Bot*. 3(1): 114–117. https://doi.org/10.3923/ijb.2007.114.117

Khalil (Manal) SM. 2019. Utilizing Some Disease Resistance Inducing Agents and Magnetized Water in the Management of Root Rot and Wilt of Faba Bean. Ph. D. *Thesis*. Faculty Agriculture, Assuit University.

Kraus J & Loper JE. 1990. Biocontrol of Phytium damping-off of cucumber by *Pseudomonas fluorescens* PF-5: mechanistic studies. In: Keel CB & Defago G (Eds.). *Plant growth promoting rhizobacteria. The Second International Workshop on Plant Growth Promoting Rhizobacteria*. pp. 172–175. Interlacen, Switzerland.

Kumar S, Pandey P, & Maheshwari DK. 2009. Reduction in dose of chemical fertilizers and growth enhancement of sesame (*Sesamum indicum* L.) with application of rhizospheric competent *Pseudomonas aeruginosa* LES4. *Eur. J. Soil Biol*. 45(4): 334–340. https://doi.org/10.1016/j.ejsobi.2009.04.002

Narasimhan A, Bist D, Suresh S, & Shivakumar S. 2013. Optimization of mycolytic enzymes (chitinase, β-1,3-glucanase and cellulase) production by *Bacillus subtilis*, a potential biocontrol agent using one-factor approach. *J. Sci. Ind. Res*. 72(3): 172–178.

Naureen Z, Price AH, Hafeez FY, & Roberts MR. 2009. Identification of rice blast disease-suppressing bacterial strains from the rhizosphere of rice grown in Pakistan. *Crop Prot*. 28(12): 1052–1060. https://doi.org/10.1016/j.cropro.2009.08.007

Nautiyal CS. 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiol. Lett.* 170(1): 265–270. https://doi.org/10.1111/j.1574-6968.1999.tb13383.x

Nielsen MN & Sørensen J. 1999. Chitinolytic activity of *Pseudomonas fluorescens* isolates from barley and sugar beet rhizosphere. *FEMS Microbiol. Ecol*. 30(3): 217–227. https://doi.org/10.1111/j.1574-6941.1999.tb00650.x

Pathak KV & Keharia H. 2013. Characterization of

fungal antagonistic bacilli isolated from aerial roots of banyan (*Ficus benghalensis*) using intact-cell MALDI-TOF mass spectrometry (ICMS). *J. Appl. Microbiol*. 114(5): 1300–1310. https://doi.org/10.1111/jam.12161

Ryu CM, Farag MA, Hu CH, Reddy MS, Kloepper JW, & Paré PW. 2004. Bacterial volatiles induce systemic resistance in Arabidopsis. *Plant Physiol*. 134(3): 1017–1026. https://doi.org/10.1104/pp.103.026583

Sarhan EAD & Shehata HS. 2014. Potential plant growth-promoting activity of *Pseudomonas* spp. and *Bacillus* spp. as biocontrol agents against damping-off in alfalfa. *Plant Pathol. J.* 13(1): 8–17. https://doi.org/10.3923/ppj.2014.8.17

Zaim S, Belabid L, & Bellahcene M. 2013. Biocontrol of chickpea Fusarium wilt by *Bacillus* spp. Rhizobacteria. *J. Plant Prot. Res*. 53(2): 177–183. https://doi.org/10.2478/jppr-2013-0027

Susilowati A, Wahyudi AT, Lestari Y, Suwanto A, & Wiyono S. 2011. Potential *Pseudomonas* isolated from soybean rhizosphere as biocontrol against soil-borne phytopathogenic fungi. *Hayati J. Biosciences*. 18(2): 51–56. https://doi.org/10.4308/hjb.18.2.51

Whipps JM. 2001. Microbial interactions and biocontrol in the rhizosphere. *J. Exp. Bot*. 52(1): 487–511. https://doi.org/10.1093/jexbot/52.suppl_1.487