Journal of Phytopathology and Pest Management 3(2): 15-25, 2016
pISSN:2356-8577 eISSN: 2356-6507
Journal homepage: http://ppmj.net/

Evaluation of certain Nitrogen-Fixing Bacteria against *Fusarium* spp. infected peanut

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Abstract

In this study, the inhibition of soil borne pathogenic fungi *Fusarium* spp. that cause root and pod rot diseases in peanut (*Arachis hypogaea*) was investigated by using soil-isolated Plant Growth Promoting Rhizobacteria (PGPR), (*Azotobacter and Azospirillum*) and root-nodule *Brady- Rhizobium* as biological agents. Twenty seven isolates of *Azotobacter* and 26 isolates of *Azospirillum* obtained from rhizosphere of peanut plants, The efficiency of the nitrogenase activity was estimated and the best four *Azotobacter* isolates including (A3, A7, A11, and A20) and three *Azospirillum* isolates including (AZ2, AZ14 and AZ18) were selected as the most efficient in nitrogenase activity. These isolates were subjected to test their ability in solubilizing zinc and phosphate and their strength in direct antagonism. The isolates A7 and Az18 were more efficient in solubilizing Phosphate and zinc. *Azospirillum sp.* AZ18, *Azotobacter sp.* A7 and *Brady-Rhizobium sp.* B-Rh1 achieved the highest reduction percentage in mycelia linear growth of pathogenic fungi *in vitro*. Under greenhouse conditions, *Azotobacter sp.* A7 recorded the highest disease reduction percentage of peanut root rot (44.38 %). Furthermore, *Brady-Rhizobium sp.* B-Rh1 recorded the highest disease reduction of peanut pod rot (50.6%) followed by *Azotobacter sp.* A7 (47.62%). In addition, our results showed that inoculation with the tested nitrogen fixing bacteria gave remarkable increase in the yield parameters of peanut plants such as number and weight of pods and increased the vegetative biomass overall.

Key words: PGPR, nitrogen-fixing bacteria, *Fusarium* spp., root rot, pod rot, peanut.

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Introduction

Peanut (*Arachis hypogaea*) is one of the most important legume and oilseed crop. In Egypt, the cultivated area is 65,000 ha producing 209000 ton with productivity 32154 hg ha^{-1} (FAO, 2013). The roots and pods of peanut are subjected to attack with numerous soil born fungi causing quantitative and qualitative losses**.** Root and pod rot diseases of peanut are serious worldwide diseases (Hilal et al., 1990). *Fusarium* spp. is known as a pathogen causing different symptoms of infected roots and pods (Hussin- Zeinab, 2011; Mahmoud 2004; Marei, 2000). Also, it has ability to cause wilt disease on a wide range of plants including peanut (Rasheed et al., 2004; Mayee, 2005; Gordon & Martyn, 1997). Various strategies were developed for controlling such diseases. Plant Growth Promoting Rhizobacteria (PGPR) is a group of bacteria that actively colonize plant roots and increase plant growth, yield and play a major role in the biocontrol of plant pathogens as they can suppress a broad range of bacterial and fungal diseases (Bin Zakaria, 2009; Kumer et al., 2002; Banasco et al., 1998). *Azotobacter***,** *Azospirillum* and *Rhizobium* are the important plant growth-promoting rhizobacteria that benefit plants in multiple ways including indole acetic acid, HCN and siderophore production, solubilization of inorganic phosphate and fixation of atmospheric nitrogen (Maheshwari et al., 2012; Verma et al., 2001). In recent studies, they showed strong antagonistic effect against *Fusarium oxysporum* (Maheshwari et al., 2012). *Azospirillum* sp. is the most studied PGPR bacteria (Dobbelaere et al. 2001). Many species of rhizobia can promote plant growth and inhibit the growth of various soil-borne pathogens including *Fusarium* spp. infected leguminous plants (El-Batanony et al. 2007; Sharif et al. 2003). This study aims to determine the efficiency of certain nitrogen-fixing bacteria strains for nitrogen fixation and against *Fusarium* spp. infected peanut.

Materials and methods

Isolation and identification of the causal pathogens: Peanut plants showing typical symptoms of root and pod rot diseases caused by *Fusarium* spp. were collected from different localities of Assiut, Sohag and Minia Governorates, Egypt during 2013 season. Pure cultures of developing fungi were obtained by hyphal tip technique. The growing fungal cultures were kept on PDA slant in refrigerator at 4°C for further studies. Pure cultures of presumptive isolates were identified using the morphological characters of mycelium and spores as described by Barnet and Hunter (1977) and Domsch et al. (1980) and confirmed by Assiut University Mycological Center (AUMC), Assiut, Egypt.

Pathogenicity tests: Pathogenicity capability of 45 isolates was carried out on peanut plants (cultivar Giza 6) under greenhouse conditions at the farm of Agriculture Faculty, Al-Azhar University (Assiut Branch), Egypt. Inocula of isolates were prepared by growing them in sterilized conical flasks (1000 ml) containing sand and sorghum medium, then incubated at 27°C for 21 days. Sterilized plastic pots (30 cm in diameter) were filled with sterilized sand

clay soil and infested with each isolate at the rate of 2% w/w, mixed well, thoroughly irrigated and left 7 days to ensure establishment of the tested isolates in soil. Non infested soil was used as control. Seeds of peanut were sterilized by dipping in 2% sodium hypochlorite solution for 2 minutes followed by washing with sterilized water and planted. Disease severity of root and pod rot diseases was recorded after 90 days, from sowing date. The arbitrary disease index scale as described by Grunwald et al., (2003).

Isolation and Identification of Nitrogen-Fixing Bacteria: Target bacteria were collected from peanut rhizosphere. Rhizospheric soils samples of peanut crop were collected from different locations of Minia, Assiut and Sohag Governorates, Egypt. Azotobacter isolates were isolated on modified Ashby medium (AbdEl-Malek & Ishac, 1968). Each isolate showing characteristic growth, pigmentation and biochemical reactions as described in Bergy's Manual of Determinative Bacteriology for *Azotobacter* sp. was purified. Similarly, *Azospirillum* isolates were isolated on Dobereiner medium (Dobereiner et al., 1976). Purified isolates were maintained on the same isolation media at 4°C. Sub-culture of the purified isolates was carried out every month for *Azotobacter* and every 15 days for *Azospirillum* isolates. Root nodules samples from peanut were collected randomly. Healthy root nodules were washed with tape water thrice before streaking on agar plate (Ben-Gweirif et al., 2005). Pink colored Gram negative rods were observed. Well isolated typical single colonies were re-streaked on freshly prepared Yeast Extract Mannitol Agar plates in order to obtain pure cultures. Bacterial isolates were biochemically characterized for Gram reaction, carbohydrate fermentation, H_2S production, NO_3^- reduction, oxidase test, starch hydrolysis and gelatin liquefaction as per the standard methods (Cappuccino & Sherman, 1992).

Determination of nitrogenase activity of *Azotobacter* and *Azospirillum* isolates: The nitrogen fixing capability of twenty seven isolates of *Azotobacter* and twenty six isolates of *Azospirillum* were growing on liquid N– free Modified Ashby′s medium and liquid N-deficient semi solid medium, respectively (Dobereiner et al., 1976; Abdelmalek & Ishac, 1968). The isolates were achieved using the ambient assay of N-ase activity according to Postage (1972). The highly nitrogen fixing capability of *Azotobacter* and *Azosirillum* isolates were selected for further studies.

Determination of phosphate and zinc solubilizing ability of certain nitrogen-fixing bacteria: Phosphate-solublizing ability of four isolates of *Azotobacter*, three isolates of *Azospirillum* and three isolates of *Bradyrhizobium* were tested. The isolates were tested by the dissolution of precipitated tricalcium phosphate [$Ca_3(po_4)_2$] in an agar medium as described by Rodriguez et al., (2004). Zinc-solubilizing ability of the isolates was tested by the dissolution of precipitated zinc oxide (ZnO) in an agar medium as reported by Saravanan et al., (2003). A pinpoint inoculation of the bacterial isolates was made on surface dried plates. Then, the plates were

incubated at 28°C for 7 days. Solubilization index (SI) was calculated according to the ratio of the total diameter (colony + halo zone) to the colony diameter (Edi- Premona et al., 1996).

***In vitro* antagonistic effect of certain nitrogen-fixing bacteria against liner growth of *Fusarium* spp.:** For studying antagonistic effect of *Azotobacter*, *Azospirillum* and *Bradyrhizobium* against *Fusarium* spp., plates containing PDA medium were streaked with the bacterial inoculation obtained from two days old cultures at opposite sides of the periphery plates by using a needle. One disk of the pathogen was placed at the center of each plate. Plates were incubated at 28°C. Plates were infested with pathogen only were used as control. When growth of the pathogen covered the control plates' surface, antagonistic effect was determined by calculated the inhibition percentage according to Dennis and Webstar (1971) as follow:

$$\text{Inhibition Percentage (\%)} = \frac{A_1 - A_2}{A_1} \times 100$$

Where $\mathbf{A_1}$= area covered by fungus in the control. $\mathbf{A_2}$= area covered by fungus in the dual culture.

Evaluation of certain nitrogen-fixing bacteria against root, pod rot and Fusarium wilt of peanut under greenhouse conditions: One isolate of *Azotobacter*, *Azospirillum* and *Bradyrhizobium* was evaluated under greenhouse conditions. Isolates of antagonistic bacteria were grown on nutrient agar medium and incubated at 28°C for 48 hrs. After incubation period, suspended in sterile distilled water and an optical density of 0.1 at 600 nm wavelength (using spectrophotometer model 6405UV/VIS), approximately (10^8 CFU/ml) was adjusted. Seed treatment was applied for 30 min. for each treatment and then the seeds were air dried. Sterilized plastic pots (30 cm in diameter) were filled with sterilized sand clay soil. Infested soil with pathogenic fungi was done as mentioned before. Non-treatment seeds were used as control. Disease severity of root and pod rot diseases were recorded after 120 days from sowing as mentioned before. The disease reduction percentage of root and pod rot diseases was calculated as follow:

$$\text{Disease reduction (\%)} = \frac{A - B}{A} \times 100$$

Where: A= disease reduction of control; B= disease reduction of treatment.

Statistical analysis: Data were subjected to statistical analysis using analysis of variance and means were compared using the LSD test according to Gomez and Gomez (1984).

Results

Isolation and identification of the causal pathogens: All fungal isolates were obtained from peanut plants showing typical symptoms of root and pod rot diseases collected from different location of Assiut (10 isolates), Minia (9 isolates) and Sohag (7 isolates) Governorates. Fungal isolates were identified using the morphological feature of mycelia spores as described by Barnet and Hunter (1977), Booth (1977) and Domsch et al. (1980) as *Fusarium*

solani (8 isolates), *F. moniliforme* (5 isolates), *F. semitectum* (5 isolates), *F. oxysporium* (3 isolates) and *F. equesti* (5 isolates).Identification of fungal isolates was confirmed by Assiut University Mycological Center (AUMC), Assiut University, Egypt.

Pathogenicity tests: Data in Table (1) showed that *F. solani* M2 and *F. solani* S1 exhibited the highest percentage of peanut root rot with the same recorded (77%) followed by *F. oxysporium* A1 (72%). While, *F. oxysporium* A2 exhibited the lowest root rot percentage (26%). Also, *F. solani* S1 exhibited significantly the highest pod rot percentage (60%). While, *F. oxysporium* A2 exhibited significantly the lowest percentage (15%). Based on the previous results, *F. oxysporium* A1, *F. solani* M2 and *F. solani* S1 were used in the following experiments.

Isolation and identification of nitrogen fixing bacteria: Fifty six bacterial isolates were obtained from rhizosphers of peanut plants collected from different locations of Assiut, Minia and Sohag Governorate, Egypt. Pure cultures of these isolates were identified by using biochemically characterized for Gram reaction, carbohydrate fermentation, H_2S production, NO_3^- reduction, oxidase test, starch hydrolysis and gelatin liquefaction as per the standard methods according to Cappuccino and Sherman, (1992). Bacterial isolates were identified as *Azotobacter sp.* (27 isolates), *Azospirillum sp.* (26 isolates) and *Brady-Rhizobium sp.* (3 isolates).

Determination of nitrogenase activity of *Azotobacter* and *Azospirillum* isolates: Results in Table (2) showed that four isolates of *Azotobactersp*. (A3, A7, A11 and A20) and three isolates of *Azospirillum sp*. (AZ2, AZ14 and AZ18) exhibited the highest nitrogenase enzyme activities and they were selected for the following studies.

Table 1: Pathogenicity tests of *Fusarium* spp. on peanut cultivar Giza 6 under greenhouse condition.

Isolates No.	Disease severity (%)*	
	Root rot	Pod rot
F. solani A1	68	33
F. solani A2	49	44
F. solani A3	54	39
F. solani M1	67	22
F. solani M2	77	53
F. solani M3	45	26
F. solani S1	77	60
F. solani S2	53	25
F. moniliforme A1	69	27
F. moniliforme M1	64	41
F. moniliforme M2	56	32
F. moniliforme M3	66	42
F. moniliforme S1	68	28
F. semitectum A1	69	18
F. semitectum A2	59	36
F. semitectum M1	45	27
F. semitectum M2	57	28
F. semitectum S1	48	39
F. oxysporium A1	72	50
F. oxysporium A2	26	15
F. oxysporium S1	44	22
F. equesti A1	57	32
F. equesti A2	59	34
F. equesti M1	46	29
F. equesti S1	63	33
F. equesti S2	50	29
Negative Control	0	0
LSD (P=0.05)	23.59	4.73

Determination of phosphate and zinc solubilizing ability of *Azotobacter* and *Azospirillum* isolates: Phosphate and zinc solubilizing ability of four isolates of *Azotobacter sp*. (A3, A7, A11 and A20) and three isolates of *Azospirillum sp*. (AZ2, AZ14 and AZ18) were determined. *Azotobacter sp*. A7 showed the highest ability of zinc and phosphate solubilizing with 4.2 and 4.86, respectively. While, *Azospirillum sp.*

AZ14 recorded the lowest ability of zinc and phosphate solubilizing (Table 3).

Table 2: Nitrogenase enzyme activity of *Azotobacter* and *Azospirillum* isolates obtained from different locations of Assiut, Minia and Sohag Governorate, Egypt.

Azospirillum isolates	Nitrogenase Enzyme activity (n mole C_2H_4/ml/hr)	Azotobacter isolates	Nitrogenase Enzyme activity (n mole C_2H_4/ml/hr)
AZ1	0.1	A1	10.4
AZ2	2.1	A2	12.4
AZ3	0.4	A3	211.4
AZ4	0.52	A4	20.7
AZ5	0.62	A5	2.3
AZ6	1.00	A6	39.4
AZ7	1.00	A7	435.3
AZ8	0.62	A8	207.3
AZ9	0.52	A9	82.9
AZ10	0.31	A10	126.5
AZ11	0.00	A11	248.7
AZ12	0.62	A12	10.4
AZ13	0.73	A13	41.5
AZ14	1.14	A14	0.05
AZ15	0.62	A15	82.9
AZ16	0.52	A16	31.1
AZ17	0.4	A17	10.4
AZ18	1.00	A18	60.2
AZ19	0.73	A19	18.7
AZ20	0.1	A20	352.4
AZ21	0.83	A21	103.7
AZ22	0.93	A22	24.9
AZ23	0.21	A23	20.7
AZ24	0.52	A24	0.00
AZ25	0.4	A25	33.2
AZ26	0.93	A26	82.9
		A27	12.4

***In vitro* antagonistic effect of certain nitrogen fixing bacteria against linear growth of *Fusarium* spp.:** Table (4) showed that *Azospirillum sp.* AZ18, *Azotobacter sp*. A7 and *Brady-Rhizobium sp.* B-Rh1 isolates achieved the highest reduction percentage of mycelia linear growth of the pathogenic fungi.

Evaluation of certain nitrogen fixing bacteria against Fusarium root and pod rot diseases of peanut under greenhouse condition: Data in Table (5) indicated that *Azotobacter sp.* A7 achieved the highest disease reduction percentage of peanut root rot (44.38 %). Also, *Brady-Rhizobium sp.* B-Rh1 achieved the highest disease reduction of peanut pod rot (50.6%) followed by *Azotobacter sp.* A7 (47.62%). While, *Azospirillum sp.* AZ18 achieved the lowest percentage of disease reduction.

Table 3: *In vitro* determination of phosphate and zinc solubilizing ability of *Azotobacter sp.* and *Azospirillum sp.* isolates.

Bacterial isolates	Solubilizing index (SI)*	
	Phosphate	Zinc
Azotobacter *sp*. A3	3 ^{d}	2.86 ^{f}
Azotobacter *sp*. A7	4.86 ^{a}	4.2 ^{a}
Azotobacter *sp*. A11	3.2 ^{c}	3.66 ^{c}
Azotobacter *sp*. A20	3.2 ^{c}	4.1 ^{b}
Azospirillum *sp.* AZ2	3.66 ^{b}	3.1 ^{e}
Azospirillum *sp.* AZ14	2.4 ^{e}	2.86 ^{f}
Azospirillum *sp.* AZ18	3.66 ^{b}	3.55 ^{d}
Negative control	1 ^{f}	1 ^{g}

*Means within the same column followed by different letters are significantly different (P≤0.05) based on LSD.

Yield: Table (6) showed that *Brady-Rhizobium sp.* B-Rh1 achieved the highest increase of pods fresh weight 43 gm/pot followed by *Azospirillum sp.* AZ18 that recorded 40.6 gm/pot. While, *Azotobacter sp.* A7caused the lowest weight with 28.3 gm/pot. All treatments increased both number of pods/pot and weight average of single pod (gm) compared with control.

Table 4: *In vitro* antagonistic effect of certain nitrogen fixing bacteria against *Fusarium* spp.

Bacterial isolates	Mycelial growth inhibition (%)		
	F. oxysporium A1	*F. solani* M2	*F. solani* S1
Azotobacter sp. A3	40 ^{bc}	34.63 ^{bc}	24.5 ^{e}
Azotobacter sp. A7	46.75 ^{ab}	39.63 ^{ab}	33 ^{bc}
Azotobacter sp. A11	45.75 ^{ab}	28.13 ^{cde}	25.25 ^{de}
Azotobacter sp. A20	34.25 ^{cd}	26.88 ^{de}	30 ^{cd}
Azospirillum sp. AZ2	32.5 ^{d}	15 ^{f}	4.25 ^{f}
Azospirillum sp. AZ14	36.75 ^{cd}	22 ^{ef}	4.25 ^{f}
Azospirillum sp. AZ18	50.75 ^{a}	43.25 ^{a}	36 ^{ab}
Brady-Rhizobium sp. B-Rh1	47 ^{ab}	35.25 ^{bc}	40.25 ^{a}
Brady-Rhizobium sp. B-Rh2	40.25 ^{bc}	33.25 ^{bcd}	37.75 ^{ab}
Brady-Rhizobium sp. B-Rh3	44 ^{ab}	30.75 ^{cd}	34.75 ^{bc}
Control	0 ^{e}	0 ^{g}	0 ^{f}

*Means within the same column followed by different letters are significantly different (P≤0.05) based on LSD.

Discussion

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and increasing plant growth and yield of the peanut crop. The action mechanisms of PGPRs can be divided into direct and indirect ones. Direct mechanisms include N2 fixation, soil mineral solubilization, production of plant growth- promoting substances (auxins, cytokinins or gibberellins) and reduction of ethylene levels that reflected in field experiment in which vigor of PGPR inoculated plants was clear. Indirect mechanisms include repressing the growth of plant pathogenic microorganisms (Marulanda et al., 2010; Lugtenberg et al., 2009). Results showed that *Azotobacter* and *Azospirillum* isolates were able to increase nitrogenase activity which works to increase the content of Nitrogen. It is worth noting that *Azotobacter sp.* isolates gave high values of the acetylene reduction activity (ARA), which registered a higher 453 nmole C_2H_4/1 ml/h culture. Similarly results with Tejera et al., (2005) surveyed efficient nitrogen fixation *A. chroococum* isolates from soil rhizosphere sample with the range of 79.6 to 329.5 nmole C_2H_4/1 ml/h culture.

Table 5: Evaluation of certain nitrogen fixing bacteria against *Fusarium* root and pod rot of peanut under greenhouse conditions*.

Treatments	Disease reduction of Root rot (%)				Disease reduction of Pod rot (%)			
	F. oxysporium A1	*F. solani* M2	*F. solani* S1	Mean	*F. oxysporium* A1	*F. solani* M2	*F. solani* S1	Mean
Azotobacter sp. A7	38.93 ^{a}	46.23 ^{a}	46.13 ^{a}	43.76	50 ^{a}	40.45 ^{a}	49.25 ^{a}	46.57
Azospirillum sp. AZ18	46.56 ^{a}	41.78 ^{a}	40.65 ^{a}	43.00	55.45 ^{a}	30 ^{b}	47.39 ^{a}	44.28
Brady-Rhizobium sp. B-Rh1	35.50 ^{a}	34.93 ^{a}	47.42 ^{a}	39.28	55.90 ^{a}	40.91 ^{a}	50.75 ^{a}	49.19
Control	0 ^{b}	0 ^{b}	0 ^{b}	0	0 ^{b}	0 ^{c}	0 ^{b}	0

*Means within the same column followed by different letters are significantly different (P≤0.05) based on LSD.

Table 6: Effect of certain nitrogen fixing bacteria on yield of peanut infected with *Fusarium* spp. under greenhouse condition*.

Treatments	Pod number/ pot				Fresh weight of pod/ pot				Weight average of single pod(gm)			
	F. oxysporium A1	*F. solani* M2	*F. solani* S1	Mean	*F. oxysporium* A1	*F. solani* M2	*F. solani* S1	Mean	*F. oxysporium* A1	*F. solani* M2	*F. solani* S1	Mean
Azotobacter sp. A7	13 ^{b}	15 ^{bc}	11 ^{b}	13	27 ^{bc}	33 ^{a}	28.3 ^{b}	29.4	2.11 ^{a}	2.28 ^{a}	1.95 ^{a}	2.11
Azospirillum sp. AZ18	20 ^{a}	18 ^{ab}	28 ^{a}	22	40 ^{ab}	37 ^{a}	40.6 ^{a}	39.2	2.04 ^{a}	2.18 ^{a}	1.89 ^{a}	2.04
Brady-Rhizobium sp. B-Rh1	23 ^{a}	20 ^{a}	23 ^{a}	22	47 ^{a}	39 ^{a}	43 ^{a}	43	1.98 ^{a}	1.84 ^{b}	1.89 ^{a}	1.90
Control	10 ^{b}	11^{c}	7 ^{c}	9.3	18 ^{c}	20 ^{b}	17 ^{b}	18.3	1.8 ^{a}	1.75 ^{b}	1.73 ^{a}	1.76

*Means within the same column followed by different letters are significantly different (P≤0.05) based on LSD.

In this study, we investigated the potential of phosphate and zinc solubilization by isolates of *Azotobacter, Azospirillum* and *Brady-Rhizobium*. As previously reported by Garg et al., (2001), the *Azotobacter* isolates were able to dissolve inorganic and organic phosphate compounds. Also, Kumer et al., (2012)**,** who reported that a large number of bacteria including species of *Azotobacter, Azospirillum, Rhizobium, Bacillus* and *Enterobacter* enhanced plant growth by their different plant growth promoting activities including phosphate solubilization. On other side, *Azotobacter* and *Azospirillum* isolates achieved the ability to dissolve zinc which increases in plant parameters and yield, similarly results were obtained with Shaukat et al., (2010). This is because the mechanisms of zinc solubilizing by microbes which include excretion of metabolites such as organic acids, proton extrusion, or production of chelating agents Nahas, (1996). In addition, production of inorganic acids such as sulphuric acid, nitric acid, and carbonic acid could also facilitate the solubilization Seshadre, (2002). In this study, the results showed the ability of *Azotobacter, Azospirillum* and *Brady-rhizobium* isolates to inhibit the liner growth of *Fusarium* spp.that causes peanut root rot and pod rot diseases *in vitro*, similarly results recorded by Tortora et al., (2011). In an experiment, field inoculation with Plant Growth Promoting Rhizobacteria (PGPR). *Azotobacter* (A7), *Azospirillum sp*.Az18 and *Brady-rhizobium sp*. B-Rh1 remarkable increase in weight, number of pods and increased the vegetative biomass. This is due to the specific studies showed that PGPR either directly or indirectly promote plant growth and yield. The direct growth promoting mechanisms includes (i) N2 fixation; (ii) solubilization of mineral phosphate and zinc; (iii) sequestration of iron by production of siderophores; (iv) production of phytohormones such as auxins, cytokinins and gibberellins; (v) production of the enzyme 1-aminocyclopropane -1- carboxylate (ACC) deaminase, which hydrolyses ACC, the immediate precursor of ethylene in plants. Lowering of ethylene concentration in seedlings results in stimulating seedlings root length (Bashan et al., 2005). PGPR also support plant growth indirectly, by improving growth restricting conditions via (i) production of antibiotics; (ii) depletion of iron from

the rhizosphere; (ii) production of fungal cell wall lysing enzymes ß-(1,3)-glucanase and chitinase; (iii) synthesis of antifungal metabolites such as cyanide; (iv) competition for infection sites on roots; (v) induction of systemic resistance (Saraf et al., 2014; Aeron et al., 2011).

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