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EVALUATION OF SOME RHIZOBACTERIA AS POTENT BIOLOGICAL CONTROL AGENTS *IN VITRO*

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ABSTRACT

Rhizobacteria are known to influence plant by direct and indirect mechanisms. Some strains of bacteria namely, *Azotobacter chroococcum, Pseudomonas fluorescens, Paenibacillus polymyxa, Bacillus megaterium* var *phosphaticum, B. subtilis, B. coagulans, and Azospirillum lipoferum* were screened for their beneficial mechanisms to use them as plant growth-promoting rhizobacteria and biological control agents against two important soil-borne root infecting plant pathogens, i.e. *Fusarium oxysporum* f. sp *lycopersici* and *F. solani*. The obtained data revealed that, *B. megaterium* and *P. polymyxa* were more potent for phosphate solubilization while *A. chroococcum, P. polymyxa and A. lipoferum* were the most strains to grow on N-free media. All strains were able to produce indole-3- acetic acid (IAA) with higher production in the presence of tryptophan especially *B. megaterium*, *P. polymyxa, A. chroococcum* and *A. lipoferum*. On the other hand, *Ps. fluorescens, P. polymyxa* and *B. subtilis* are more potent to produce catechol-type siderophores, chitinase, cellulase and protease and they were more efficient for inhibition of fungal growth, either by using dual culture technique or by using filtrates of their respective cultures. Culture filtrates of *Ps. fluorescens, P. polymyxa* and *B. subtilis* were slightly exhibited antagonism against some bacterial strains used in the current study. By using 10 antibiotics to test the sensitivity for antibiotics, *A. chroococcum* and *B. coagulans* were the most sensitive bacteria to all the tested antibiotics, while *Ps. fluorescens* and *P. polymyxa* were the most resistant.

Key words: rhizobacteria, mycolytic enzymes, siderophores, antagonism, biocontrol agents

INTRODUCTION

Some bacteria are associated with roots of crop plants and exert beneficial effects on their hosts and are referred to as plant growth-promoting rhizobacteria (PGPR) (Siddiqui, 2006). PGPR inhabit the rhizosphere, the volume of soil under the immediate influence of the plant root system, and favors the establishment of a large amount of active microbial population. PGPR can directly stimulate plant growth in several different ways. They can fix atmospheric nitrogen, synthesize several plant hormones, solubilize minerals, synthesize enzymes that can modulate plant hormone levels. A particular plant growth promoting bacterium may possess one or more of these mechanisms. The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effects of one or more phytopathogenic organisms by producing siderophores that limit the available iron to the pathogen, producing antibiotics that kill the pathogen and/or inducing systemic resistance in plant. PGPRs and biocontrol agents (BCAs) still form most important yet most fragile component of integrated pest management (IPM) leading to discouragement of IPM initiative throughout the world (Fravel, 2005). This situation tempted us to screen for newer and region specific strain(s).

Many bio-control strains of bacteria have shown potential for some degree of

control of several plant pathogens, but a potent rhizobacterium that could provide the best control of *Fusarium* diseases and also have potential for effective implementation in commercial agriculture has yet not been reported (Bolwerk *et al*., 2003 and Trivedi and Pandey, 2008). Indeed, control of *Fusarium* sp., even with chemical fungicide combined with field management strategies, is difficult in practice; hence, the continual existence and high prevalence of several wilt diseases have prompted scientists to search for biological alternatives that are efficient, reliable and safe for the environment (Asante *et al*., 2008). Therefore, we set up our experiment with a wider scope, to search for bacterial strain that could inhibit the growth of *Fusarium*.

Our main objectives were to evaluate many rhizobacteria and selected the active bacterial strains that possess beneficial mechanisms and to investigate the reactions of the active bacterial strains in their culture fluids against *Fusarium*.

MATERIALS AND METHODS

Source of microorganisms and cultural conditions

Bacterial strains

Bacterial strains assessed as potential biocontrol agents in this study were previously isolated and identified at Fac. Agric., Moshtohor, Benha University except *Azospirillum lipoferum* as follow:

Bacterial strains	Maintenance media	Characteristics	Source and References
- Azotobacter chroococcum	Ashby's modified medium (Abd EL-Malek and Ishac, 1986)	Cytokinins, gibberellins and IAA production	Isolated and identified by El-Mehiy (2007)
Bacillus megaterium var. phosphaticum	Nutrient agar (Atlas, 1995)		
- Pseudomonas fluorescens	King's medium B (Atlas, 1995)	Phosphatase production	Isolated and identified by Abd El-Rahman (2004)
- Paenibacillus polymyxa	Nutrient agar (Atlas, 1995)	Phosphate solubilizer (Abou-Aly *et al*., 2006)	
Bacillus subtilis **M14**	Nutrient agar (Atlas, 1995)	Proteases production	Isolated and identified by Neweigy *et al.* (2003)
- Bacillus coagulans **M53**			
- Azospirillum lipoferum	Semi-solid malate medium (Hegazi *et al*.,1979)	N_2-fixer	Microbiological Unit, Desert Research Center

Fungal strains

The fungal strains *Fusarium oxysporum* f. sp *lycopersici* and *Fusarium solani* used in this study were obtained from Plant Pathology Branch, Fac. of Agric., Benha University. Fungal strains were maintained on potato dextrose agar (PDA).

Phosphate solubilization

Phosphate solubilization by bacterial strains was detected by growing 0.1 ml of each culture on Pikovskaya's agar plates (Pikovskaya, 1948) [containing per liter tricalcium phosphate 2.5 g, glucose 13 g, $(NH_4)_2SO_4$ 0.5 g, NaCl 0.2 g, $MgSO_4.7H_2O$ 0.1 g, KCl 0.2 g, yeast extract 0.5 g, $MnSO_4$ trace, $FeSO_4.7H_2O$ trace, agar 15 g and pH adjusted to 7.2]. Plates were observed after 48 h incubation at 30º C for development of zone of clearance around colony. Solubilization Index was measured using following formula (Edi-Premono *et al.,* 1996):

$$\text{Solubilization Index} = \frac{\text{Colony diameter + halozone diameter}}{\text{Colony diameter}}$$

Total nitrogen accumulation

The capability of strains to fix nitrogen was determined by their ability to grow in nitrogen free broth media. Bacterial strains were cultured one day in nitrogen free solid malate-sucrose medium (Xie *et al.,* 2003) with shaking for 24 h at 28ºC. The content of the culture filtrate was subjected to kjeldahl digestion and total N was determined.

Indolacetic acid (IAA) production

A modfied colorimetric method was used for determination of IAA (Asghar *et al.,* 2000). The selected rhizobacteria strains were grown on broth of their respective media (25 ml) with and without (5 mL) L-TRP (0.5%) solution and shaked at 28°C for 48 h and then centrifuged for 10 min. One milliliter culture supernatant was placed in a test-tube and mixed with 2 ml Salkowsky reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M $FeCl_3$ solution). After 20-25 min, the colour absorbance was read using spectrophotometer at 535 nm. Concentration of IAA produced by cultures was measured with the help of standard graph of IAA obtained in the range of 10-100 µg/ml.

Detection of catechol-type siderophores

The culture supernatant was tested to catechol-type siderophores according to Carson *et al.* (1992). The assay include combining 4.0 ml culture supernatant with 0.25 ml 2M HCl and 0.5 ml nitrate-molybdate reagent. The identity of catecol-type siderophore is indicated by the formation of a yellow color.

Enzymes assay

Cellulase activity was determined according to the method of Mandels *et al*. (1976). Briefly, adding 0.5 ml of culture filtrate to 0.5 ml of 1.0% carboxymethyl cellulose in 0.05 M citrate-phosphate buffer (pH 4.8). The reaction mixture was incubated at 50º C for 30 min. The released reducing sugars were determined according to Miller *et al.* (1960) One unit of enzyme activity was defined as the amount of enzyme producing one µM of glucose per min. Chitinase activity was determined by adding 1 ml culture filtrate and 1 ml suspension of colloidal chitin (0.1 % in 50 mM sodium acetate buffer, pH 5.0) was incubated at 37ºC in a water bath with constant shaking. After 2 hr, the release of N-acetylglucosamine in reaction mixture was estimated by the method of Reissig *et al.* (1955). One unit of chitinase activity was defined as the amount of enzyme, producing 1 µM of N-acetylglucosamine from colloidal chitin in the reaction mixture/ml. Protease activity was determined by the method of Keay and Wildi (1970). The reaction mixture consisting of 1 ml diluted enzyme and 1 ml of casein solution for 10 min, then the reaction was stopped by the addition of 2.0 ml 24% trichloroacetic acid. After 30 min at room temperature, the undigested casein was removed by filtration. The amount of trichloroacetic acid-soluble casein breakdown fragments were determined using the method of Hindazlothink *et al*. (1983) One unit of enzyme activity was defined as the amount of enzyme producing one µg of tyrosine per min.

In vitro antagonistic activity of bacterial strains against fungal growth.

Presumptive antagonistic bacteria were screened on nutrient agar medium for their ability to inhibit fungal growth in dual petri dish culture test as described by Landa *et al*. (1997). Briefly, bacterial strain was streaked at equidistant points along the perimeters of the each perti dishs, then incubated at 28º C for 2 days. Plates were inoculated at the center with a 5 mm diameter plug of the 7 days old *Fusarium oxysporum* f. sp *lycopersici* or *F. solani*. The plates were incubated at 28º C for 3-4 days. Plates without bacteria were used as control. When the fungal mycelium had reached the edge of the plate in control, the radius of fungal growth towards bacteria and control were measured and % inhibition of the fungal growth was calculated in relation to the control.

Culture filtrates of bacterial strains.

All bacterial strains were grown on broth of their respective media in shaking incubator (200 rpm) for 3 days at 28 – 30º C. The cells were then harvested by centrifugation at 6000 rpm for 20 min and the supernatants were filter sterilized through 0.45µm bacterial filter.

Effect of bacterial culture filtrates on fungal biomass.

25 ml filtrate of each bacterial culture was separately added in 175 ml Czapek-Dox liquid medium in conical flask. Flasks containing the medium without bacterial filtrates served as control. Flasks were then inoculated with *Fusarium oxysporum* f. sp *lycopersici* or *F. solani* and incubated at 28º C. After 3 and 7 days the fungal mats were harvested through Whatman filter paper No.2, then gently pressed between the folds of blotting paper to remove the excess amount of water and weighed.

Antagonism between bacterial culture filtrates and the tested bacterial growth.

Disc diffusion method was used for determining the antibacterial activity of cultture filtrates against the tested bacteria. Sterilized paper discs were dipped in culture filtrate of each bacteria, placed on the surface of seeding plates of the bacteria, incubated at 30°C for 2 days. After incubation the inhibition zone was measured.

Antibiotic sensitivity/resistance pattern for the tested bacteria.

Antibiotic sensitivity was determined according to National Committee for Laboratory Standards (2003) by inoculation the selected rhizobacteria strains on their respective media. Then a disc containing one of the following antibiotics (ampicillin, gentamycin, cephalexin, chloramphenicol, streptomycin, tetracycline, amoxicillin, erythromycin, novobiocin and oxytetracycline)) with specific concentration was placed on the surface of the culture. Plates without antibiotic discs served as control. The plates were incubated at 30º C for 4 days and the zone of inhibition around each antibiotic disc was observed, measured to determine the resistance and sensitivity of the tested bacteria.

The data thus obtained were statistically analyzed using Mstatc software-program to detect if significant differences exist for various parameters. LSD was also determined.

RESULTS AND DISCUSSION

Phosphate solubilization.

Results showed that all the tested bacteria can solubilize insoluble tricalcium phosphate (Fig. 1). *B. megaterium* and *P. polymyxa* were the most efficient phosphate solubilizers on Pikovskaya's medium and they were considered as phosphate-solubilizing rhizobacteria. Whereas *B. coagulans* showed the lowest solubilization index. Similar results were found by Abou-Aly *et al*. (2006) and Muthukumar and Udaiyan (2006). They found that *P. polymyxa* plays an important role in phosphate solubilization beside *B. megaterium*.

Total nitrogen accumulation

All bacterial strains were able to grow in nitrogen-free broth and increased the total nitrogen in the growth culture (Fig.1). The amount of nitrogen produced varied between the strains. *Az. lipoferum, A. chroococcum* and *P. ploymyxa* produced higher amount of nitrogen, while *B. megaterium*, *B. subtilis* and *B. coagulans* produced very low amount of nitrogen. This presumptive test indicates the N_2 fixing activity of the bacterial strains. Yasmin *et al*. (2007) found that many of rhizobacteria can be able to grow in N free broth medium.

Indolacetic acid (IAA) production

Data in Fig. 2 show that all bacterial strains were able to produce IAA eithr with or without tryptophan as a precursor. The presence of tryptophan gave higher production of IAA by all strains. Concentration of IAA were varies with strains, the highest IAA was obtanied by *B. megaterium* followed by *Az. lipoferum*, *P. polymyxa* and *A. chroococcum* when grown in media with tryptophan. This shows that some strains are dependent on tryptophan precursor and probably synthesized IAA through tryptophan pathways. Also, addition of tryptophan to the media increased the auxin production by several folds. Under the natural condition, plant roots excrete organic compounds including tryptophan which can then be utilized by the rhizobacteria for IAA biosynthesis. Perrig *et al*. (2007) and Trivedi and Pandey (2008) found that pro-

duction of indole acetic acid demonstrated in broth assays was another important plant growth promoting character by *Azospirillum* and *Bacillus megaterium.*

Catechol-type siderophores and lytic enzymes assay

Data in Table (1) show catechol-type siderophore detection in culture filterates of bacterial strains. Catechol-type siderophore production, as determined by the color change, was variable among strains. Although *B. megaterium* appeared not to produce catechol-type sidrophore, *Ps. fluorescens* followed by *P. polymyxa* and *B. subtilis* produced the greatest amount of siderophore. *A. chroococcum*, *B. coagulans* and *Az. lipoferum* produced only trace amount of siderophore, yet they are still considered positive siderophore-producing strains.

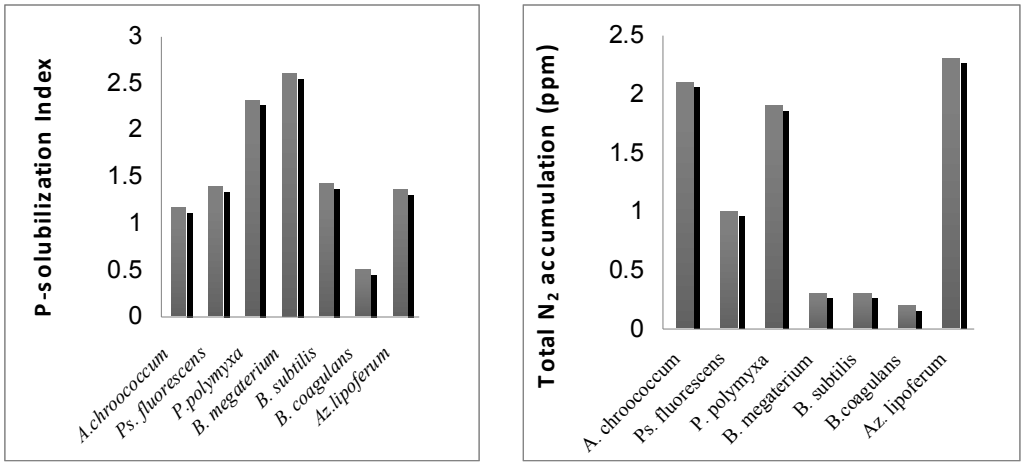

Fig. (1): Phosphate solubilization index and total nitrogen accumulation of the selected rhizobacteria.

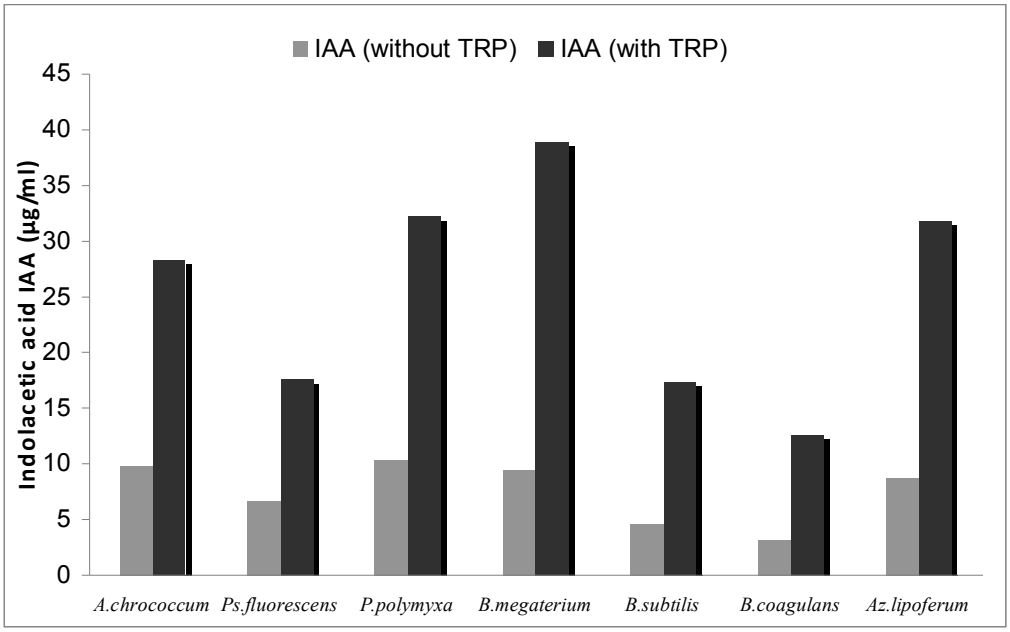

Fig. (2): Indol acetic acid produced from selected rhizobacteria (with and without tryptophan TRP)

Table (1): Siderophores and mycolytic enzymes in the culture filtrate of the tested bacterial strains.

Culture filtrates	Catechol-type siderophores	Chitinase (U/ml)	Cellulase (U/ml)	Protease (U/ml)
A. chroococcum	+	0.76	3.73	14.92
Ps. fluorescens	+++	4.73	11.48	34.32
P. polymyxa	++	3.05	13.60	36.11
B. megaterium	-	1.43	7.82	23.81
B. subtilis	++	3.62	8.69	66.97
B. coagulans	+	2.38	7.84	50.16
Az. lipoferum	+	1.12	6.71	13.54
LSD at 1%		0.97	2.21	6.47

Significant quantity of chitinase was produced by *Ps. fluorescens, P. polymyxa* and *B. subtilis*, while *A. chroococcum* and *Az. lipoferum* exhibited the lowest activities of chitinase. On the other hand, *Ps. fluorescens* and *P. polymyxa* produced higher quantity of cellulase when compared to the other strains. Unlike to the other two enzymes, protease production was higher by *B. subtilis* and *B. coagulans* followed by *Ps. fluorescens* and *P. polymyxa*. From the previous data, lytic enzymes produced by the bacterial strains are very important in biocontrol technology. There are many reports on production of lytic enzymes by microorganisms (Gohel *et al*., 2004 and Diby *et al*., 2005). It can be inferred that those lytic enzymes produced by the tested strains can be involved in pathogen suppression.

Antifungal activity in plate assay

The bacterial strains were assayed for *in vitro* antagonism against *Fusarium oxysporum* f. sp *lycopersici* and *F. solani* (Table 2). Each fungus showed a different level of sensitivity toward the biological control agents. In a dual culture assay, inhibition was clearly discerned by limited growth or by the complete absence of fungal mycelium in the inhibition zone surrounding a bacterial colony. *Ps. fluorescens*, *P. polymyxa* and *B. subtilis* showed the strongest antagonistic activity among the tested bacterial strains against the both fungi. This may be attributed to the ability of those bacteria to produce siderophores and mycolytic enzymes (chitinase, cellulose and protease) which was confirmed in this study (Table 1). Muleta *et al*. (2007) and Abdel-Salam *et al*. (2007) found similar observations for *Pseudomonas* and *Bacillus* on suppression of *Fusarium* growth. On the other hand, bacteria that showed the greatest plant promotion potential (*B. megaterium*, *Az. lipoferum* and *A. chroococcum*) were not necessarily the best at inhibiting both pathogens. However *P. polymyxa* was the only isolate that showed strong PGPR qualities and pathogen inhibition in this study.

Table (2): Inhibition of *Fusarium* mycelia growth by the tested strains of rhizobacteria.

Bacterial strains	*Fusarium oxysporum*		*Fusarium solani*	
	Fungal growth (mm)	Inhibition (%)	Fungal growth (mm)	Inhibition (%)
Control	55.6	0.0	56.3	0.0
A. chroococcum	42.3	23.9	34.3	39.0
Ps. fluorescens	18.6	66.5	20.0	64.4
P. polymyxa	27.3	50.8	20.6	63.4
B. megaterium	29.6	46.7	33.3	40.8
B. subtilis	24.0	56.8	25.3	55.0
B. coagulans	34.0	38.8	38.6	31.4
Az .lipoferum	29.6	46.7	35.6	36.7
LSD at 1%	7.52	--	4.86	--

Effect of bacterial culture filtrates on fungal biomass

The bacterial filtrates of the different tested bacteria were evaluated to their effect on suppression of *Fusarium oxysporum* f. sp *lycopersici* and *Fusarium solani* growth after 3 and 7 days (Table 3). The tested culture filtrates were different in their influence on fungal growth depending on type of culture. The maximum reduction in mycelial weight was observed after 3 and 7 days by using filtrates of *Ps. fluorescens, P. polymyxa* and *B. subtilis*. The toxicity of culture filtrates of these bacteria may be attributed to the production of certain toxic metabolities, siderophores and lytic enzymes in the culture media. These results confirm this paper data, that is, the efficacy of *Ps. fluorescens, P. polymyxa* and *B. subtilis* for production of toxic metabolites (Table 1) and the antagonistic activity of them against the fungal pathogens (Table 2). The present findings are corroborated with those of Dijksterhuis *et al*. (2003) and Wang *et al*. (2007) who reported the efficacy of bacterial culture filtrates against *Fusarium*. Furthermore, culture filtrate of *Az. lipoferum* slightly exhibited antagonism activity against the fungal growth but still more potent than *A. chroococcum*, *B. megaterium* and *B. coagulans*.

Antagonism between bacteria

Culture filtrates of the tested bacteria were evaluated to their effect on growth of all bacterial strains to determine the antagonistic activity among them (Table 4). Culture filtrates of *A. chroococcum*, *B. megaterium*, *B. coagulans* and *Az. lipoferum* did not exhibit any antagonistic effect against all the tested bacteria. Whereas, culture filtrate of *Ps. fluorescens* slightly exhibited antagonistic effect against *B. megaterium* and *B. coagulans*, also, *P. polymyxa* filtrate had antimicrobial activity against *B. megaterium*. In addition, filtrate of *B. subtilis* recorded antagonistic activity against *A. chroococcum*, *Ps. fluorescens* and *B. coagulans*.

From data in Table (5), *Ps. fluorescens*, *B. subtilis* and *Az. lipoferum* were resistant to ampicillin, amoxicillin and oxytetracycline. Also, all tested bacteria were sensitive to gentamycin and novobiocin, while *A. chroococcum* and *B. coagulans* were sensitive to all the tested antibiotics. On the other hand, *Ps. fluorescens* was the most resistant bacteria which exhibited resistance to five antibiotics, followed by *P. polymyxa* that not affected by four antibiotics. Brown and Balkwill (2008) found that many bacterial strains were resistance to more antibiotics. Also, they reported that resistance to ampicillin was noted most frequently.

Table (3): Effect of culture filtrates of the tested bacteria on fungal biomass (gl^{-1}) of *Fusarium* after 3 and 7 days

Culture filtrate	*Fusarium oxysporum*				*Fusarium solani*			
	F.B. 3 days	Reduction (%)	F.B. 7 days	Reduction (%)	F.B. 3 days	Reduction (%)	F.B. 7 days	Reduction (%)
Culture filtrate free	3.74	0.0	11.31	0.0	2.85	0.0	9.87	0.0
A. chroococcum	3.25	13.10	9.34	17.41	2.67	6.31	9.25	6.28
Ps. fluorescens	1.27	66.04	4.32	61.80	1.81	36.49	6.53	33.83
P .polymyxa	1.91	48.93	5.12	54.73	2.15	24.56	6.72	31.91
B. megaterium	3.15	15.77	8.71	22.98	2.61	8.42	7.98	19.14
B. subtilis	1.51	59.62	4.92	56.49	1.76	38.24	5.83	40.93
B. coagulans	2.72	27.27	6.83	39.61	2.31	18.94	7.76	21.37
Az .lipoferum	2.68	28.34	6.58	41.82	2.19	23.15	7.08	28.26
LSD at 1%	0.72	--	2.47	--	0.94	--	3.01	--

F.B.: fungal biomass.

Table (4): Effect of bacterial culture filtrates on the bacterial growth.

Culture filtrates	Inhibition zone (mm)						
	A. chroococcum	*Ps. fluorescens*	*P. polymyxa*	*B. megaterium*	*B. subtilis*	*B. coagulans*	*Az. lipoferum*
A. chroococcum	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ps. fluorescens	0.0	0.0	0.0	2.9	0.0	3.8	0.0
P. polymyxa	0.0	0.0	0.0	3.7	0.0	0.0	0.0
B. megaterium	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B. subtilis	7.6	1.2	0.0	0.0	0.0	4.1	0.0
B. coagulans	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Az. lipoferum	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table (5): Antibiotic sensitivity pattern of the tested bacteria grown at 30º C for 4 days.

Antibiotics	Inhibition zone (mm)						
	A. chroococcum	*Ps. fluorescens*	*P. polymyxa*	*B. megaterium*	*B. subtilis*	*B. coagulans*	*Az. lipoferum*
Ampicillin	8.4	0.0	4.6	3.7	0.0	12.5	0.0
Gentamycin	5.7	8.3	3.5	4.2	3.6	6.9	10.3
Cephalexin	8.9	0.0	3.8	7.4	10.3	4.5	5.7
Chloramphenicol	10.4	1.7	0.0	0.0	3.6	2.6	3.8
Streptomycin	3.9	0.0	0.0	9.4	5.2	6.7	6.2
Tetracycline	11.8	6.4	0.0	0.0	4.8	3.0	1.7
Amoxicillin	6.5	0.0	5.6	3.5	0.0	2.6	0.0
Erythromycin	6.8	4.2	0.0	6.3	4.3	6.8	7.2
Novobiocin	3.6	3.1	4.2	2.5	4.9	7.1	1.8
Oxytetracycline	8.2	0.0	5.4	7.8	0.0	7.3	0.0

From the present study, it can be concluded that rhizobacteria showed variation in their activities either as PGPR or biocontrol agents. *Ps. fluorescens*, *B. subtilis* are the dominant antagonists in soil. Those bacteria exhibited more than two or three biological control trails. Also, many of the tested bacteria can play a great role as plant growth-promoting bacteria especially *Az. lipoferum*. However *P. polymyxa* was the only isolate that showed strong PGPR qualities and pathogen inhibition in this study.

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تقييم بعض ميكروبات التربة كعوامل فعالة للمقاومة الحيوية تحت الظروف المعملية

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تعرف ميكروبات التربة بتأثيرها المباشر وغير المباشر على تشجيع نمو النبات ومقاومته للأمراض. لذلك تم اختيار مجموعة من البكتريا المعزولة من التربة وهى *Azotobacter chroococcum, Pseudomonas fluorescens, Paenibacillus polymyxa, Bacillus megaterium* var *phosphaticum, B. subtilis, B. coagulans, and Azospirillum lipoferum* وتم تقييمها للاستخدام كميكروبات مشجعة لنمو النبات وكذلك كعوامل مقاومة حيوية ضد اثنين من فطريات التربة.

وقد أشارت النتائج إلى أن أفضل هذه الميكروبات فاعلية فى إذابة الفوسفات كانت *P. polymyxa* و *B. megaterium* var *phosphaticum* بينما استطاعت كل من *A. chroococcum, P. polymyxa A. lipoferum* النمو فى بيئة خالية من النيتروجين. كذلك فإن كل البكتريا المستخدمة وخاصة *B. megaterium*, *P. polymyxa, A. chroococcum* and *A. lipoferum* استطاعت انتاج اندول حمض الخليك بكميات عالية فى وجود التربتوفان.

وعلى الجانب الآخر فإن أكثر الميكروبات فاعلية فى إنتاج السيدروفور وكذلك الإنزيمات المحللة مثل الشيتينيز والسليوليز والبروتييز كانت بكتريا *Ps. fluorescens, P. polymyxa*, *B. subtilis* وكذلك كانت تلك البكتريا أكثر كفاءة فى تثبيط الفطريات المستخدمة سواء باستخدام المزارع البكتيرية أو رواشحها. ولقد أظهر استخدام رواشح كل من *Ps. fluorescens, P. polymyxa, B. subtilis* نشاط تضادى بسيط ضد بعض أنواع البكتريا المستخدمة فى الدراسة.

ولدراسة حساسية البكتريا المستخدمة للمضادات الحيوية استخدمت عشرة أنواع من المضادات الحيوية وكانت بكتريا *B. coagulans* و *A. chroococcum* الأكثر حساسية لكل أنواع المضادات المستخدمة بينما كانت *Ps. fluorescens, P. polymyxa* الأكثر مقاومة لتلك المضادات الحيوية