

Minia J. of Agric. Res. & Develop. Vol. (37), No. 3, pp. 463-476, 2017

FACULTY OF AGRICULTURE

EVALUATION OF SOME PEPPER RHIZOSPHERE MICROFLORA AS PLANT GROWTH PROMOTERS AND ANTIFUNGAL TRAITS

A. R. Abdullah, A .M .M Hammad, O. A. O. Saad and N. H .I. Hussien

Agric.Microbiology Department,Faculty of AgricIture,Minia University

Received: 16 September (2017) Accepted: 9 November (2017)

ABSTRACT

Pot experiments were carried out to select and evaluate efficient isolates of Azotobacter, Azospirillum, Pseudomonas sp. and Bacillus sp. from rhizosphere soil of pepper plants, for plant growth promoting traits and phytopathogen antagonist. Total counts of bacteria, Bacillus sp. ,Azospirillum, Azotobacter and Pseudomans sp were higher in rhizosphere soil, positive rhizosphere effects particularly at the early periods of plant growth (15, 30 and 45 days) were recorded. Eighty isolates representative to Azotobacter. Azospirillum, Bacillus sp. and Pseudomonas sp. (twenty from each) tested towards their efficiency for some traits related to plant growth promoter. Nitrogenase activity for Azotobacter isolates ranged from 3.28 to 48.17 nmoles, C₂H₄/ 1ml culture/hr.,the production of C₂H₄ by isolates Azospirillum ranged from 3.78 to 57.21 .Moreover isolates of Pseudomonas sp. production C_2H_4 ranged from 2.20 – 27.92 .With regard to phosphate solubilization out of twenty isolates of Azotobacter 15 had positive effect on solubilizing. Solubilizing index (SI) ranged from 1.3-4.6. Isolates of Azospirllum, Pseudomonas sp. and Bacillus sp. out of the tested isolates 13, 15 and 15 had positive effect on melting phosphorus. Soluble index (SI) were recorded 1.3-5.0, 1.3-6.0 and 1.3-6.0 respectively. Moreover for zinc solubilizing out of the twenty isolates which tested, 14,13,13 and 16 for Azotobacter, Azospirillum, Pseudomonas and Bacillus had positive effect on zinc solubilizing with SI ranged (1.3-5.0), (1.3-5.3), (1.3-6.3) and (1.3-5.6) respectively. The obtained results showed that the tested isolates

produced varies antifungal traits and inhibit growth of two different soil borne plant pathogenic fungi. *Rhizoctonia solani* and *Fusarium oxysporum*. Generally, *Pseudomonas sp.* achieved high recorded in reducing mycelial growth of pathogenic fungi (*F.oxysporum*) followed by *Bacillus sp.*, and *Azospirillum* isolates. With regard of growth of *R.solani* the highest inhibition percentage values on mycelia growth were achieved by isolates *Bacillus sp.*, followed by *Pseudomonas sp*, then *Azotobacter* and *Azospirillum* isolates. The recorded inhibition were 74.2%, 71.6%, 64.3% then 43.6% for isolates Bac12, PS14, Azt14 then Azo14 for *R.solani*, while in case of *F.oxysporum* the inhibition effect reached 83.6%, 82.14%, 73.52% and 52.6% respectively.

INTRODUCTION

Gray and Smith (2005) showed that the Plant Growth Promoting Rhizobacteria (PGPR) associations range in the degree of bacterial proximity to the root and intimacy of association. These can be separated into extracellular (PGPR), existing in the rhizophere, on the rhizoplane, or in the spaces between cells of the root cortex, and intracellular (PGPR), which exist inside root cells. generally in specialized nodular structures.

Forlani *et al*, (1995) showed that several bacterial strains of the genera *Azotobacter*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Kebsilla*, *Sarcina* and *Pseudomonas* were isolated from the rhizosphere of various crops and they were able to produce auxins.

Vessey, (2003) showed that Plant Growth Promoting Rhizobacteria (PGPR) comprise a diverse group of rhizosphere-colonizing bacteria and diastrophic microorganisms, when grown in association with a plant, stimulate growth of the host as well as PGPR can affect plant growth indirectly or directly.

Sood (2003) studied the freenitrogen fixers like other PGPR species, without reference to nitrogen fixation using the nitrogen-fixer Azotobacter chroococcum and Pseudomonas fluorescens. The chemotaxis of these two PGPR towards roots of mycorrhizal tomato plants (Glomus *fasciculatum*) was an important step of communication for root colonization

Mahmoud *et al*,. (2002) showed that *Azotobacter* inoculation caused highly significant increases in all measured of pepper plant growth parameters and acetylene reduction activity of roots as well as in total and bulb yields in comparison with the inoculated treatments.

Introducing selected PGPR strains during sowing or transplanting, the rhizosphere can be enriched with beneficial bacteria which create a biological barrier for plant pathogens and pests detrious to root health (Cook and Baker, 1983).

Many bacterial and fungal antagonists were found to have substantial effect against many soil-

- 464 -

borne pathogens. (Benhamou et al, 1996, and Khalifa and liddell, 1996). The aim of this work to evaluate the efficiency of different isolates of *Azotobacter*, *Azospirillum*, *Pseudomonas* and *Bacillus* for some traits related to plant growth promoters as the antagonistic microorganisms and studing the efficacy as bio-controlling agents against pepper diseases.

MATERIALS AND METHODS

For counting and isolation of certain rhizosphere microorganisms of pepper plants, pots experiments were carried out at Faculty of Agricultural Minia University. Three kg portions of clay loam soil were placed in plastic pots (30 cm in diameter, five pepper transplants (variety Kanza) were planting as each pot. Samples were taken after 15, 30, 45, 60 and 75 days from transplanting for total counts of bacteria, Bacillus, Pseudomonas, Azotobacter and Azospirillum. Removal and treatment of rhizosphere soil samples as well as determination was carried out as described by (Abdallah et al, 2015). The plate count method was prepared to determine the total count of bacteria, Bacillus sp. and Pseudomonas sp. using nutrient agar medium (Oxoid Manual 1965) for Bacillus sp, King B medium (King et al 1954) for Pseudomonas. The most probable numbers (MPN) method was used for counting Azotobacter and Azospirillum using Ashby's liquid medium modified by Abdel-Malek and Ishac (1986) and Dobereiner and Day(1976), respectively. Isolation, purification and maintenance of

Bacillus sp., Pseudomonas sp, Azotobacter sp. and Azospirillum sp. were carried out as described by (Abdelmagid 2016).

The nitrogen fixing capability of the isolates was achieved using the ambient assay of nitrogenase activity according to Postage (1972).

Phosphate and zinc solubilizing ability of the isolates were tested by the dissolation of precipitated tricalcium phosphate in an agar medium as described by Rodrigues *et al.*, (2004), or zinc oxide in an agar medium as reported by **Saravanan** *et al.*, (2003). Solubilizing index (SI) was calculated according to ratio of the total diameter (colony halo zone) to the colony diameter (Edi-premono *et al* (1986).

Preparation of Fungal inoculum

Inoculum of isolates of *Rhizoctonia solani* and *Fusarium oxysporum* were prepared using sorghum, coars sand water as described by (Abdelmaged, 2016)

Preparation of bacterial inoculum

Bacterial suspension $(1x10^{\circ})$ CFU or MPN were prepared by dilution plate assay as described by Abdelmagid (2016).

Antagonistic effect of isolated microorganisms against *F. oxysporum* and *R. solani*

Five isolates from Azotobacter. Azospirillum, Bacillus sp. and Pseudomonas sp which showed more efficient in N₂-fixation. Phosphate and solubilizing zinc were tested. Azotobacter isolates were Azt14, Azt19, Azt20, Azt15 and Azt8, Azospirillum Azo.14, Azo12, Azo10, Azo8 and Azo9, Pseudomonas sp Ps14,

- 465 -

Ps7, Ps11, Ps5 and Ps8 and *Bacillus sp* isolates Bac12, Bac6, Bac19, Bac9 and Bac3) on growth of *Fusarium* oxysporium and *Rhizoctonia solani* were used to study the antagonistic effect against the fungal growth of *F.* oxysporum and *R. solani*.

Plates were streaked with the bacterial growth of the tested microorganisms obtained from 2 days old culture at opposite sides at the periphery by using needle. At the same time, one disc of the pathogen was placed at the center of each plate. Inoculated plates were incubated at 20°C. Four replicates for each treatment were used. When growth of the pathogen covered the plate surfaces (9.0 cm in diameter) of control treatment. antagonistic or mycoparasitic effect was determined by measuring the free inhibition zone, then percentage of mycelial growth inhibition was calculated according to formula as follows: Percentage of mycelial growth inhibition % = [A-B/A] $\times 100$

- A= Length of the control hyphal growth.
- B= Length of the treated hyphal growth.

RESULTS AND DISCUSSION

Data presented in Table (1) generally showed that counts of bacteria Azotobacter, *Azospirillum*, *Pseudomonas* and *Bacillus sp.* in rhizosphere of pepper plants were higher than in control non-rhizosphere one. Accordingly, positive rhizosphere effects, particularly at the early periods of plant growth (15,30,45 and 60 days) were recorded. The observed changes in numbers of rhizosphere population during different growth phases of pepper plant may be partly or largely due to changes in the amount and chemical composition of root exudates during plant growth.

These results are in agreement with those reported by Besada (1981) who found that total bacteria. actinomycetes, fungi and free nitrogen fixers showed positive rhizosphere soil (R/S) ratio in the rhizosphere of thirteen xerophytic plants, commonly found in Egyptian desert. Manv investigators reported that the rhizosphere of plants supports greater population of bacteria, which are physiologically more active types than non-rhizospheric microorganism (Hussien, 2016 Abdelmagid, and 2016).

Testing the efficiency of different isolates for some traits related to plant growth.

purified isolates Eighty representing of Azotobacter. Azospirillum, Pseudomonas and Bacillus sp. (twenty from each) were isolated at random from rhizosphere of pepper plants grown in clay loam soil. These isolates were picked up, purified by repeated planting and coded these isolates were tested towards their efficiency for some traits related to plant growth promoting.

1) Screening the isolates for nitrogenase activity:

The obtained data show that high values of nitrogen fixation were recorded for *Azospirillum* isolates followed by *Azotobacter* then

- 466 -

Pseudomonas sp, for isolates Azo14, Azt14, Ps5, the obtained figures were 57.21, 48.17 and 27.92 nmole/C₂H₄/1ml culture/hr, respectively

The results in Table (2) represent the nitrogenase activity of *Azotobacter* isolates wherever the production of C_2H_4 ranged from 3.28 to 48.17 n.moles/ C_2H_4 /1ml culture/hr. It is clear that the best isolate with the highest recorded values was the isolate (Azt.14, Azt.19, Azt.15, Azt.8 and Azt.20) with values of 48.17, 39.16, 34.19, 33.19 and 33.15nmoles/ C_2H_4 /1ml culture/hr., respectively. With regard to *Azospirillum* isolates the best isolates for nitrogenase activity which attained the highest values were (Azo.14, Azo.9, Azo.12, Azo.20 and Azo.10) with values of 57.21, 30.78, 30.61, 22.14 and 21.21 n.moles/ $C_2H_4/1ml$ culture/hr respectively.

The producing of C_2H_4 by *Pseudomonas* isolates ranged from 2.20 – 27.92 n moles/ C_2H_4 /1ml culture/hr. The highest N₂ fixation was recorded for isolates (Ps.5, Ps.14, Ps.7, Ps.12 and Ps.18).The obtained figures were 27.92, 22.61, 22.22, 19.04 and 17.21 n moles/ C_2H_4 /1ml culture/hr., respectively.

Table (1): Counts of bacteria *Azotobacter*, *Azospirillum*, *Pseudomonas* and *Bacillus* in rhizosphere and non-rhizosphere soil of pepper plants.

Days after	Sam-	Bacte (tota		Bac	illus	Pseudo	omonas	Azotol	bacter	Azospi	irillum
transp- lanting	pling	10 ⁵	R/S	10 ³	R/S	10^{4}	R/S	10 ³	R/S	10 ⁴	R/S
15	R	129.92	4.27	14.21	2.3	19.6	3.16	210.62	2.9	8.6	3.18
15	S	30.38	4.27	6.13	2.5	6.2	5.10	70.31		2.7	
30	R	162.11	5.04	15.63	³ 1.88.0	23.6	2.56	250.11	3.04.0	9.7	3.34
30	S	32.11	5.04	8.3		9.2	2.50	82.22		2.9	5.54
45	R	192.22	6.38	16.22	1.87.0 56	56.6	6.21	322.84	3.33.0	18.2	5.05
45	S	30.11	0.58	8.66	1.67.0	9.1	0.21	96.66	5.55.0	3.6	5.05
60	R	57.40	2.02	14.22	2.0	69.2	5.76	310.71	3.4	21.6	4.69
00	S	28.32	2.02	7.11	2.0	12.0	5.70	100.22	5.4	4.6	4.09
75	R	43.66	1 60	18.61	2.05.0	59.6	7 25	255.15	2 1 9 0	20.1	4 70
	S	27.11	1.68	6.3	2.95.0	8.1	7.35	70.66	3.18.0	4.2	4.78
D 11	-		a		1			D/G 1		00	

R: rhizosphere soil, **S:** non-rhizosphere soil,

1) Screening the isolates for phosphate dissolving

It is clear from data presented in Table (3 and 4) that the screening and the tested isolates for phosphate dissolving. Twenty isolates of *Azotobacter* have been tested for their impact of melting phosphors, 15 **R/S:** rhizosphere effects.

isolates of them were found to have positive effects and the other 5 isolates had negative effects. Solubilization index (SI) ranged from 4.6– 1.3. The isolates varied on their abilities to solubilize P indicated by differences in solubilization index. Solubilizing index (SI) was calculated according to ratio

- 467 -

of the total diameter (colony halo zone) to the colony diameter (Edi-premono *et al* (1986), .The results indicated that the highest values of the isolates of *Azotobacter* in solubilize P were Azt14, Azt8, Azt20,Azt5 and Azt19 ,the obtained figures were 4.6, 4.0, 4.0, 3.6and 3.3 mm respectively. With regard to *Azosprillium* isolates twenty isolates were tested out of them, 13 isolates were found to have positive effects and the other 7 isolates had negative effects. Solubilization index (SI) ranged from 5.0 - 1.3. The more efficient 5 isolates of *Azospirillum* in solubilize P were Azo14, Azo10, Azo12, Azo8 and Azo9, the obtained figures were 5.0, 4.6, 4.6, 4.3 and 3.6 mm, respectively.

Table (2): Nitrogenase activity *of Azotobacter, Azospirillum* and *Pseudomonas sp* isolates which were isolated from rhizosphere of pepper plants (nmoles/C₂H₄/ 1 ml culture/hr)

No	Azotobacter	No	Azospirillum	No	Pseudomonas
Azt1	6.31	Azo1	12.14	Ps1	2.20
Azt2	12.69	Azo2	3.78	Ps2	6.02
Azt3	3.28	Azo3	8.14	Ps3	10.29
Azt4	4.23	Azo4	12.63	Ps4	8.19
Azt5	5.90	Azo5	3.85	Ps5	27.92
Azt6	16.24	Azo6	7.92	Ps6	7.74
Azt7	12.25	Azo7	6.07	Ps7	22.22
Azt8	33.19	Azo8	18.71	Ps8	15.29
Azt9	19.71	Azo9	30.78	Ps9	3.03
Azt10	5.90	Azo10	21.21	Ps10	14.30
Azt11	16.11	Azo11	7.35	Ps11	12.96
Azt12	20.41	Azo12	30.61	Ps12	19.04
Azt13	8.12	Azo13	15.64	Ps13	12.54
Azt14	48.17	Azo14	57.21	Ps14	22.61
Azt15	34.19	Azo15	14.51	Ps15	8.14
Azt16	5.65	Azo16	8.75	Ps16	6.61
Azt17	22.16	Azo17	12.14	Ps17	2.21
Azt18	14.82	Azo18	7.92	Ps18	17.21
Azt19	39.16	Azo19	20.17	Ps19	13.55
Azt20	33.15	Azo20	22.14	Ps20	15.90

Twenty isolates of *Bacillus sp.* were tested for their impact of melting phosphors, 15 isolates of them were found to have positive effects. Solubilization index (SI) ranged from 6.0-1.3. The more efficient isolates of *Bacillus sp.* in solubilize P were Bac12, Bac3, Bac6, Bac9 and Bac19, the obtained figures were 6.0, 5.3, 5.3, 5.0 and 4.3 mm respectively.

Phosphate dissolving by *Pseudomonas* isolates, twenty isolates was tested for their impact of melting phosphors, 15 isolates of them were found to have positive effects and the other 5 isolates had negative effects.

- 468 -

Solubilization index (SI) ranged from 6.0-1.3. The results indicated that the highest values of the more efficient 5 isolates of *Pseudomonas sp.* in solubilize P were No. Ps14, Ps7, Ps5, Ps8 and Ps11, the obtained figures were 6.0, 5.6, 5.3, 5.3 and 5.0 mm respectively.

These results are accordance with those of (Kumar *et al.*, 2012) who reported that a large number of bacteria including species of *Pseudomonas sp.*, *Azospirillum*, *Azotobacter*, *Bacillus sp.*, *Rhizobium* and *Serratia* enhanced plant growth by their different plant growth promoting activities including phosphate solubilization. Trivedi and Sa, (2008) reported that gluconic acid Table (3): Phosphate solubilization by is the principal organic acid produced by phosphate-solubilizing bacteria such as *Pseudomonas sp.*, *Erwinia herbicola*, *Pseudomonas cepacia* and *Burkholderia cepacia*.

Phosphorus is commonly diligent in most natural soil since it is fixed as insoluble iron and aluminum phosphate in acidic soil (pH lower than 5), calcium phosphate in alkaline soil (pH above 7.0) in Egyptian soils. However, insoluble calcium phosphate can be dissolved and made available to plants rhizosphere by soil and microorganisms via a mechanism that is thought to involve the release of organic acid (Cunningham and Kuiack, 1992).

Table (3): Phosphate solubilization by different isolates of *Azotobacter* and *Azospiriluum*.

	Azotobacter			Azospirillum	
IsolatesNo.	Colony diameter + clear zone	(SI)	IsolatesNo.	Colony diameter + clear zone	(SI)
Azt1	5	1.6	Azo1	4	1.3
Azt2	3	1.0	Azo2	8	2.6
Azt3	4	1.3	Azo3	3	2.0
Azt4	3	-	Azo4	5	1.6
Azt5	11	3.6	Azo5	3	-
Aztó	6	2	Azo6	4	1.3
Azt7	0 7	2.3	Azo7	3	1.5
Azt8	12	4	Azo8	13	4.3
Azt9	9	3	Azo9	15	3.6
Azt10	4	1.3	Azo10	14	4.6
Azt11	5	1.6	Azo11	8	2.6
Azt12	3	1.0	Azo12	14	4.6
Azt12 Azt13	3	-	Azo12 Azo13	3	4.0
Azt13	14	4.6	Azo13 Azo14	15	5
Azt14	9	3	Azo15	3	5
Azt15 Azt16	4	1.3	Azo15 Azo16	5 7	2.3
Azt10 Azt17	7	2.3	Azo17	4	1.3
Azt17 Azt18	3	2.3	Azo18	4	1.5
Azt18 Azt19	10	- 3.3	Azo18 Azo19	3	-
Azt19 Azt20	10	3.3 4	Azo19 Azo20	5	1.6
AL120	12	4	AL020	5	1.0

- 469 -

Pseud	lomonas.				
	Bacillus			Pseudomonas	
No.	Colony diameter + clear	(SI)	No.	Colony diameter + clear	(SI)
isolates	zone	(31)	isolates	zone	(31)
Bac1	6	2	Ps1	3	-
Bac2	4	1.3	Ps2	7	2.3
Bac3	16	5.3	Ps3	4	1.3
Bac4	12	4	Ps4	8	2.6
Bac5	8	2.6	Ps5	16	5.3
Bac6	16	5.3	Ps6	3	-
Bac7	3	-	Ps7	17	5.6
Bac8	6	2	Ps8	16	5.3
Bac9	15	5	Ps9	3	-
Bac10	3	-	Ps10	5	1.6
Bac11	3	-	Ps11	15	5
Bac12	18	6	Ps12	4	1.3
Bac13	7	2.3	Ps13	9	3
Bac14	3	-	Ps14	18	6
Bac15	8	2.6	Ps15	3	-
Bac16	4	1.3	Ps16	3	-
Bac17	3	-	Ps17	5	1.6
Bac18	9	3	Ps18	10	3.3
Bac19	13	4.3	Ps19	12	4
Bac20	10	3.3	Ps20	9	3

Table (4) : Phosphate solubilization by different isolates of *Bacillus* and *Pseudomonas*.

SI: Solubilizing Index

 $SI = \frac{colony + halozone}{colony + halozone}$

colony diameter

Colony diameter = 3mm

2) Screening the isolates for zinc dissolving

Data in Table (5and 6) show the screening and the tested isolates for zinc dissolving . Twenty isolates of *Azotobacter* were studied for their impact on melting zinc. There are 14 isolates out of them found to have positive effect. Solubilizing index (SI) was calculated according to ratio of the total diameter (colony halo zone) to the colony diameter (Edi-premono *et al* (1986). It was found that SI ranged from 5.0-1.3. The results indicated that

the highest solubilizing index (SI) achieved were 5.0 for isolates Azo20. Moreover the most efficient 5 isolates were

Azt20,Azt15,Azt19,Azt14andAzt5, the obtained figures were 5.0, 4.6, 4.6, 4.3and 4.0 mm respectively.

With regard to *Azospirillum* isolates, (SI) ranged from 5.3-1.3. The more active isolates on zinc solubilizing was isolates Azs14. Out of the twenty *Azospirillum* isolates,13 isolates were found to have positive effect.

- 470 -

Moreover, SI for *Bacillus sp.* isolates ranged from 5.6 to 1.3. Out of twenty isolates of *Bacillus sp.* 14 isolates have positive effect on zinc solubilizing. The most efficient 5 isolates of *Bacillus* were Bac19, Bac6, Bac12, Bac3 then Bac9; the obtained figures were 5.6, 5.3, 5.3, 5.0 and 4.3 mm, respectively.

The same data in Table (6) show that out of the twenty isolates of *Pseudomonas sp.*, which investigated for their impact on melting zinc 13 isolates found to have positive effect. The SI ranged from 6.3–1.3. The most efficient isolates of *Pseudomonas sp.* in zinc solubilizing are Ps14 followed by isolates Ps7, Ps11, Ps5 and Ps8.

The role of zinc in nutrition and physiology of microorganisms in widely especially studied, its importance for activity of many enzymes, since zinc is limiting factor in crop production, exogenous application of soluble zinc source, similar to fertilizer application has been advocated to various crop. This causes transformation of about 96-99% of applied available zinc to various microorganisms. Unavailable can be reverted back to available from bacterial enucleating with strains capable of solubilizing at (Saravanan et al, 2003).

3) Screening the tested isolates for antagonism towards pathogenic fungi.

Biological control of the plant pathogens has been the focus of many studies in plant protection that search for alternative or complementary methods to the use of chemical pesticides.

The most efficient 5 isolates from either *Pseudomonas sp., Bacillus sp., Azotobacter* and *Azospirillum.*, which show the more efficient in N2-fixation, P and Z solibilition were selected to test their effect for antagonism toward plant pathogenic fungi. (i.e. *F.oxysporum* and *R.solani*).

The results presented in Table (7) indicated that the tested isolates were shown exhibit a wide range of antagonistic activity against both *Fusarium oxysporum* and Rhizoctonia solani

Results exhibited that the highest inhibition percentage values on mycelial growth of *F. oxsoporum* were achieved by *Pseudomonas, Bacillus, Azotobacter* and *Azospirillum* isolates with inhibition effect 83.6, 82.14, 73.5 and 52.6 for isolates Ps14, Bac12, Azt14 and Azo14, respectively.

With regard of *R.solani* the highest inhibition percentage values on mycelial growth were achieved by *Bacillus*, followed *by Pseudomonas sp.*, then *Azotobacter* and *Azospirillum* isolates. The recorded inhibition were 74.2%, 71.6%, 64.3% then 43.6% for isolates Bac12, PS14, Azt14 then Azo14, respectively.

The results obtained are in agreement with those of Pandey and Kumar (1990) Verma, et al (2001) and Pandy, *et al* (2009) who reported that *Azotobacter chroococcum* and *Azospirillum brasilense* had inhibitory effect of 14 rhizosphere fungi. El. Mougy *et al* (2011), examined the influence of antagonistic isolates of *B*.

- 471 -

subtilis and *P.fluorescens* against soil borne root rot, the tested isolates reduced the linear growth of fungal pathogens, and all isolates were effective against *F.solani* and might be very useful as potential biological control. Similar results were obtained by Khalifa and Liddell (1996), Hussein (2016) and Abdelmagid, (2016).

Table (5): Zinc solubilization by different isolates of *Azotobacter* and *Azospirillum*

	Azotobacter			Azospirillum	
No.	Colony diameter +		No.	Colony diameter +	
isolates	clear zone	(SI)	isolates	clear zone	(SI)
Azt1	8	2.6	Azo1	5	1.6
Azt2	4	1.3	Azo2	7	2.3
Azt3	3	-	Azo3	3	-
Azt4	3	-	Azo4	4	1.3
Azt5	12	4	Azo5	3	-
Azt6	4	1.3	Azo6	3	-
Azt7	5	1.6	Azo7	3	-
Azt8	10	3.3	Azo8	12	4
Azt9	9	3.0	Azo9	10	3.3
Azt10	3	-	Azo10	15	5
Azt11	7	2.3	Azo11	8	2.6
Azt12	3	-	Azo12	13	4.3
Azt13	3	-	Azo13	3	-
Azt14	13	4.3	Azo14	16	5.3
Azt15	14	4.6	Azo15	4	1.3
Azt16	3	-	Azo16	6	2
Azt17	6	2.3	Azo17	3	-
Azt18	5	1.6	Azo18	3	-
Azt19	14	4.6	Azo19	5	1.6
Azt20	15	5.0	Azo20	8	2.6

Table(6): Zinc solubilization by different isolates of Bacillus and Pseudomonas						
	Bacillus			Pseudomonas		
No.	Colony diameter +	(SI)	No.	Colony diameter +	(SI)	
isolates	clear zone		isolates	clear zone		
Bac1	5	1.6	Ps1	3	-	
Bac2	3	-	Ps2	5	1.6	
Bac3	15	5	Ps3	3	-	
Bac4	4	1.3	Ps4	6	2	
Bac5	6	2	Ps5	17	5.6	
Bac6	16	5.3	Ps6	4	1.3	
Bac7	4	1.3	Ps7	18	6	
Bac8	8	2.6	Ps8	15	5	
Bac9	13	4.3	Ps9	3	-	
Bac10	7	2.3	Ps10	6	2	
Bac11	3	-	Ps11	17	5.6	
Bac12	16	5.3	Ps12	3	-	
Bac13	9	3	Ps13	7	2.3	
Bac14	3	-	Ps14	19	6.3	
Bac15	7	2.3	Ps15	3	-	
Bac16	3	-	Ps16	6	2	
Bac17	5	1.6	Ps17	3	-	
Bac18	8	2.6	Ps18	8	2.6	
Bac19	17	5.6	Ps19	10	3.3	
Bac20	10	3.3	Ps20	3	-	

Table(6) · Zinc solubilization by different isolates of *Bacillus* and *Pseudomonas*

SI: Solubilizing Index

SIcolony diameter

Colony diameter = 3mm

Table (7): Inhibitory effect of Aztobacter, Azospirillum, Pseudomonas sp. and Bacillus sp. Isolates against Fusarium oxysporum and Rhizoctonia solani.

	sarium oxysporum	Rhizoctonia solani.
Bacterial isolates	Mycelial growth inhibition (%)	Mycelial growth inhibition(%)
Azt.8	31.3	22.5
Azt.20	42.5	35.8
Azt. 19	62.3	56.1
Azt.14	73.5	64.3
Azt.15	37.0	28.1
Azo.9	32.0	12.3
Azo.8	36.0	10.2
Azo.10	43.1	38.6
Azo.12	48.2	30.3
Azo.14	52.6	43.6

Ps.7	75.6	50.3
Ps.11	62.3	64.2
Ps.5	53.1	44.1
Ps.8	42.6	40.3
Ps.14	83.6	71.6
Bac.12	82.1	74.21
Bac.3	36.3	50.4
Bac.19	72.1	63.2
Bac. 9	54.3	35.1
Bac.6	79.1	67.3

A. R. Abdullah et al., 2017

REFERENCES

- Abdallah, A.R.; EL-Dahtory, Th. El.; Abdel Moneem, A.A.; Koriem, S. O. and Hussien G.A. (2015):The effect of plant growth promoting rhizobacteria and mycorohyza as againt antiganistic sclerotium cepivorum on different varieties of onion. Minia International Conference "Agriculture and Irrigation in Nile Basin Countries" 2015, 168-176 Minia, Egypt.
- Abdelmagid, W.M.A (2016): Biological control of some plant diseases via certain soil microorganism .M.Sc.Thesis, Fac. Agric. Minia Univ. Egypt.
- Abdel-Malek, Y. and Ishac, Y. Z.(1968) Evaluation of methods used in conting Azotobacter. J. Appl. Bat. 31: 269-275.
- Benhamou, N, Kloepper, J.W.I Quadt. Hallmanns and Tuzun, S. (1996): Induction of defence related ultrastructure modification in pea root tissues inoculated with endophytic bacteria. Plant physiology, 112: 919-929.
- Besada, Y.B. (1981) Rhizosphere Microflora and their role in nutrition of desert plants. M. Sc.

Thesis, Fac. Agric Cairo Univ. Egypt.

- Cook, R.J. and Baker, K.F.(1983). The Nature and practice of biological control of plant pathogens. Amer. Phytopathol. Soc. St. Paul, Minn. (C.F. Plant Dis., 1982, 66: 357-364).
- Cunningham, J.E. and Kuiack, C (1992): Production of citric acid and oxalic acid and solubilization of calcium phosphate by *Penicillum bilaii*. Applied and Environmental Microbiology 58, 1454, 1458.
- Dobereiner, j and Day, J.H. (1976) :Associative symbioses in tropical grasses characterization of microorganism and nitrogen fixing sile. In Proceeding of First International Symposium on Nitrogen Fixation. E.D W.E. Newton and C.Y. Nyman vol2.
- El-Mougy, N.S. Abdel-Kader, M.M and Alhabeb, R.S. (2011). *In vitro* antifungal activity of chitinolytic enzymes produced by bio-agents against root rot pathogenic fungi. Archives of Phytopathology and Plant Protection, 44(7): 613-622.
- Edi-premono, H., Moawad, A.M. and Viek, P.L.G. (1986) Effect of

Phosphate solubilizing *Pseudomonas putida* on growth of Mango and its survival in the rhizosphere. Indonesian Journal of Crop Science 11, 13-23.

- Forlani, G.; Pastorelli, R.k; Branzoni, M. and Favillim, F. (1995): Root colonization efficiency, plant growth-promoting activity and potentially related propertiesassociated bacteria. j. Gene. And Breed. 49(4): 343-351.
- Gray, E.J. and smith, D.L. (2005): intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol. Biochem. 37: 395-412.
- Husseen, G.A.H., (2016) Effect of plant growth promoting rhizobacteria (PGPR) on growth and yield of different variation of onion .Ph.D. Thesis, Fac. Agric. Minia. Univ. Egypt .
- Khalifa, E.Z. and Liddell, C.M. (1996).
 Biological management of *Macrophomina phaseolina* by *Trichoderma harziaum*. Minufiya J. Agric. Res., 21(5)
- King, E.O. Ward, M.K and Raney, D.E.(1954): Two simple media for the demonstration of pycyanin and fluorescin. Journal of Laboratory and Clinical Medicine 44, 301-307.
- Kumar, A.; Devi, S.; Patil, S.; Chandani, P. and Nagi, S. (2012): Isolation, screening and characterization of bacteria from rhizospheric soils from different plant growth promotion activites: as *in vitro* study. Recent research

in science and technology. 4(1): 01-05.

- Mahmoud, S.A.Z.; El-Sawy, M.; Ishac,
 Y.Z and El-Safty, M.M (2002):
 The effect of salinity and alkalinity
 on the distribution and capacity of
 N2- fixation by *Azotobacter* in
 Egyptian soil. In: Granhall U. (ed)
 Environmental Role of Nitrogenfixing Blue-green Algae and A
 symbiotic Bacteria, Uppsala,
 Sweden, p.p 99-109.
- Oxoid Manual of Culture Media Ingredients and Other Laboratory Services (1965). Oxoid Limited, London.
- Pandya, J.R. Joshi, D.M. and Sabalpara, A.N. (2009): Biological control of muskmelon (*Cucumis melo L.*) wilt *Fusarium solani*. Journal of Plant Disease Science, 4(2): 232-234.
- Pandy, A.and
 - Kumar,S.(1990):Inhibitory effect of *Azotobacter chrooccum* and *Azospirillum brasilense* on a range of rhizosphere fungi. Indian J. Exp. Biol., 28(1): 52-54
- Postage, J.R. (1972): The acetylene reduction test for nitrogen fixation. Meth. Microbial. 66:343.
- Rodrigues, H; Gonzales, T, Igoire, I and Bashan, Y (2004) Gluconic acid production and phosphate solubilizing by plant growth promoting bacterium *Azospirillum sp.* Naturwissneschaften 91, 552-555.
- Saravanan, V.S.; Subramoniam, S.R. and Raj, S.A (2003): Assessing *in vitro* solubilization potential of different zinc solubilizing bacterial

(ZSB) isolates. Brazilian Journal of Microbiology 34, 121-125.

- Sood, S. G., (2003): Chemotactic response of plant-growthpromoting bacteria towards roots of vesicular-arbuscular mycorrhiza tomato plants, FEMS Microbial Ecol 45: 219-227.
- Trivedi, P. and Sa, T.M. (2008). *Pseudomonas corrugate* (NRRL B-30409) mutants increased phosphate solubilization, organic acid production and plant growth

at lower temperatures. Current Microbiology 56: 140-144.

- Verma, S, Kumar, V, and Narula, N (2001): Studies on *in vitro* production of anitmi Rubstances by *Azotobacter Chrooccum* isolates (mutants) Zeitschrifl fur. *P. flanzen* Krankheiten and *P. flanzenschutz,*. 108 (2). 152-165.
- Vessey, K.J. (2003): Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255, 571-586.

تقييم بعض ميكروبات منطقة جذور الفلفل لبعض سمات منظمات نمو النبات ومضادات الفطريات

احمد رفعت عبدالله - عادل محمود محد حماد -عمر عبداللطيف عمر سعد نورهان حجاج ابراهيم حسين قسم الميكروبيولوجي- كليه الزراعه – جامعه المنيا

في تجربة أصص تم عزل وتقييم بعض عزلات في منطقة ريز وسفير الفلفل لبعض سمات منظمات النمو ومضادات الفطريات وقد وجد أن ميكروبات الأزوتوباكتر ،الأزوسبيريللم ،سيدوموناس وباسيلس تزداد في منطقة الريز وسفير مما أعطى تأثير ريز وسفير موجب خاصة في بداية نمو النباتات حتى 60 يوم . تم عزل 80 عزلة من هذه الميكروبات لتقييم كفاءتها في تثبيت النيتروجين، واذابه كل من الفوسفات والزنك.

كانت عزلات الازوسبيريلليم أعلي العزلات كفاءة في ثتبيت النيتروجين، يليها في التأثير عزلات الازوتوباكتر ثم عزلات سيدوموناس بينما جاءت أعلي قيم إذابة للفوسفات من عزلات السيدوموناس و عزلات باسيلس ثم عزلات الأزوسبيريلليم وأخيرا عزلات الأزوتوباكتر حيث كان معامل الإذابة (1.3 – 6 مم) ، (1.3 – 6 مم)، (1.3 – 5 مم) ، (1.3 – 6.4 مم) علي الترتيب. كانت قيم الإذابة الزنك لعزلات السيدوموناس (1.3 – 6.3 مم)، ثم عزلات الباسيليس (1.3 – 5.6 مم) ثم عزلات الأزوسبيريلليم (1.3 – 5.7 مم) وأخيرا الأزوتوباكتر (1.3 – 5 مم).

َنَّمُ أَخْتَبَار التَأْثَير المضَادُ لعز لات الأزوتوباكتر، الأزوسبيريللم، باسيليس وسيدوموناس علي فطريات فيوزاريوم اوكسيسبورم، ريزوكتونيا سولاني وذلك بقياس النسبة المئوية لتثبيط نمو الميسليوم وبينت النتائج أن أعلي نسبة تثبيط للفيوزاريوم كان لعزلات سيدوموناس ثم عزلات باسيلس ثم الأزوسبيريلليم وأخيرا الأزوتوباكتر.

أما بالنسبة لفطر ريزوكتونيا فكان أعلي تثبيط من باسيلس ثم السيدوموناس يليها الأزوتوباكتر ثم الأزوسييريلليم.