Interaction effect between growth regulators producing bacteria root-rot fungi on tomato growth plants

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ABSTRACT

The current study was carried out to study the effect of plant growth promoting rhizobacteria (PGPR) on growth performance of tomato. *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum* were used in this research . Obtained results showed that *A. chroococcum* and *B. megaterium* var. *phosphaticum* gave high suppression against tomato roots pathogenic fungi i.e *Fusarium oxysporum* f.sp *lycopersici* and *Fusarium solani* . *In vitro*, clear zones around PGPR growth were showed . Such clear zones are likely to be due to the production of antibiotics-like substances , siderophores and cyanogens by PGPR strains.

Tomato inoculation with the mixture of *A. chroococcum* and *B. megaterium* var. *phosphaticum* appeared lower percentage of infected plants than those inoculated with them individually. Growth characteristics , macro-nutrients content, endogenous phytohormones and photosynthetic pigments of tomato were significantly increased in the inoculated treatments with PGPR mixture compared by that inoculated with either *A. chroococcum* or *B. megaterium* var. *phosphaticum* singly .

Key words: PGPR , Growth characteristics **,** macro-nutrients **,** endogenous phytohormones , photosynthetic pigments **.**

INTRODUCTION

Tomato (*Lycopersicom esculentum* Mill) is one of the most important vegetable crops in Arab Republic of Egypt. It is well known that, several fungal diseases attack tomato plants during all stages of growth causing a considerable reduction in both yield quality and quantity. Damping-off, root rots and wilting are among the important diseases. Root rot pathogens such as *Rhizoctonia solani* and *Sclerotium rolfsii* attack the roots and stem base of tomato **(Wokocha 1990; Ristaino *et al* 1991).**

Plant growth promoting rhizobacteria (PGPR) can produce direct or indirect effects on the host plants , indirect effects are these related to the production of metabolites such as antibiotics , siderophores or cyanogen which increase plant growth by decreasing the activities of pathogens. PGPR can produce direct effects on plant growth by producing metabolites such as plant growth regulators (PGRs) that directly promote plant growth or by facilitating nutrient uptake by the plant **Salamone *et al* (2001**) ; **Ahmad *et al* (2005) and Teixeira *et al* (2007)**

Currently, There are several PGPR inoculants commercialized those seem to promote plant growth through at least one mechanism, suppression of plant disease (termed Bioprotectants), improved nutrient acquisition (termed Biofertilizers) or phytohormones production (termed Biostimulants) **Tenuta (2006)**.

Albuquerque *et al* (2003) studied the effects of endophytic and epiphytic PGPR *Bacillus* sp on controlling of Fusarium wilt in banana caused by *F. oxysporum* f.sp *cubense*. These bacteria colonize plant organs epiphytically or endophytically caused enhancing development, yield and protecting and/or inducing resistance against pathogens. **Zaghloul *et al* (2007)** showed that the highest records of plant growth and macro-nutrients contents were observed in the treatment of tomato inoculation with *A. chroococcum* in combination with *B. subtilis* and *T. harzianum*.

The aim of this research is to study the effect of inoculation with *A. chroococcum* and/or *B. megaterium* var. *phosphaticum* in presence of tomato roots pathogenic fungi (*F. oxysporum* f.sp *lycopersici* and *F. solani*) on growth performance of tomato .

MATERIALS AND METHODS

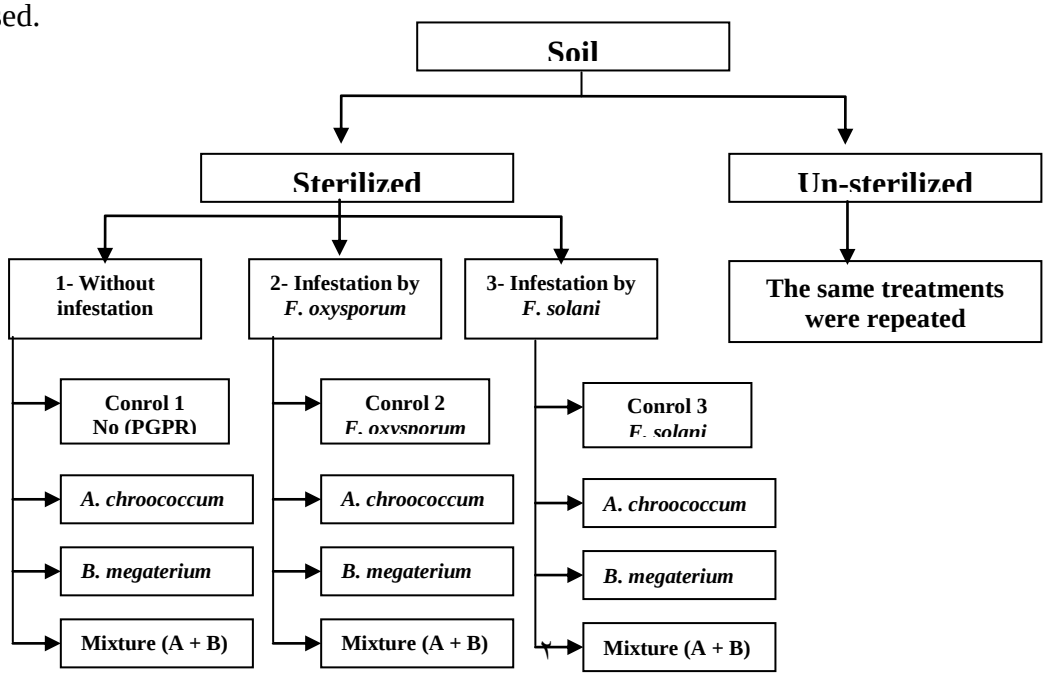
This experiment was carried out under greenhouse conditions to evaluate the efficiency of *A. chroococcum* and *B. megaterium* var. *phosphaticum* as a plant growth promoting rhizobacteria on growth performance in presence of *F. oxysporum* f.sp *lycopersici* and *F. solani*.

Antagonistic activity of PGPR

Antagonistic effect of *A. chroococcum* and *B. megaterium* var. *phosphaticum* against two soil-borne pathogenic fungi *F. oxysporum* f.sp *lycopersici* and *F. solani* was studied in vitro . Pathogenic fungi were initially grown in Petri dishes containing PDA medium and incubated at 28°C for 48 hrs . Then 0.5 cm disks were cut from the edge of the active growth colonies . One disk was transferred to the center or in one half of Petri dish containing the previous mixture of media. Bacterial strains were added with circular shape around the fungus disk or linearly in the other half dish .Then , the dishes were incubated at 28°C for 7 days. Inhibition zones in fungal growth by bacterial strains were observed .

Experimental design

A pot experiment designed to study the inoculation activity with PGPR (*A. chroococcum* and/or *B. megaterium* var. *phosphaticum*) on tomato plants growth in infested and un-infested soil with pathogenic fungi (*F. oxysporum* f.sp *lycopersici* or *F. solani*) in sterilized and un-sterilized soil . The treatments were distributed in greenhouse using randomized complete design. Three replicates were used.



Physical and chemical analyses of the experimental soil are shown in Table (1) .

Table 1. Physical and chemical analyses of the experimental soil .

Parameters	Unit	Values	Parameters	Unit	Values
A. Mechanical analysis			B. Chemical analysis		
Coarse sand	(%)	3.91	Organic matter	(%)	1.52
Fine sand	(%)	24.04	$CaCO_3$	(%)	0.55
Silt	(%)	25.22	Total nitrogen	(%)	0.23
Clay	(%)	44.14	Total phosphorus	(%)	0.12
Textural class	(%)	Clayey loam	Total potassium	(%)	0.27
			pH		8.2

Preparation of pathogenic inocula and soil infestation

F. oxysporum f.sp. *lycopersici* and *F. solani* inocula were prepared by growing on potato dextrose broth medium . After incubation period , growth was decanted and mycelial mats were blended in a warring blender. The spores density was counted using a haemocytometer slide and adjusted to contain about 10^7 spore/ml recommended by (**Zaghloul *et al* 2007**). The sterilized soil was infested by mixing 100 ml of spore suspension per Kg soil. Then pots were carefully irrigated and kept under greenhouse conditions for 7 days for fungi activation.

Preparation of PGPR inocula

A. chroococcum and *B. megaterium* var. *phosphaticum* inocula were prepared on modified Ashby's and Modified Bunt and Rovira broth media, respectively under optimal conditions .

Cultivation process

Super strain B tomato cultivar was used in this experiment. Before cultivation, tomato seedlings were soaked by dipping the root system in mixture of sucrose solution (40 %) as an adhesive for inocula, and cell suspension of either *A. chroococcum* (8×10^7 cfu/ml) 4 days-old or *B. megaterium* var. *phosphaticum* (9×10^8 cfu / ml) 2 days-old for 60 minutes before planting. The same prepared inocula were added to the pots three times throughout the growing season at a rate of 100 ml. pot^{-1}.

Diseases assessment

Estimation the percentage of infected and survived plants was determined after 30 and 45 days from planting .

Growth characteristics

The following characteristics were determined at flowering stage (120 days after planting):

1. Plant height , number of branches and number of leaves .
2. Number of flowers and fruits .

Estimation of hormones in plants

Endogenous indole acetic acid (IAA), gibberellic acid (GA_3) and cytokinins in plants was achieved by the method of (**Sadeghian, 1971**) .

Estimation of photosynthetic pigments

Photosynthetic pigments (chlorophyll A & B and carotenoids) were determined spectrophotometrically according to **Nornal (1982)** and calculated as mg. g^{-1} fresh weight of leaves.

Determination of macro-elements

Total nitrogen , phosphorus and potassium of tomato shoots contents were determined according to the methods described by **A.O.A.C (1980); A.P.H.A. (1992**) and **Dewis and Freitas (1970)** respectively.

While, rhizosphere soil samples were taken for total and available nitrogen and phosphorus contents according to the method described by **Black *et al* (1982)** .

Statistical analysis

Statistical analysis was carried out according to **Snedecor and Cochran (1989)** .The differences between the means value of various treatments were compared by Duncan's multiple range test (**Duncan's, 1955)** .

RESULTS AND DISSCUSION

Antagonistic activity of PGPR

The antagonistic effect of *A. chroococcum* and *B. megaterium* var. *phosphaticum* against soil-borne pathogenic fungi was observed . Obtained results in **Figs (1; 2; 3 & 4)** shows the suppression effect of PGPR strains on pathogenic fungi . Also, obtained results emphasized that a clear zones around PGPR strains . Such clear zones are likely to be due to the production of antibiotics like substances by PGPR (*A. chroococcum* and *B. megaterium* var. *phosphaticum*). Siderophores and cyanogenes are the main compounds produced by most PGPR strains **(Albuquerque *et al,* 2003 and Somers *et al,* 2005**). Such substances reduced the mycelium formation and spore germination of *F. oxysporum* f.sp *lycopersici* (**Al-Kahal *et al,* 2003**).

In addition, Fusarium wilt was suppressed through the activity of PGPR strains. The disease suppressive mechanisms by PGPR include siderophores (mediated competition for iron) **(Raaijmakers *et al,* 1995)** . Meanwhile, other investigators attributed the disease suppressive mechanisms by PGPR to the competition for nutritional substances or induction of systemic resistance (**Fuchs *et al,* 1997; Van Loon *et al,* 1998**).

Nevertheless, **Albuquerque *et al* (2003)** reported that the production of HCN by PGPR strains (*Bacillus* sp) showed antibiosis against soil borne pathogenic fungi. Also, they reported that the PGPR colonize plant organs epiphytically or endophytically and caused enhancing development, protecting the roots from soil borne pathogens and inducing resistance against pathogens.

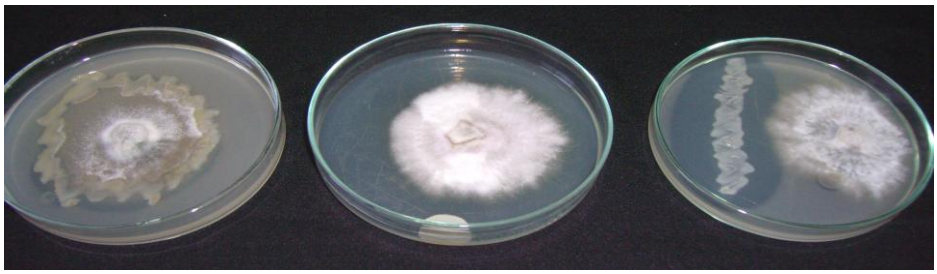


Fig 1. Antagonistic effect of *Azotobacter chroococcum* against *Fusarium oxysporum* f.sp *lycopersici.*

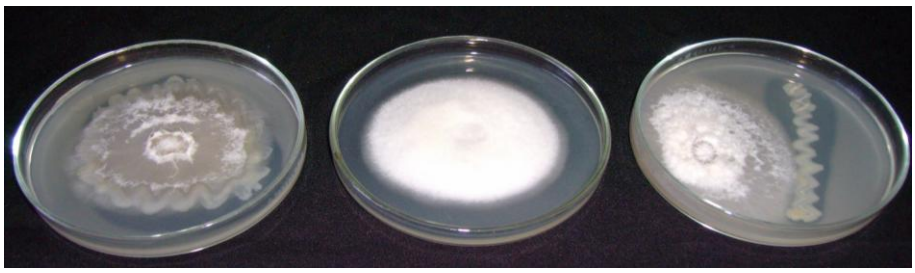


Fig 2. Antagonistic effect of *Azotobacter chroococcum* against *Fusarium solani.*

Fig 3. Antagonistic effect of *Bacillus megaterium* var. *phosphaticum* against *Fusarium oxysporum* f.sp *lycopersici* .

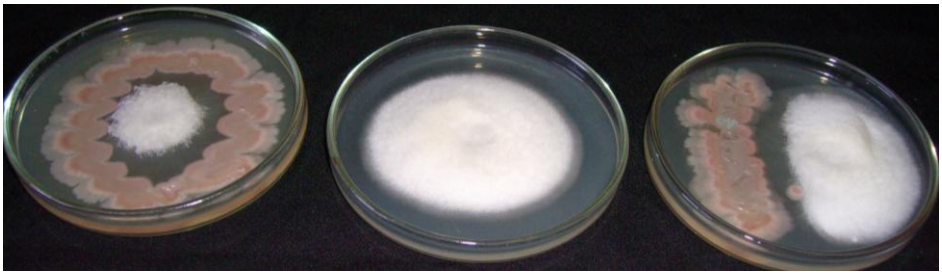


Fig 4. Antagonistic effect of *Bacillus megaterium* var. *phosphaticum* against *Fusarium solani* .

Effect of inoculation with PGPR on infected and survived plants of tomato

Data in **Table (2)** showed that the inoculation of tomato with PGPR (*A. chroococcum* or *B. megaterium* var. *phosphaticum)* significantly decreased the percentage of infected tomato plants compared to the un-inoculated ones. While , the percentage of survived plants significantly increased with tomato inoculated with PGPR . Inoculation of tomato with *B. megaterium* var. *phosphaticum* gave lower percentage of infected plants rather than that inoculatied with *A. chroococcum*. Similar trend was observed with either sterilized or un-sterilized soil treatments.

Moreover, inoculated tomato with a mixture of *A. chroococcum* + *B. megaterium* var. *phosphaticum* inoculum showed lowest percentage of infection as compared to the inoculation with either *A. chroococcum* or *B. megaterium* var. *phosphaticum* individually.

This result is in accordance with **Hassouna *et al* (1998)** who found that the growth promoting N_2-fixing bacteria *A. chroococcum* exhibited antagonistic activity and reduced damping-off by 56% for some pathogenic fungi *F. oxysporum* f.sp *lycopersici* , *F. solani* and *Pythium* sp which cause root diseases .

Soil infestation with either *F. oxysporum* f.sp *lycopersici* or *F. solani* gave high percentage of infected plants. Also, the infestation of soil with *F. oxysporum* f. sp *lycopersici* show higher percentage of infected plants rather than that infested with *F. solani* . This result could be attributed to the higher virulence of *F. oxysporum* f.sp *lycopersici* for tomato root infection rather

Table 2. Effect of inoculation with PGPR on infected and survived tomato plants.

Parameters / Treatments				Sterilized soil				Un-sterilized soil			
				First period (30 days)		Second period (45 days)		First period (30 days)		Second period (45 days)	
				Damping-Off (%)	Survived Plants (%)	Damping-Off (%)	Survived Plants (%)	Damping-Off (%)	Survived Plants (%)	Damping-off (%)	Survived Plants (%)
Untreated plants with PGPR				21.3^{bcd}	78.7^{def}	25.0^{bcde}	75.0^{cdef}	18.0^{c}	82.0^{de}	18.7^{bc}	81.3^{cd}
***A. chroococcum* (A)**				19.3^{bcde}	80.7^{cdef}	20.0^{cdef}	80.0^{bcde}	7.7^{def}	92.3^{abc}	8.7^{cdef}	91.3^{ab}
***B. megaterium* var. *phosphaticum* (B)**				17.7^{cdef}	82.3^{bcde}	18.7^{def}	81.3^{bcd}	5.7^{ef}	94.3^{ab}	7.3^{def}	92.7^{ab}
Mixture (A) + (B)				7.3^{fg}	92.7^{ab}	8.30^{g}	91.7^{a}	3.3^{f}	96.7^{a}	5.0^{ef}	95.0^{a}
***Fusarium oxysporum* f.sp. *lycopersici* (F.O)**				54.0^{a}	46.0^{g}	58.3^{a}	41.7^{g}	58.7^{a}	48.0^{f}	55.7^{a}	44.3^{e}
A. chroococcum	+	**(F.O)**		28.0^{bc}	72.0^{ef}	29.7^{bc}	70.3^{ef}	15.7^{cd}	84.3^{cde}	15.7^{bcd}	81.0^{d}
B. megaterium* var. *phosphaticum	+	**(F.O)**		13.3^{defg}	86.7^{abcd}	15.3^{efg}	84.7^{abc}	11.7^{cdef}	88.3^{abcd}	13.3^{bcdef}	86.7^{abcd}
Mixture (A) + (B)	+	**(F.O)**		10.7^{defg}	89.3^{abcd}	11.3^{fg}	88.7^{ab}	5.7^{ef}	94.3^{ab}	7.3^{def}	92.7^{ab}
***Fusarium solani* (F.S)**				48.3^{a}	51.7^{g}	55.0^{a}	45.0^{g}	46.0^{b}	54.0^{f}	48.3^{a}	51.7^{e}
A. chroococcum	+	**(F.S)**		26.0^{bc}	74.0^{ef}	27.0^{bcd}	73.0^{def}	14.7^{cde}	85.3^{bcde}	15.0^{bcde}	85.0^{bcd}
B. megaterium* var. *phosphaticum	+	**(F.S)**		9.3^{efg}	90.7^{abc}	11.0^{fg}	89.0^{ab}	8.0^{def}	92.0^{abc}	10.0^{bcdef}	90.0^{abc}
Mixture (A) + (B)	+	**(F.S)**		3.0^{g}	97.0^{a}	5.30^{g}	94.7^{a}	3.7^{f}	96.3^{a}	4.3^{f}	95.7^{a}

than *F. solani* . But , inoculation of tomato seedlings with PGPR in presence of either *Fusarium oxysporum* f.sp *lycopersici* or *F. solani* dipressed the percentage of infected plants as compared to un-inoculated tomato which planting in infested soil with either pathogenic fungi individually.

From data in **Table (2)** can observe that, the lowest percentage of infected plants was obtained when tomato was inoculated with the mixture of *A. chroococcum* + *B. megaterium* var. *phosphaticum* in presence of soil infestation with *F. solani* . It is important to mention that there are several mechanisms by which plant growth promoting rhizobacteria inhibit soil borne pathogen including the iron-chleating siderophores , antibiotics and HCN which reduce the population of root pathogenic fungi. As well as, plant growth promoting rhizobacteria have also shown promise as a potential biological control agents for many soil borne root diseases (**Gupta *et al,* 1995**).

Also, **Buchenauer (1998)** reported that the rhizobacteria might be associated with the control of soil borne fungi since such bacteria excrete of lytic enzymes such as chitinase. The lowest percentages of survived plants of tomato were observed with soil infestation treatments with either *F. oxysporum* f.sp *lycopersici* or *F. solani*. Whereas , the highest percentage of survival tomato plants was observed with tomato inoculated with the mixture of *A. chroococcum* + *B. megaterium* var. *phosphaticum* in presence of soil infestation with *F. solani*. Similar trend of results was observed with either sterilized or un-sterilized soil treatments.

These results are in agreement with those obtained by **Khalifa (2005)** who reported that *F. oxysporum* f.sp *lycopersici* was more specific than *R. solani* and *R. rolfsii* to infect tomato plants (Super strain-B cultivar) and reduced the percentage of pre and post emergence damping-off being 33.3 and 26.7%, respectively .

Effect of inoculation with PGPR on tomato growth characteristics

Data in **Table (3)** showed that infestated soil with either *F. oxysporum* f.sp *lycopersici* or *F. solani* significantly decreased the growth characteristics of tomato. This result was observed in both, sterilized and un-sterilized soil treatments.

Growth characteristics of tomato were significantly increased with the inoculation with PGPR compared to un-inoculated ones. Inoculated tomato with the mixture of PGPR (*A. chroococcum* + *B. megaterium* var. *phosphaticum*) gave higher growth characteristics rather than those inoculated with either *A. chroococcum* or *B. megaterium* var. *phosphaticum* singly.

The beneficial effect of N_2-fixers and phosphate dissolving microorganisms on plant growth was also observed by **Buchenauer (1998)** who concluded that the mechanisms by which PGPR stimulate plant growth via the production of IAA and cytokinins as well as by lowering ethylene level in plants. Also, PGPR induce systemic resistance against root pathogens.

Data in **Table (3)** showed that tomato inoculation with PGPR either individually or dually combined with soil infestation with pathogenic fungi *F. oxysporum* f.sp *lycopersici* or *F. solani* significantly increased the growth plant characteristics compared to those un-inoculated with PGPR**.**

In sterilized soil treatments the highest values of tomato growth characteristics were observed when tomato inoculated with the mixture of PGPR inoculum and planted in soil infested

Table 3. Effect of inoculation with PGPR on growth characteristics of tomato

parameters / Treatments	Sterilized soil					Un-sterilized soil				
	Plant Height (cm)	Number of leaves	Number of branches	Number of flowers	Number of fruits	Plant Height (cm)	Number of leaves	Number of branches	Number of flowers	Number of fruits
Untreated plants with PGPR	22.7^{e}	11.0^{cdef}	2.0^{cd}	5.6^{d}	1.0^{cd}	20.3^{fg}	11.3^{ab}	1.7^{cd}	4.0^{de}	1.3^{cd}
***A. chroococcum* (A)**	28.7^{cde}	14.3^{bcd}	3.3^{bc}	7.7^{bcd}	2.3^{abcd}	28.0^{cd}	13.3^{ab}	2.3^{abc}	7.0^{bcd}	2.0^{bc}
***B. megaterium* var. *phosphaticum* (B)**	27.8^{de}	12.0^{cde}	3.0^{bc}	5.7^{d}	1.7^{bcd}	28.7^{cd}	12.7^{ab}	2.3^{abc}	7.7^{bcd}	1.7^{c}
Mixture (A) + (B)	36.0^{ab}	21.3^{a}	4.0^{abc}	10.7^{b}	4.3^{a}	35.0^{ab}	14.3^{a}	3.7^{ab}	9.7^{ab}	2.7^{bc}
***Fusarium oxysporum* f.sp *lycopersici* (F.O)**	10.7^{f}	7.0^{ef}	0.0^{d}	1.0^{e}	0.0^{d}	9.0^{h}	6.7^{b}	0.0^{d}	1.0^{ef}	0.0^{d}
A. chroococcum + **(F.O)**	30.2^{cd}	15.7^{abcd}	6.0^{a}	10.3^{bc}	2.3^{abcd}	24.0^{def}	15.7^{a}	3.3^{abc}	8.7^{abc}	2.3^{bc}
B. megaterium* var. *phosphaticum + **(F.O)**	34.0^{bc}	14.0^{bcd}	3.0^{bc}	7.0^{cd}	3.3^{abc}	26.3^{cde}	12.3^{ab}	3.3^{abc}	7.0^{bcd}	2.0^{bc}
Mixture (A) + (B) + **(F.O)**	41.0^{a}	19.3^{ab}	5.0^{ab}	19.3^{a}	4.0^{ab}	37.0^{ab}	16.0^{a}	4.0^{a}	9.7^{ab}	3.3^{b}
***Fusarium solani* (F.S)**	8.7^{f}	5.3^{f}	0.0^{d}	0.7^{e}	0.0^{d}	11.7^{h}	6.7^{b}	0.0^{d}	0.0^{f}	0.0^{d}
A. chroococcum + **(F.S)**	27.0^{de}	14.0^{bcd}	3.0^{bc}	7.0^{cd}	0.3^{d}	29.0^{cd}	14.0^{a}	2.0^{bc}	7.0^{bcd}	2.0^{bc}
B. megaterium* var. *phosphaticum + **(F.S)**	25.7^{de}	13.0^{bcde}	2.0^{cd}	6.3^{d}	1.7^{bcd}	32.0^{bc}	13.0^{ab}	3.3^{abc}	6.7^{bcd}	2.3^{bc}
Mixture (A) + (B) + **(F.S)**	37.3^{ab}	16.7^{abc}	3.7^{abc}	10.3^{bc}	3.3^{abc}	38.3^{a}	17.7^{a}	3.7^{ab}	12.3^{a}	5.0^{a}

by *F. oxysporum* f.sp *lycopersici*. While, in non-sterilized soil treatments the highest records were observed with tomato inoculated with the mixture of PGPR inoculum and planted in soil infested by *F. solani*.

The high records of growth characteristics values showed with tomato inoculated with PGPR may be attribute to the beneficial effects of PGRs produced by these microorganisms. Beneficial effects including cell division, cell enlargement, root initiation, shoot growth increase, development and formation of flowers and translocation of nutrients and organic substances **Leveau and Lindow, 2005 and Pallai, 2005).**

Effect of inoculation with PGPR on the macro-nutrients content of tomato shoots

Data in **Table (4)** revealed that un-inoculated plants with PGPR recorded lower values of nitrogen, phosphorus and potassium rather than the inoculated ones . Tomato inoculation with the mixture of PGPR significantly increased the total of macro-nutrients content (N , P and K) of tomato shoots compared to individual inoculation with either *A. chroococcum* or *B. megaterium* var. *phosphaticum*.

Data in **Table (4)** also showed that soil infestation with either *F. oxysporum* f.sp *lycopersici* or *F. solani* significantly decreased the macro-nutrients content in tomato shoots . While the inoculation with the PGPR either individually or dually combined with soil infestation with pathogenic fungi significantly increased NPK content of tomato shoots compared to those planted in soil infested with pathogenic fungi in absence of PGPR.

These results are in accordance with those reported by **Abou-Aly (2005)** who noted that the mineral content (N, P and K) in tomato plants significantly increased when plants were inoculated with yeast especially in presence of *A. lipoferum* Mn3 and *B. megaterium* var. *phosphaticum*.

Badran *et al* (2007) reported that the highest values of fresh and dry weights of tomato plants and N, P and K contents were observed in case of inoculating with *T. viridie* and *A. chroococcum*.

Effect of inoculation with PGPR on phytohormones of tomato plants grown in un-sterilized soil

Data in **Table (5)** showed that the un-inoculated tomato plants with PGPR gave lower values of phytohormones (auxins, gibberellins and cytokinins) than those inoculated . Inoculated tomato with *B. megaterium* var. *phosphaticum* showed higher values of phytohormones than that inoculated with *A. chroococcum*.

In addition, inoculation of tomato with the mixture of *A. chroococcum +B. megaterium* var. *phosphaticum* recorded higher values of phytohormones rather than those inoculated individualy with either *A. chroococcum* or *B. megaterium* var. *Phosphaticum*.

From data presented in **Table (5)** it is obviously clear that the phytohormones content in tomato plants was significantly decreased when soil was infested with either *F. oxysporum* f.sp *lycopersici* or *F. solani* individually. Moreover, obtained results clearly indicated that tomato inoculation with PGPR strains in presence of pathogenic fungi significantly increased their phytohormones content. It is worthily to mention that the dual inoculation with PGPR strains in presence of pathogenic

Table 4. Effect of inoculation with PGPR on the macro-nutrients content of tomato shoots

Treatments \ Parameters			Sterilized soil			Un-sterilized soil		
			N	P	K	N	P	K
Untreated plants with PGPR			3.43 ^{d}	0.225^{def}	2.28^{f}	3.50^{ef}	0.212^{de}	2.71^{d}
***A. chroococcum* (A)**			4.77^{abc}	0.272^{abc}	4.04^{bc}	5.53^{a}	0.316^{a}	4.87^{a}
***B. megaterium* var. *phosphaticum* (B)**			4.35^{bc}	0.286^{ab}	3.95^{bc}	4.40^{cde}	0.251^{bcde}	4.17^{bc}
Mixture (A) + (B)			5.26^{a}	0.314^{a}	5.07^{a}	5.60^{a}	.296^{ab}	4.83^{a}
***Fusarium oxysporum* f.sp *lycopersici* (F.O)**			3.03^{d}	0.213^{def}	2.10^{f}	3.30^{f}	0.203^{de}	2.80^{d}
A. chroococcum	+	(F.O)	4.23^{bc}	0.290^{ab}	3.69^{bcd}	5.27^{abc}	0.271^{abc}	3.73^{bc}
B. megaterium* var. *phosphaticum	+	(F.O)	4.40^{bc}	0.259^{bcd}	3.86^{bc}	4.06^{de}	0.203^{de}	3.99^{bc}
Mixture (A) + (B)	+	(F.O)	5.03^{ab}	0.251^{bcde}	4.27^{b}	5.24^{abc}	0.242^{bcde}	4.27^{ab}
***Fusarium solani* (F.S)**			3.00^{d}	0.198^{f}	2.59^{ef}	2.99^{f}	0.194^{e}	2.53^{d}
A. chroococcum	+	(F.S)	4.30^{bc}	0.294^{ab}	3.63^{bcd}	4.44^{bcde}	0.259^{bcd}	3.53^{c}
B. megaterium* var. *phosphaticum	+	(F.S)	4.17^{c}	0.232^{cdef}	3.73^{bcd}	4.23^{cde}	0.207^{de}	3.53^{c}
Mixture (A) + (B)	+	(F.S)	5.21^{a}	0.279^{ab}	4.24^{b}	5.47^{ab}	0.299^{ab}	4.80^{a}

Table 5. Effect of inoculation with PGPR on phytohormones of tomato plants grown in un-sterilized soil

Parameters / Treatments	Auxins		Gib berellin	Cytokinins				
	IAA	**IBA**	**GA_3**	**Z**	**KIN**	**(9R) BAP**	**(9G) BAP**	**IP**
Untreated plants with PGPR	19.6^{c}	3.1^{d}	104.9^{c}	1.4^{d}	3.2^{cd}	2.5^{d}	1.1^{d}	3.6^{c}
***A. chroococcum* (A)**	21.9^{bc}	6.5^{c}	108.1^{bc}	3.8^{b}	4.4^{c}	5.8^{bc}	1.3^{d}	4.1^{bc}
***B. megaterium* var. *phosphaticum* (B)**	26.4^{b}	7.4^{b}	109.0^{bc}	4.0^{ab}	4.9^{bc}	6.4^{b}	1.3^{d}	4.6^{b}
Mixture (A) + (B)	37.7^{a}	10.7^{ab}	122.1^{a}	4.4^{a}	5.9^{a}	7.9^{ab}	2.3^{bc}	5.4^{ab}
***Fusarium oxysporum* f.sp *Lycopersici* (F.O)**	18.2^{c}	ND	103.5^{c}	ND	ND	2.3^{d}	1.1^{d}	3.5^{c}
***A. chroococcum* (F.O)**	23.1^{bc}	7.0^{b}	114.6^{b}	2.11^{c}	3.5^{cd}	3.7^{cd}	2.0^{cd}	4.9^{b}
***B. megaterium* var. *phosphaticum* (F.O)**	25.4^{b}	8.2^{b}	112.9^{b}	2.9^{bc}	3.9^{c}	5.5^{bc}	1.9^{cd}	5.1^{ab}
Mixture (A) + (B) (F.O)	36.5^{a}	14.0^{a}	120.8^{a}	4.0^{ab}	5.4^{b}	8.5^{a}	3.8^{a}	5.9^{a}
***Fusarium solani* (F.S)**	16.3^{d}	2.6^{d}	101.5^{d}	ND	ND	ND	ND	ND
***A. chroococcum* (F.S)**	21.6^{bc}	7.4^{b}	109.0^{bc}	3.3^{b}	5.1^{bc}	4.0^{c}	2.2^{bc}	3.5^{c}
***B. megaterium* var. *phosphaticum* (F.S)**	25.0^{b}	8.0^{b}	111.7^{b}	3.6^{b}	5.6^{b}	4.4^{c}	2.4^{bc}	4.7^{b}
Mixture (A) + (B) (F.S)	30.3^{ab}	11.3^{ab}	119.6^{a}	4.7^{a}	5.9^{a}	7.3^{ab}	3.3^{b}	6.1^{a}

fungi significantly increased the phytohormones content in comparison with the individual inoculation.

Data in **Table (5)** emphasized that the plants inoculated with the mixture of PGPR strains and grown in soil infested with *F. oxysporum* f.sp *lycopersici* contained higher values of auxins and gibberellins (GA_3) than those grown in soil infested with *F. solani*. Also, the plants grown in soil infested with *F. solani* combined with the mixture strains of PGPR contained higher values of zeatin and kinetin than those grown in soil infested with *F. oxysporum* f.sp *lycopersici*.

These results are in harmony with those obtained by **Brian (2004)** who reported that the inoculation with PGPR (*B. megaterium* or *P. polymyxa*) increased the phytohormones content in tomato plants.

The combination of phosphate dissolving microorganisms (*P. polymyxa* + *B. megaterium* var. *phosphaticum*) treatment showed an increase in endogenous gibberellins , auxins and cytokinins level in squash **(Abou-Aly *et al,* 2006)** .

Effect of inoculation with PGPR on photosynthetic pigments

Data presented in **Table (6)** generally revealed that soil infestation with either *F. oxysporum* f.sp *lycopersici* or *F. solani* significantly decreased the photosynthetic pigments (chlorophyll a, b and carotenoids) in the leaves, inoculated treatments with PGPR either individually or dually increased photosynthetic pigments as compared to un-inoculated ones. Inoculation with the mixture of PGPR gave higher records of photosynthetic pigments rather than the individual inoculation.

Moreover, soil infestation with pathogenic fungi lead to decrease of photosynthic pegments whereas in presence of PGPR inoculation significantly increased the photosynthic pegments . The highest obtained values of photosynthetic pigments were observed with the treatments of soil infested by *F. solani* in combination with tomato inoculation with the mixture of *A. chroococcum* and *B. megaterium* var. *phosphaticum*.

These results are in harmony with those reported by **Abou-Aly and Gomaa (2002**) who stated that the mixed biofertilizers increased both nutrient content and leaf chlorophyll concentrations than control. **Abou-Aly *et al* (2006**) found that the inoculation of squash plants with *B. megaterium* var. *phosphaticum* or mychorriza combined with *P. polymyxa* increased the values of photosynthetic pigments . Also, **Han *et al* (2006)** stated that the dual inoculation by *B. megaterium* var. *phosphaticum* and *B. mucilaginous* improved photosynthetic pigments production in pepper and cucumber plants .

Effect of inoculation with PGPR on nitrogen and phosphorus contents in tomato rhizosphere

The illustrated results in **Table (7)** revealed that the inoculation with either *A. chroococcum* or *B. megaterium* var. *phosphaticum* significantly increased the available N and P in rhizosphere as compared to un-inoculated plants.

Table 6. Effect of inoculation with PGPR on photosynthetic pigments

Treatments \ Parameters	Sterilized soil			Un-sterilized soil		
	Chlorophyll A	Chlorophyll B	Carotenoids	Chlorophyll A	Chlorophyll B	Carotenoids
Untreated plants with PGPR	1.0^{gh}	0.6d^{ef}	0.6^{de}	1.0^{ghi}	0.5^{e}	0.7^{def}
A. chroococcum (A)	1.6^{de}	0.8^{cde}	0.9^{cde}	1.6^{cdef}	0.9^{bcde}	0.9^{cde}
B. megaterium var. *phosphaticum* (B)	1.8^{cd}	1.1^{abc}	1.2^{abc}	1.8^{cd}	1.2^{ab}	1.2^{abc}
Mixture (A) + (B)	2.2^{b}	1.2^{ab}	1.3^{ab}	2.2^{b}	1.2^{ab}	1.3^{ab}
Fusarium oxysporum f.sp *lycopersici* (F.O)	0.9^{h}	0.4^{f}	0.6^{de}	0.9^{hi}	0.5^{e}	0.6^{ef}
A. chroococcum + F.O)	1.5^{ef}	0.9^{bcd}	1.0^{bcd}	1.4^{defg}	0.9^{bcde}	1.0^{bcd}
B. megaterium var. *phosphaticum* + F.O)	1.3^{efg}	0.5^{ef}	0.7^{de}	1.3^{efgh}	0.6^{de}	0.7^{def}
Mixture (A) + (B) + F.O)	1.9^{c}	1.1^{abc}	1.2^{abc}	1.9^{bc}	1.1^{abc}	1.2^{abc}
Fusarium solani (F.S)	0.8^{h}	0.4^{f}	0.5^{e}	0.8^{i}	0.6^{de}	0.5^{f}
A. chroococcum + F.S)	1.3^{efg}	0.7^{def}	0.8^{cde}	1.3^{efgh}	0.6^{de}	0.8^{def}
B. megaterium var. *phosphaticum* + F.S)	1.6^{de}	0.9^{bcd}	1.0^{bcd}	1.7^{cde}	1.0^{bcd}	0.9^{cde}
Mixture (A) + (B) + F.S)	2.6^{a}	1.3^{a}	1.5^{a}	2.8^{a}	1.5^{a}	1.4^{a}

Table 7 . Effect of inoculation with PGPR on nitrogen and phosphorus contents in tomato rhizosphere

Parameters / Treatments	Sterilized soil				Un-sterilized soil			
	Nitrogen		Phosphorus		Nitrogen		Phosphorus	
	Total	Available	Total	Available	Total	Available	Total	Available
Untreated plants with PGPR	1410^{e}	340^{d}	1000^{c}	90^{c}	1430^{g}	450^{d}	1170^{c}	100^{c}
***A. chroococcum* (A)**	1750^{cd}	450^{bc}	1010^{c}	140^{b}	174^{d}	540^{c}	1160^{c}	150^{bc}
***B. megaterium* var. *phosphaticum* (B)**	1460^{e}	470^{bc}	1220^{b}	160^{ab}	1520^{f}	500^{cd}	1270^{b}	180^{bc}
Mixture (A) + (B)	2280^{b}	560^{a}	1370^{a}	180^{ab}	2350^{a}	590^{bc}	1390^{a}	210^{b}
***Fusarium oxysporum* f.sp *Lycopersici* (F.O)**	1130^{f}	390^{cd}	930^{d}	60^{d}	1100^{h}	420^{d}	1150^{cd}	120^{c}
A. chroococcum	2110^{b}	500^{b}	1040^{bc}	140^{b}	1530^{ef}	620^{b}	1190^{c}	160^{bc}
B. megaterium* var. *phosphaticum	1660^{cd}	460^{bc}	1220^{b}	170^{ab}	1600^{e}	580^{bc}	1240^{bc}	200^{b}
Mixture (A) + (B)	2760^{e}	540^{a}	1320^{ab}	220^{a}	2210^{b}	740^{a}	1340^{ab}	250^{ab}
***Fusarium solani* (F.S)**	660^{g}	410^{c}	990^{d}	50^{d}	1110^{h}	440^{d}	1120^{d}	110^{c}
A. chroococcum	1830^{c}	510^{ab}	1000^{c}	140^{b}	1770^{d}	590^{bc}	1120^{d}	170^{bc}
B. megaterium* var. *phosphaticum	1570de	420^{c}	1200^{b}	180^{ab}	1880^{c}	560^{c}	1220^{bc}	200^{b}
Mixture (A) + (B)	2670^{a}	530^{ab}	1300^{ab}	220^{a}	2120^{b}	720^{a}	1310^{ab}	290^{a}

On reverse, the rhizosphere of tomato plants infested with either *F. oxysporum* f.sp *lycopersici* or *F. solani* showed lower values of total nitrogen and phosphorus. This result was observed in sterilized and un-sterilized soils. The inoculation with *A. chroococcum* caused higher values of total nitrogen rather than that inoculation with *B. megaterium* var. *phosphaticum*. Reverse results were obtained with total phosphorus amounts .

The higher values of total nitrogen which observed as a result of inoculation with *A. chroococcum* could be attributed to the N_2-fixation by *A. chroococcum*. While, the high content of available phosphorus in the plants' rhizosphere inoculated by *B. megaterium* var. *phosphaticum* may be attributed to the important role of *B. megaterium* var. *phosphaticum* in increasing the available phosphorus level in inoculated soil. Also, data in **Table (7)** announced that the inoculation with the mixture of *A. chroococcum* + *B. megaterium* var. *phosphaticum* significantly increased the total and available macro-nutrient elements (N and P) in soil as compared with the inoculation with each one individually.

The high amounts of available macro-nutrients observed in the soil treated with dual inoculation may be attributed to the synergistic effect between *A. chroococcum* and *B. megaterium* var. *phosphaticum*.

Data in **Table (7)** indicated that the inoculation with *A. chroococcum* in presence of the pathogenic fungi significantly increased total and available nitrogen content in the soil rather than the inoculation with *B. megaterium var. phosphaticum*. Also, the inoculation with *B. megaterium* var. *phosphaticum* in presence of *F. oxysporum* f.sp *lycopersici* or *F. solani* significantly increased the total and available phosphorus content in the soil compared to un-inoculated plants with the above PGPR strain . The highest values of total N and P in tomato rhizosphere were occurred in inoculated treatments with the mixture of PGPR in presence of either *F. oxysporum* f.sp *lycopersici* or *F. solani*.

Generally, it is obvious that the available nitrogen and phosphorus contents in tomato rhizosphere were significantly increased in the treatments inoculated with *A. chroococcum* and *B. megaterium* var. *phosphaticum* strains respectively .

Obtained results are in harmony with those obtained by **Zaghloul (2002)** who found that the inoculation of potato tuber with *B. megaterium* var. *phosphaticum* in combination with N_2-fixers (*A. chroococcum* and *A. lipoferum*) increased the avilable macro-nutrient content (N and P) in soil.

Abou-Aly *et al* (2006) reported that significant increases in available N, P and K contents were observed when squash plants were inoculated with *P. polymyxa* individually or with *B. megaterium* var. *phosphaticum*.

CONCLUSION AND RECOMMENDATION

In view of the obtained results , it can be mentioned that the inoculation with PGPR enhance the growth performance of cultivated plants . PGPR able to fix of nitrogen, phosphate solubilization, phytohormons production as well produce many antagonistic substances such as siderophores, cyanogens and antibiotic substances. For these options , the inoculation with PGPR should be done three times at least throughout growing season .

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تأثير التفاعل بين البكتريا المنتجة لمنظمات النمو والفطريات المسببة لأعفان الجذور على نباتات الطماطم

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أظهرت النتائج المتحصل عليها فى هذا البحث أن كلا من الميكروبين *A. chroococcum* and *B. megaterium* var. *phosphaticum* لهما قدرة على تنشيط نمو الفطريات الممرضة *F. oxysporum* f.sp *lycopersici* and *F. solani* والتى تسبب مرض الذبول حيث شوهد مناطق شفافة حول الميكروبات المنتجة لمنظمات النمو مع حدوث تثبيط لنمو الميسيليوم الفطرى وذلك بالمقارنة بالأطباق الغير ملقحة بمثل هذه البكتريا . لذلك فقد أجريت تجربة لدراسة تأثير التلقيح بهذه الميكروبات على نمو نباتات الطماطم المعداه بهذه الفطريات حيث أوضحت نتائج هذه التجربة ما يلى :

- عند تلقيح بادرات الطماطم بميكروبات *B. megaterium* var. *phosphaticum* *A. chroococcum* and شوهد إنخفاض معنوى فى نسبة الإصابة بالمقارنة بتلك الغير ملقحة . أيضا عند التلقيح بمخلوط السلالتين (*A. chroococcum* + *B. megaterium* var. *phosphaticum*) شوهد إنخفاض معنوى فى نسبة الإصابة بالمقارنة بالتلقيح بكلا من السلالتين على حده .
- أوضحت النتائج أيضا أن صفات النمو التى درست قد إنخفضت معنويا عند إجراء عدوى للتربة بفطريات *F. oxysporum* f.sp *lycopersici* or *F. solani* . كذلك أوضحت النتائج أن عدوى التربة بالفطريات الممرضة مع إجراء التلقيح بميكروبات *A. chroococcum* + *B. megaterium* var. *phosphaticum* أدى إلى زيادة معنوية فى مواصفات النمو بالمقارنة بعدم التلقيح .
- أظهرت النتائج أن التلقيح المزدوج بمخلوط السلالتين أدى إلى حدوث زيادة معنوية فى محتوى الأوراق من الهرمونات النباتية و عناصر النيتروجين والفوسفور والبوتاسيوم بالمقارنة بالتلقيح الفردى بكل سلالة على حده .

- عند إجراء عدوى للتربة بفطريات *F. oxysporum* f.sp *lycopersici* or *F. solani* حدث إنخفاض معنوى فى محتوى الأوراق من صبغات (كلوروفيل أ وكلوروفيل ب والكاروتينات) . ولكن التلقيح بميكروبات *A. chroococcum* or *B. megaterium* var. *phosphaticum* أدى إلى إرتفاع محتوى الأو راق من هذه الصبغات خصوصا فى حالة التلقيح المزدوج .

- أوضحت النتائج أن عدوى التربة بالفطريات الممرضة أدى إلى إنخفاض محتوى التربة من النيتروجين و الفوسفور الميسر ولكن عند تلقيح الطماطم بميكروبات *A.chroococcum* or *B. megaterium* var. *phosphaticum* فقد لوحظت زيادة فى محتوى التربة من العناصر المغذيةخصوصا الصور الميسرة فى معاملات التلقيح المزدوج . أيضا لوحظ أن تلقيح الطماطم بميكروبات PGPR مع إجراء عدوى للتربة بالفطريات الممرضة أدى إلى زيادة محتوى التربة من هذه العناصر المغذية وذلك بالمقارنة بعدوى التربة بالفطريات الممرضة فقط .

مما سبق يتضح أن التربة المصرية غنية فى محتواها من الميكروبات المنتجة للمواد المشجعة لنمو النبات ، والتى تستطيع أن تنتج كميات لا بأس بها من الأوكسينات والجبريللينات والسيتوكينينات .وهذه الميكروبات لها القدرة على أن تثبط نشاط الفطريات الممرضة للنبات خصوصا فطريات أعفان الجذور والذبول ، وذلك يرجع إلى قدرة هذه الميكروبات على إفراز مواد مضادة للفطريات الممرضة مثل مركبات السيدروفورز وسيانيد الهيدروجين لذلك ينصح بتلقيح التربة أو النباتات المنزرعة بمثل هذه الميكروبات ثلاث مرات على الأقل خلال موسم النمو .