Efficiency Of Soil Inoculation With Growth Regulators Producing Microorganisms On Some Enzymes Activity

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Abstract

This experiment was carried out to study the efficiency of soil inoculation with *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum* on some enzymes activity in presence of tomato wilting fungi. Obtained results showed that tomato inoculation with the mixture of *A. chroococcum* and *B. megaterium* var. *phosphaticum* gave higher values of dehydrogenase activity (DHA) as compared with individual inoculation treatments .Tomato inoculation with *A. chroococcum* or *B. megaterium* var. *phosphaticum* in combination with soil infestation with either *F. oxysporum* f.sp *lycopersici* or *F. solani* significantly increased DHA compared to un-inoculated ones.Tomato inoculation with *B. megaterium* var. *phosphaticum* significantly increased the phosphatase activity rather than that inoculated with *A. chroococcum* .Dual inoculation with *A. chroococcum* + *B. megaterium* var. *phosphaticum* gave significant increase in phosphatase activity rather than the individual inoculation with either *A. chroococcum* or *B. megaterium* var. *phosphaticum* . Tomato inoculation with *A. chroococcum* only significantly increased N_2–ase activity as compared to other investigated treatments. Also , soil infestation with either *F. oxysporum* f.sp *lycopersici* or *F. solani* in combination with the mixture of two studied plant growth promoting rhizobacteria (PGPR) showed higher records of N_2-ase activity than that inoculated with *A. chroococcum* only .

Soil infestation with either *F oxysporum* f.sp *lycopersici* or *F. solani* significantly decreased the peroxidase and polyphenol oxidase content in tomato plants. Tomato inoculation with PGPR significantly increased the peroxidase and polyphenol oxidase content in tomato plants compared to the un-inoculated ones . Tomato inoculation with PGPR combined with soil infestation with pathogenic fungi significantly increased the content of peroxidase and polyphenol oxidase as compared to soil infestation with pathogenic fungi only.

Key words: **dehydrogenase , phosphatase , nitrogenase , peroxidase , polyphenoloxidase , *Azotobacter* , *Bacillus* , tomato .**

Introduction

Dehydrogenase activity (DHA) was indicated to a criterion of respiration rate and total microbial activity. **Abou-Aly (2005)** provided that the combined inoculation of tomato plants with *Azospirillum* and *Bacillus megaterium* var. *phosphaticum* increased the activity of DHA at all growth stages . **Abou-Aly *et al.* (2006)** provided that combination of mychorriza or *Bacillus megaterium* var. *phosphaticum* with *Paenibacillus polymyxa* recorded the highest DHA either with or without single application in squash plants.

Phosphatase activity was indicated to the important role in organic phosphorus compounds hydrolysis. **Ponmurgan and Gopi (2006)** reported that the phosphatase activity of phosphobacteria *Pseudomonas* sp

which was isolated from groundnut rhizosphere had higher activity . Also, there was a positive correlation between phosphate solubilizing bacteria and phosphatase activity .

Soil nitrogenase activity (N_2-ase) was indicated to a criterion of atmospheric nitrogen fixation by diazotrophs . **Zaghloul *et al.* (2007)** indicated that tomato inoculated with *Azotobacter chroococcum* individually or in combination with biocontrol agents *Trichoderma harzianum* and *Bacillus subtilis* significantly increased N_2-ase activity compared to un-inoculated treatments. Similarly, dehydrogenase activity showed higher values in cases of tomato inoculation with *A. chroococcum* combined with either *Streptomyces aureofaciens* or *Bacillus subtilis* than individual inoculation by each of them .

Concerning the effect of inoculation with PGPR on resistance enzymes content , **Gamil (1995)** proved that the inoculation with *Bacillus polymyxa (Paenbacillus polymyxa)* was induced peroxidase and polyphenol oxidase content of squash leaves after inoculation . Increase of peroxidase and polyphenol oxidase content in the PGPR (*Pseudomonas* sp) treated plants may play either a direct or indirect role in the suppression of pathogen development in the host **(Chen *et al.* , 1998)**. The induction of peroxidase and polyphenol oxidase by PGPR (*Pseudomonas fluorescens*) treatment was in turn correlated with the percentage root rot suppression in pepper plants **(Diby *et al.*, 2001)**. Similary, **Martinez *et al.* (2001)** observed that the inoculation of melon cotyledons with *Trichoderma longibrachiatum* increased peroxidase activities. **Gailite *et al.* (2005)** reported that the content of both peroxidase and polyphenol oxidase increased in bean leaves after the treatment with growth regulators producing bacteria or fungi .

Materials and Methods

Experimental design

A pot experiment designed to study the role of inoculation with PGPR (*Azotobacter chroococcum* and/or*Bacillus megaterium* var. *phosphaticum*) on tomato plants growth in infested and un-infested soil with pathogenic fungi (*Fusarium oxysporum* f.sp *lycopersici* or *F. solani*) under sterilized and un-sterilized soil conditions. This experiment was carried out in plastic pots containing clay loam soil (3kg / pot).

The treatments were distributed in greenhouse using randomized complete block design. Three replicates of each treatment were used.

Pots and experimental soil Sterilization

Plastic pots (20 cm in diameter) were sterilized by immersing in 5 % formalin solution for 15 minutes and covered overnight with plastic sheets , then left to dry in the open air. Soil sterilization was carried out by autoclaving at 15 1b/inch^2 for two hours. The physical and chemical analyses of the experimental soil are shown in **Table (1)** .

Preparation of pathogen inocula and soil infestation

The inoculum of either fungus (*Fusarium oxysporum* f.sp. *lycopersici* or *Fusarium solani*) was prepared by growing in conical flasks (500 ml) individually. Each flask containing 250 ml potato dextrose broth medium was inoculated with 0.5 cm diameter agar discs bearing mycelium of each fungus , then the flasks were incubated at 28°C for two weeks . 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 with each inoculum by mixing 100 ml of spore suspension per Kg soil. Then pots were carefully irrigated and kept under greenhouse conditions for 7 days to activate the fungi before planting.

Preparation of PGPR inocula

The inocula of *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum* were prepared in modified Ashby's and Modified Bunt and Rovira broth media, respectively under optimal conditions of growth

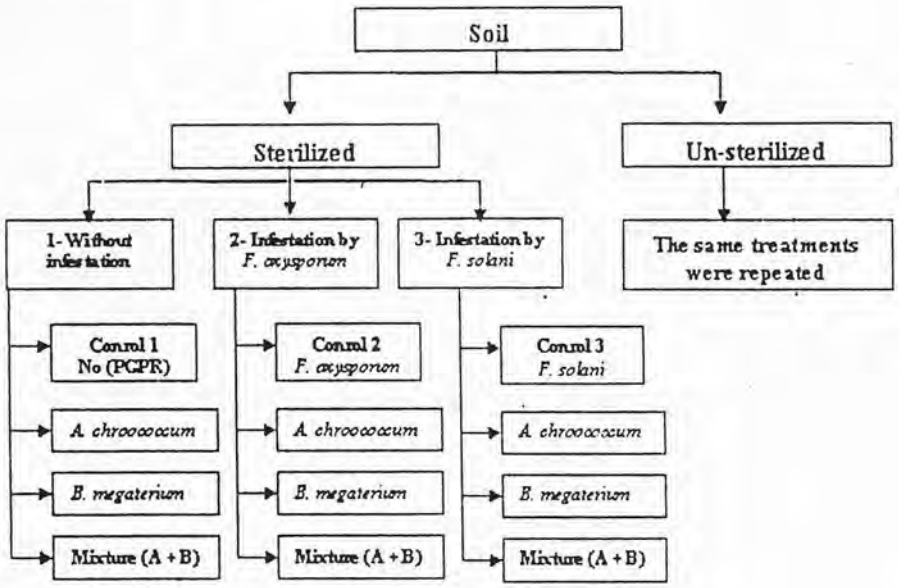

Table 1. Physical and chemical analyses of the experimental soil

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

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 *Azotobacter chroococcum* (8 x 10^7 cfu/ml) 4 days-old or *Bacillus megaterium* var. *phosphaticum* (9 x 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}.

Enzymes determination

Assessment of dehydrogenase activity (DHA)

Dehydrogenase activity in soil was assayed according to Glathe' and Thalmann (1970). DHA was estimated at 30 and 60 days after cultivation.

Assessment of phosphatase activity

Phosphatase activity was estimated two times as mentioned before in DHA according to Drobrikova (1961).

Assessment of nitrogenase activity (N_2 – ase)

Nitrogenase activity was measured by using the acetylene reduction technique given by Diloworth (1970) .

Peroxidase and Polyphenol oxidase assessment

Peroxidase and Polyphenol oxidase activity were determined according to the methods described by Allam and Hollis (1972) and Matta and Dimond (1963) , respectively .

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

Effect of inoculation with PGPR on dehydrogenase activity

Data in Table (2) showed that the sterilized soil treatments gave lower values of DHA rather than un-sterilized ones. This result is likely be due to the sterilization effect, since the sterilization lead to getting rid of native (indigenous) soil microorganisms. Dehydrogenase activity which was observed with sterilized treatments due to the activity of introduced inocula only .

Obtained results clearly indicated that tomato inoculation with the mixture of *A. chroococcum* + *B. megaterium* var. *phosphaticum* gave higher values of DHA as compared to individual inoculation treatments. Similar trend of results was observed with sterilized and un-sterilized soil treatments.

The higher values of DHA which was observed with the application of PGPR mixture could be attributed to the synergistic effect of the two strains .

Data in **Table (2)** also revealed that soil infestation with either *F. oxysporum* f.sp *lycopersici* or *F. solani* significantly decreased the DHA specially in un-sterilized soil treatments .

Tomato inoculation with *A. chroococcum* or *B. megaterium* var. *phosphaticum*) in combination in infested soil either with *F. oxysporum* f.sp *lycopersici* or *F. solani* significantly increased DHA compared to un-inoculated soil treatments.

With the soil infestation treatment with either *F. oxysporum* f.sp *lycopersici* or *F. solani*. Similar trend of results was observed with both determination periods.

Table 2. Effect of inoculation with PGPR on dehydrogenase activity (µg TPF. g dry soil $^{-1}$. 24 hrs^{-1}) in tomato rhizosphere in presence of *Fusarium* spp .

Treatments	Sterilized soil		Un-sterilized soil	
	First period (30 days)	Second period (60 days)	First period (30 days)	Second period (60 days)
Untreated plants with PGPR	ND	ND	29.5^{f}	35.5^{g}
A. chroococcum (A)	33.7^{ef}	40.3^{g}	57.3^{c}	73.8^{d}
B. megaterium var. *phosphaticum* (B)	36.4^{d}	42.0^{g}	58.8^{c}	83.2^{b}
Mixture (A) + (B)	42.8^{b}	51.3^{d}	76.2^{a}	88.6^{a}
Fusarium oxysporum f.sp *Lycopersici* (F.O)	20.8^{g}	23.3^{j}	29.3^{f}	31.3^{h}
A. chroococcum + (F.O)	41.9^{bc}	46.8^{ef}	46.8^{d}	64.6^{e}
B. megaterium var. *phosphaticum* + (F.O)	44.4^{b}	47.3^{e}	47.3^{d}	77.4^{c}
Mixture (A) + (B) + (F.O)	50.9^{a}	62.5^{b}	62.5^{b}	82.5^{b}
Fusarium solani (F.S)	23.3^{g}	27.0^{i}	25.0^{f}	37.0^{g}
A. chroococcum + (F.S)	39.5^{c}	44.5^{f}	44.5^{d}	72.0^{d}
B. megaterium var. *phosphaticum* + (F.S)	41.9^{bc}	57.2^{c}	58.2^{c}	64.6^{e}
Mixture (A) + (B) + (F.S)	49.1^{a}	64.6^{a}	74.6^{a}	80.5^{b}

Table 3. Effect of inoculation with PGPR on Phosphatase activity (µg inorganic phosphate . g^{-1} . day) in tomato rhizosphere in presence of *Fusarium* spp .

Treatments	Sterilized soil		Un-sterilized soil	
	First period (30 days)	Second period (60 days)	First period (30 days)	Second period (60 days)
Untreated plants with PGPR	ND	ND	20.00^{e}	50.23^{e}
A. chroococcum (A)	7.26^{cd}	14.14^{de}	24.57^{de}	58.30^{d}
B. megaterium var. *phosphaticum* (B)	9.00^{b}	15.75^{cd}	26.53^{cd}	73.78^{b}
Mixture (A) + (B)	9.51^{b}	19.31^{a}	29.43^{bc}	88.66^{a}
Fusarium oxysporum f.sp *lycopersici* (F.O)	6.34^{d}	36.78^{f}	21.38^{e}	49.22^{e}
A. chroococcum + (F.O)	8.73^{bc}	14.06^{de}	21.56^{de}	59.13^{d}
B. megaterium var. *phosphaticum* + (F.O)	9.08^{b}	17.19^{abc}	24.70^{de}	61.10^{d}
Mixture (A) + (B) + (F.O)	8.53^{bc}	18.44^{ab}	24.53^{de}	71.20^{bc}
Fusarium solani (F.S)	6.95^{d}	8.49^{f}	21.39^{e}	43.54^{f}
A. chroococcum + (F.S)	12.29^{ab}	16.74^{bc}	32.34^{b}	47.25^{ef}
B. megaterium var. *phosphaticum* + (F.S)	12.28^{ab}	16.12^{bcd}	40.72^{ab}	60.27^{d}
Mixture (A) + (B) + (F.S)	13.36^{a}	16.37^{bcd}	43.92^{a}	66.23^{c}

The lower DHA which was observed with soil infested by pathogenic fungi may be due to the antagonistic effect of such fungi against *soil microflora* . Generally, data recorded in **Table (2)** clearly indicated that non-sterilized soil treatments gave higher values of DHA rather than sterilized ones. Higher records of DHA in case of un-sterilized soil treatments could be attributed to the presence of native (indigenous) soil microorganisms besides the introduced inocula

These results were in harmony with those obtained by **Abou-Aly (2005)** who found that the combined inoculation of tomato plants with *Azospirillum* and *Bacillus megaterium* var. *phosphaticum* increased the DHA at all growth stages. **Zaghloul *et al.* (2007)** indicated that tomato seedlings inoculated with *Azotobacter chroococcum* individually or in combination with biocontrol agents *Trichoderma harzianum* and *Bacillus subtilis* significantly increased . **Abou-Aly *et al.* (2006)** reported that combination of

mychorriza or *Bacillus megaterium* var. *phosphaticum* with *Paenibacillus polymyxa* recorded the highest DHA either with or without single application in squash plants.

Effect of inoculation with PGPR on phosphatase activity

Obtained results in **Table (3)** emphasized that sterilized soil treatments gave lower values of phosphatase activity as compared to un- sterilized ones. This result is expected and could be attributed to the sterilization effect as mentioned with DHA.

Data presented in **Table (3)** also showed that tomato inoculated with *B. megaterium* var. *phosphaticum* significantly increased the phosphatase activity rather than tomato inoculated with *A. chroococcum*. This was true with sterilized and non-sterilized soil treatments.

Concerning the effect of tomato inoculation with PGPR mixture on phosphatase activity, data in **Table (3)** revealed that tomato inoculated with the mixture of PGPR and growing in sterilized soil in presence of soil infestation by *F. solani* gave significant increase of phosphatase activity compared with the individual PGPR inoculation. But, no significant difference in phosphatase activity was observed with the application of PGPR mixture combined with *F. oxysporum* f.sp *lycopersici* as compared to individual PGPR inoculation.

As regard to the effect of non-sterilized soil treatments on phosphatase activity, data in **Table (3)** announced that dual inoculation with PGPR recorded significant increase in phosphatase activity rather than the individual inoculation with either *A. chroococcum* or *B. megaterium* var. *phosphaticum*. Similar trend of results was observed in the two determination periods. Higher values of phosphatase activity which was observed in case of dual inoculation with PGPR could be attributed to the synergistic effect.

Synergistic effect may lead to proliferation of rhizosphere soil microorganisms and consequently increased phosphatase activity. In addition, tomato inoculation with PGPR either individually or dually in non-sterilized soil and presence of root–rot pathogenic fungi (*F. oxysporum* f.sp *lycopersici* or *F. solani*) increased the phosphatase activity compared to soil infested with either *F. oxysporum f.sp*

lycopersici or *F. solani* . Generally, non-sterilized soil treatments showed higher records of phosphatase activity as compared to sterilized soil. This likely may be due to the presence of indigenous (native) soil microorganisms besides the introduced inocula.

These results were in harmony with those obtained by **Bopaiah and Shetty (1991)** who mentioned that enzymatic activities of microflora and microbial biomass in the rhizosphere soil were greater than those in non rhizosphere. Dehydrogenase and phosphatase activities showed variable trends in the root regions and rhizosphere of the different crops.

Also, **Kuklinsky –Sobral *et al.* (2004)** found during initial colonization of soybean roots with phosphate solubilizing PGPR that the phosphate availability and phosphatase activity were increased. **Ponmurgan and Gopi (2006)** reported that there was a positive correlation between phosphate solubilizing bacteria and phosphatase activity. Also, **Abou-Aly *et al.* (2006)** reported that dual inoculation especially with *Paenibacillus polymyxa* and mychorriza gave maximum values of phosphatase activity .

Effect of inoculation with PGPR on nitrogenase activity

Data in **Table (4)** showed that un-sterilized soil treatments gave higher values of N_2-ase rather than sterilized ones . This result may be attributed to the sterilization effect .

The N_2-ase activity which was observed with sterilized treatments was due to the activity of introduced PGPR inocula only.

In sterilized soil treatments, data presented in **Table (4)** clearly indicated that tomato inoculation with *A. chroococcum* only increased significantly N_2–ase activity as compared to other investigated treatments.

Also , soil infestation with either *F. oxysporum* f.sp *lycopersici* or *F. solani* in combination with the PGPR mixture showed higher N_2-ase activity than the individual inoculation with *A. chroococcum* only.

The high N_2-ase activity obtained in dual inoculation treatment with PGPR may be attributed to the synergistic effect between the both *A. chroococcum* and *B. megaterium* var. *phosphaticum*.

Moreover, tomato inoculation with the mixture of *A. chroococcum* +*B. megaterium* var. *phosphaticum* in presence of soil infestation with *F. solani* gave higher records of N_2-ase activity rather than those in presence of soil infestation with *F. oxysporum* f.sp *lycopersici* .

Data in **Table (4)** show high N_2-ase activity in un-sterilized soil as compared to sterilized ones. This result is likely be due to the activity of native microorganisms in un-sterilized soil treatments beside the introduced inocula

Also, data in **Table (4)** emphasized that the tomato inoculation with the mixture of *A. chroococcum* + *B. megaterium* var. *phosphaticum* gave higher records of N_2-ase activity rather than the individual inoculation . Soil infestation with either *Fusarium oxysporum* f.sp *lycopersici* or *Fusarium solani* decreased N_2-ase activity. While infested soil with pathogenic fungi combined with PGPR inoculation increased N_2-ase activity.

Table 4. Effect of inoculation with PGPR on nitrogenase activity (µg C_2H_4 . hr^{-1} . g dry $soil^{-1}$) in tomato rhizosphere in presence of *Fusarium* spp .

Treatments	Sterilized soil		Un-sterilized soil	
	First period (30 days)	Second period (60 days)	First period (30 days)	Second period (60 days)
Untreated plants with PGPR	ND	ND	7.69^{h}	11.2^{h}
A. chroococcum (A)	26.3^{a}	34.8^{a}	38.4^{a}	49.3^{a}
B. megaterium var. *phosphaticum* (B)	ND	ND	20.0^{f}	38.1^{d}
Mixture (A) + (B)	25.8^{b}	33.0^{b}	39.2^{a}	50.5^{a}
Fusarium oxysporum f.sp *Lycopersici* (F.O)	ND	ND	11.3^{g}	13.6^{g}
A. chroococcum + (F.O)	20.4^{d}	27.3^{d}	34.6^{c}	43.7^{c}
B. megaterium var. *phosphaticum* + (F.O)	ND	ND	21.4^{d}	30.3^{e}
Mixture (A) + (B) + (F.O)	21.3^{d}	30.7^{c}	38.2^{a}	47.4^{b}
Fusarium solani (F.S)	ND	ND	10.0^{g}	13.8^{g}
A. chroococcum + (F.S)	19.7^{d}	25.2^{e}	33.2^{c}	47.0^{b}
B. megaterium var. *phosphaticum* + (F.S)	ND	ND	22.3^{d}	39.8^{d}
Mixture (A) + (B) + (F.S)	23.1^{c}	32.2^{b}	36.3^{b}	46.2^{bc}

Table 5. Effect of inoculation with PGPR on peroxidase and polyphenol oxidase activity (as absorbance . g^{-1} . fresh leaves) of tomato plants in presence of *Fusarium* spp .

Treatments	Sterilized soil		Un-sterilized soil	
	Peroxidase	Polyphenyl oxidase	Peroxidase	Polyphenyl oxidase
Untreated plants with PGPR	2.629^{g}	0.184f	2.44^{hi}	0.177^{hi}
A. chroococcum (A)	3.608^{ef}	0.323^{d}	3.659^{de}	0.269^{fg}
B. megaterium var. *phosphaticum* (B)	3.882^{de}	0.312^{d}	3.380^{fg}	0.220^{gh}
Mixture (A) + (B)	4.633^{bc}	0.587^{a}	4.322^{b}	0.437^{bc}
Fusarium oxysporum f.sp *lycopersici* (F.O)	1.140^{i}	0.147^{g}	1.304^{k}	0.110^{j}
A. chroococcum + (F.O)	3.726^{de}	0.305^{de}	3.724^{d}	0.319^{ef}
B. megaterium var. *phosphaticum* + (F.O)	3.133^{fg}	0.361^{c}	3.242g	0.309^{ef}
Mixture (A) + (B) + (F.O)	5.558^{a}	0.359^{c}	5.255^{a}	0.534^{a}
Fusarium solani (F.S)	1.782^{h}	0.150^{g}	1.831^{j}	0.124^{ij}
A. chroococcum + (F.S)	4.184^{cd}	0.276^{e}	2.944^{h}	0.367^{de}
B. megaterium var. *phosphaticum* + (F.S)	4.788^{b}	0.296^{de}	3.747^{d}	0.385^{cd}
Mixture (A) + (B) + (F.S)	5.654^{a}	0.446^{b}	4.115^{c}	0.448^{b}

These results were in harmony with those obtained by **Zaghloul (1999)** reported that the highest values of CO_2 evolution and nitrogenase activity in rhizosphere of maize plants were obtained with vesicular arbuscular mycorrhiza combined with *Azospirillum lipoferum* inoculation as compared to either phosphate solubilizing bacteria or un-inoculated ones.

Shalaby (2001) reported that the interactive effect of arbuscular mycorrhiza (*Glomus mosseae*) and *Azospirillum lipoferum* was positive on rhizosphere microflora .Coupling both organisms significantly increased bacteria, actinomycetes and azospirilla counts as well as nitrogenase activity in the rhizosphere of tomato plants.

Effect of inoculation with PGPR on peroxidase and polyphenol oxidase content

Data recorded in **Table (5)** clearly indicated that the soil infestation with either *Fusarium oxysporum* f.sp *lycopersici* or *F. solani* significantly decreased the content of peroxidase and polyphenol oxidase in tomato plants. Soil infestation with *F. oxysporum* f.sp *lycopersici* gave lower values of both peroxidase and polyphenol oxidase rather than soil infestation with *F. solani*. This result could be attributed to the more virulent *F. oxysporum* f.sp *lycopersici* for tomato root infection rather than *F. solani*.

Tomato inoculation with PGPR significantly increased the peroxidase and polyphenol oxidase content of tomato plants as compared to un-inoculated ones . Also, tomato inoculation with the mixture of *A. chroococcum* and *B. megaterium* var. *phosphaticum* as PGPR gave higher records of peroxidase content and polyphenol oxidase in comparison with tomato inoculated with either *A. chroococcum* or *B. megaterium* var. *phosphaticum* individually. In addition, tomato inoculation with PGPR combined with soil infestation with pathogenic fungi significantly increased the content of peroxidase and polyphenol oxidase as compared to soil infestation with pathogenic fungi alone.

From data presented in **Table (5)** it is worthily to mention that tomato inoculation with the mixture of PGPR in sterilized soil infested by *F. solani* gave higher records of peroxidase and polyphenol oxidase rather than soil infested with *F. oxysporum* f.sp *lycopersici*. On the contrary, tomato inoculation with the mixture of PGPR in un- sterilized soil infested with *F. oxysporum* f.sp *lycopersici* gave higher records of peroxidase and polyphenol oxidase rather than soil infested with *F. solani*.

These results were in harmony with those stated by **Gamil (1995)** proved that the inoculation with *Bacillus polymyxa (Paenbacillus polymyxa)* increased peroxidase and polyphenol oxidase content of squash leaves . Increasing the content of peroxidase and polyphenol oxidase in the PGPR (*Pseudomonas* spp) treated plants may be play either a direct or indirect role in the suppression of pathogen development in the host **(Chen *et al.*, 1998)**.

Similar results of elevated levels of peroxidase and polyphenol oxidase have been reported in cucumber plants treated with PGPR strains (*Pseudomonas* spp), which peaked 2-4 days after root treatment **(Chen *et al.*, 2000).** The induction of peroxidase and polyphenol oxidase by PGPR (*Pseudomonas*

fluorescens) treatment was in turn correlated with the percentage root rot suppression in pepper plants (**Diby *et al.* , 2001**).

In general , in view of the obtained results it could be mentioned that the inoculation with plant growth promoting rhizobacteria increased the activity of dehydrogenase , phosphatase and nitrogenase in rhizosphere . Neverthless, also the inoculation with PGPR increased the content of resistance enzymes such as peroxidase and polyphenol oxidase.

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الملخص العربى

كفاءة تلقيح التربة بالميكروبات المنتجة لمنظمات النمو على نشاط بعض الإنزيمات

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تأثير التلقيح بالميكروبات المنتجة لمنظمات النمو على نشاط بعض الإنزيمات فى التربة

إشتملت هذه التجربة على دراسة تأثير تلقيح الطماطم بميكروبات *Azotobacter chroococcum* و *Bacillus megaterium* var. *phosphaticum* فى وجود بعض الفطريات المسببة للذبول فى الطماطم على نشاط إنزيمات الديهيدروجينيز والفوسفاتيز والنيتروجينيز بالتربة ولقد أوضحت النتائج ما يلى:

تلقيح للطماطم بميكروب *Azotobacter chroococcum* أو *Bacillus megaterium* var. *phosphaticum* فى وجود الفطريات المسببة لذبول الطماطم أدى إلى زيادة نشاط إنزيم الديهيدروجينيز بالمقارنة بالمعاملات غير الملقحة . وقد أوضحت النتائج أنه عند التلقيح بمخلوط السلالتين كان نشاط إنزيم الديهيدروجينيز أعلى بالمقارنة بالتلقيح الفردى .

بخصوص تأثير التلقيح على نشاط إنزيم الفوسفاتيز فقد أوضحت النتائج أن التلقيح بميكروب *B. megaterium* var. *phosphaticum* أدى إلى زيادة فى نشاط الإنزيم بالمقارنة بالتلقيح بميكروب *A. chroococcum* . كذلك أوضحت النتائج أن نشاط الإنزيم قد إزداد فى حالة التلقيح المزدوج بالمقارنة بالتلقيح الفردى. ولوحظ من النتائج أيضا أن التلقيح بميكروب *A. chroococcum* أعطى زيادة فى نشاط إنزيم النيتروجينيز فى كلا التربة المعقمة والغير معقمة أيضا أوضحت النتائج أن التلقيح المزدوج بكلا السلالتين أعطى نتيجة أعلى من التلقيح الفردى بأى من السلالتين .

عند التلقيح بميكروب *B. megaterium* var. *phosphaticum* منفردا لم يعطى أى نشاط لهذا الإنزيم وذلك فى التربة المعقمة ، ولكن فى التربة الغير معقمة أظهر التلقيح الفردى نشاط فى إنزيم النيتروجينيز وقد عزى ذلك إلى الميكروبات الموجودة طبيعيا فى التربة.

تأثير التلقيح بالميكروبات المنتجة لمنظمات النمو على محتوى النبات من إنزيمى البيروكسيدز والبولى فينول أوكسيديز.

أوضحت النتائج أن عدوى التربة بفطريات *Fusarium oxysporum* f.sp *lycopersici* or *F. solani* أدى إلى إنخفاض معنوى فى محتوى النبات من كلا الإنزيمين . ولكن عند تلقيح الطماطم بكلا من *Azotobacter chroococcum* or *Bacillus megaterium* var. *phosphaticum* أدى إلى زيادة معنوية فى محتوى النبات من كلا الإنزيمين .

كذلك أوضحت النتائج أن التلقيح بمخلوط *Azotobacter chroococcum* + *Bacillus megaterium* var. *phosphaticum* فى وجود العدوى بالفطريات الممرضة قد زاد من محتوى النبات من هذه الإنزيمات .