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BIOFERTILIZATION INFLUENCE OF TOMATO SEEDS AND SEEDLINGS BY NON-SYMBIOTIC N_2-FIXERS ON TOMATO PLANT GROWTH AND *FUSARIUM* DISEASE SEVERITY

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ABSTRACT

Tomato seeds and seedlings were inoculated by using non-symbiotic N_2-fixers *Azospirillum brasilense* and *Azotobacter chroococcum* to investigate the biofertilization influence on dehydrogenase activity, plant growth-promoting bacterial population, total nitrogen, phosphorus and potassium in soil. Fruits physical characteristics and percentage of total phenoles and indoles in fruits were also determined. Moreover, disease incidence of damping-off, in the presence of *Fusarium oxysporum* pathogen in the presence of biofertilizers were evaluated.

Obtained data showed that, inoculation of seedlings (seeds+ seeding roots) gave the highest values of dehydrogenase activity, azospirilla and azotobacters proliferation, fruits quality and reduction of disease incidence.

Also, results revealed that mixed inoculation of non-symbiotic N_2-fixers increased all tested parameters and reduced disease incidence than single inoculation of either seed inoculation or seedling inoculation by diazotrophs.

INTRODUCTION

Bacteria of the genera *Azotobacter* and *Azospirillum* are free-living, N_2-fixing organisms, which live in close association with plant roots. These kind of plant bacterial interaction often results in plant growth-promotion (Rodelas *et al.*, 1997). Several mechanisms by which plant growth-promoting rhizobacteria (PGPR) promote plant growth or inhibit soilborne plant pathogens by production of extra-cellular growth-promoting chemical substances that act as potential biological control agents for many soilborne root diseases (Kloepper, 1992). Studies suggested that N_2-fixation, hormonal effects improvements of root development, minerals and water up take and stimulation of nitrate assimilation by plants are results of non-symbiotic N_2-fixers biofertilization (Bashan *et al.*, 1988). Sivan *et al.* (1987) found that inoculation of seeds and of transplants (seedlings) gave a protection action against tomato *Fusarium* pathogen. Due to the importance of *Azospirillum* and *Azotobacter* as plant growth-promoting and plant protection agents they consedered to be necessary to involve as inoculants of tomato seeds or seedlings.

The purpose of the present investigation is to evaluate the biofertilization influence by non-symbiotic N_2-fixing rhizobacteria for seeds and seedlings on soil microbial activity, plant parameters (plant height + fruit quality) and reduction ability of *Fusarium* disease incidence in tomato plants (*Lycopersicon esculentum* L.) cv. Super Marmande.

MATERIALS AND METHODS

A pot experiment was carried out in summer season 1997 under greenhouse conditions at the Fac. of Agric., Moshtohor, to investigate the effect of biofertilization by using non-symbiotic N_2-fixers, *Azospirillum brasilense* and *Azotobacter chroococcum* on tomato plants and also, to evaluate the effect of inoculation method on the growth of tomato plant in the presence of *Fusarium oxysporum* pathogen, and ability to reduce the disease incidence of damping-off. The experimental soil was obtained from the Fac. of Agric., Moshtohor Farm. Chemical and mechanical analysis of the experimental soil according to Black *et al.* (1982) and Jackson (1973), are presented in Table (1). All pots were supplemented with calcium super phosphate (15.5% P_2O_5) and potassium sulphate (48% K_2O) at a rate of 30 Kg P_2O_5 and K_2O/fed in two equal doses at 30 and 60 days after sowing.

Table (1): Chemical and mechanical analysis of the experimental soil.

A- Chemical analysis					
Organic matter %	**pH**	**T.N %**	**T.P %**	**E.C m mhos/cm**	**$CaCO_3$ %**
1.82	7.91	0.45	0.22	0.84	1.40
B-Mechanical analysis					
Coarse sand %	Fine sand %	Silt %	Clay %	TexturalClass	
3.66	18.52	22.16	55.66	Loamy sand	

T.N. Total nitrogen **T.P. Total phosphorus**
E.C. Electric conductivity.

Biofertilizers:

Source. *Azospirillum brasilense* and *Azotobacter chroococcum* strains were provided from Microbiological Unit, Desert Research Center, Mataria, Cairo.

Preparation of N_2-fixers inocula:

For preparation of *Azospirillum brasilense* and *Azotobacter chroococcum* inocula, modified Dobereiner's malate medium (Baldani *et al.*, 1986) and modified Ashby's medium (Abdel-Malek and Ishac, 1968) were inoculated with *A. brasilense* and *A. chroococcum*, respectively and incubated at 30ºC for 7 days till the number of bacteria reached about 1×10^8 cfu ml^{-1} and then, the inocula were used for inoculation of tomato seeds or tomato seedlings.

Pathogen fungus:

A strain of *Fusarium oxysporum* (a pathogen for tomato plants) was provided from Botany Dept. (Plant pathology), Fac. of Agric., Moshtohor. The pathogenic fungus was maintained on potato dextrose agar medium (PDA) at 24ºC, then grown on Czapek medium for 7 days at 28ºC. Conidia were suspended in distilled water and diluted to give 2×10^6 spores/ml. one ml spore suspension was added to sterilized 100 grams corn bran in 500 ml

conical flasks. Inoculated flasks were incubated at 28ºC for 10 days (Martyn, 1986) to give fungal growth inoculum to infeste the experimental soil.

Cultivar:

Tomato seeds (*Lycopersicon esculentum* L.) cv. Super Marmande, susceptible to *F. oxysporum* were obtained from Preservation and Utilization of Primitve Germplasm Laporatory of Dept. of Horticulture, Fac. of Agric., Moshtohor.

Seed and Seedlings bacterization:

Tomato cultivar seeds were bacterized by the method of Weller (1988). Seven days old culture of modified Dobereiner's medium containing 1 x 10^8 cfu ml^{-1} of *A. brasilense* and Ashby's medium containing about 1 x 10^8 cfu ml^{-1} of *A. chroococcum* were centrifuged for 5 min., washed twice in sterilized distilled water. Washed cells of *A. brasilense* and *A. chroococcum* were mixed separately with 16% gum arabic. Seeds were sterilized with 96% ethanol for 1 min., washed, dipped in 0.1% $HgCl_2$ for 10sec. and then washed 2-3 times in sterilized distilled water (El-Abyad *et al.*, 1993). Sterilized seeds were placed in gum arabic bacterial inoculum. Coated seeds were air dried for 12 hr at 25-28ºC. Seeds treated with 16% gum arabic without bacteria cells were used as a control treatment.

Seed-inoculated tomato seedings were procedure was carried out by root inoculation (Gupta *et al.*, 1995) as follows:

Seven days old inoculated seedings were uprooted, washed carefully 2-3 times in sterilized distilled water and their roots were immersed in a bacterial cell suspension (10^8 cfu ml^{-1}) for 5 min. Such seedlings were considered as seed + root-inoculated seedings. After inoculation plants repotted again. Seedlings without root inoculation were used as control.

Soil infestation:

All pots were infested by 3% of ten days old culture on corn bran of *F. oxysporum,* except control without infestation. All pots were irrigated daily for 7 days to stimulate the fungal growth and to ensure its distribution within the soil before cultivation.

Cultivation process.

Seventy five cm pots were filled with infested loamy sand soil. Five replicates for each treatment were used in a randomized complete block design. Ten seeds were sown in each pot after inoculation pretreatment. Seeds were inoculated with individual *A. brasilense* or individual *A. chroococcum* or with a mixture of both of them.

The experiment included the following treatments:

I = Control (without inoculation).

II = Single biofertilizer contained non-symbiotic N_2fixer *Azospirillum brasilense.*

III = Single biofertilizer contained non-symbiotic N_2-fixer *Azotobacter chroococcum.*

IV = Mixed biofertilizer contained a mixture of *A. brasilense* (II) + *A. chroococcum* (III).

All treatments were studied in the presence or absence of *Fusarium oxysporum* the causal pathogen of damping-off and wilt diseases of tomato plants.

Sampling.

Rhizosphere soil samples at seedling, flowering and fruiting stages were taken for microbiological determinations. Also, tomato plants at flowering stage and tomato fruits at fruiting stage were obtained for chemical analysis and to determine plant parameters.

Determinations:

Microbiological analyses.

1. Dehydrogenase activity in the soil was assayed by the method described by Casida *et al.* (1964).
2. Densities of azotobacters and azospirilla in soil were determined on modified Ashby's medium (Abdel-Malek and Ishac, 1968) and modified Dobereiner's malate medium (Baldani *et al.*, 1986), respectively using the most probable densities technique.

Damping-off percentage:

Percentage of pre and post emergence damping-off were calculated after 2 and 4 weeks respectively after planting and survival plants was calculated at fruiting stage (Maturity).

Chemical analysis:

Total nitrogen was determined according to Kjeldahl method (1983) and Jackson (1973). Total phosphorus was colourimetrically determined according to APHA, (1989). Total potassium was determined according the method described by Gough (1981) in soil and in plant dry matter of shoots at flowering stage.

The phenolic compounds percentage were determined in fresh matter of tomato fruits as described by APHA, (1989). Also, total Indoles percentage was determined according to Singh (1982) in fresh matter of tomato fruits.

Plant parameters:

Fruit fresh weight (g/fruit), fruit length (cm) fruit diameter (cm) and plant height (cm) were determined at fruiting stage.

Statistical analysis:

Variance analysis (ANOVA) was used to determine the significant difference between treatments according to Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Effect of seed inoculation and seed + root–inoculation on total NPK in soil during different growth stages:

Data presented in Table (2) indicated that the inoculation with a mixture of *A. brasilense* and *A. chroococcum* gave more total NPK in each of seed inoculation and seed + root–inoculated seedlings but, total NPK with seed + root-inoculated (seedlings) was more pronounced than seed inoculation only. The highest values of total NPK were recorded at flowering stage in seed + root-inoculated seedlings with mixed inoculation by non-symbiotic N_2-fixers in absence of *Fusarium* pathogen. This may be due to the beneficial effect of root exudates that increased during flowering stage. These results are in agreement with those reported by Rodelas *et al.* (1997). Total NPK in soil increased gradually from the seedling stage, till flowering stage and decreased after flowering stage. Nitrogen content in soil at flowering stage raised with inoculation with mixed diazotrophs in seed + root-inoculated seedlings application over another inoculation treatments. Also total N in soil was superior with control (without *Fusarium)* than with *F. oxysporum* (infested soil).This could be attributed to the competition between *Fusarium* pathogen and diazotrophs which gave a decrease in rhizosphere bacteria whereby, nitrogen content decreased in soil in interaction with pathogen. Results in Table (2) also emphasized that the content of NPK in soil was more increased in both of control and *F. oxysporum* infested seed + root-inoculated seedlings application than both of control and *Fusarium* in seed inoculation. It is obvious from data that application with non-symbiotic N_2 - fixers for seed or seed + root-inoculation gave higher content of nitrogen, phosphorus and potassium.

Table (2): Effect of tomato seed and seedlings biofertilization by non- symbiotic N_2-fixers on the total NPK in rhizosphere soil during growth stages.

Treatments	Seedling stage											
	Seed inoculation						Seed + root inoculated Seedlings					
	Control			*F. oxysporum*			Control I			*F. oxysporum*		
Stage I	N	P	K	N	P	K	N	P	K	N	P	K
	(ppm)			(ppm)			(ppm)			(ppm)		
I	411	287	486	410	260	335	520	310	450	426	315	435
II	1160	331	664	916	315	615	1315	641	765	1154	375	687
III	937	325	622	893	276	574	1283	470	618	1036	346	624
IV	1026	355	783	971	295	673	1372	650	986	1211	377	798
Stage II	Flowering stage											
I	712	421	577	673	345	518	840	483	654	717	452	648
II	1466	595	728	1334	385	638	1630	864	1015	1435	584	894
III	1270	482	684	1213	316	534	1452	648	842	1316	517	710
IV	1495	543	839	1422	419	718	1721	923	1032	1569	664	994
Stage III	Fruiting stage											
I	518	363	489	410	351	467	645	412	568	554	408	538
II	1229	478	615	983	417	516	1311	588	996	1280	560	680
III	927	635	649	815	493	588	1435	732	728	1100	572	765
IV	1100	683	843	947	435	662	1513	742	1013	1291	624	883

I = Control (without inoculation).
II = Single biofertilizer contained non-symbiotic N_2- fixer *Azospirillum brasilense.*
III = Single biofertilizer contained non-symbiotic N_2-fixer *Azotobacter chroococcum.*
IV = Mixed biofertilizer contained a mixture of *A. brasilense* + *A. chroococcum*.

Effect of seed inoculation and seed + root-inoculated seedings on the rhizosphere soil non-symbiotic N_2-fixer proliferation:

1. Changes in azotobacters densities during the various plant growth stages:

Data presented in Table (3) clearly indicate that the population of azotobacters in rhizosphere soil increased in all inoculated treatments compared to control. The seed + root-inoculated seedlings was superior of seed inoculation in most treatments. The highest densities of azotobacters were observed in the mixed biofertilizer of seed + root-inoculation seedlings at flowering stage in control treatment (without pathogen). Data showed that the counts of azotobacters increased at flowering stage than seedling stage and fruiting stage with all treatments and particularly increased in the mixed biofertilization of seed + root-inoculated seedlings. The seed-root-inoculated seedlings showed a reduction of the disease incidence by *F. oxysporum* in all treatments. The highest reduction of disease incidence by *F. oxysporum* was recorded with mixed biofertilization of seed-root-inoculation, this result may be attributed to the increase of azotobacters densities in rhizosphere

Table (3): Effect of seed and seedlings biofertilization by non-symbiotic N_2-fixers on the azotobacters densities (x 10^4 cell/g dry soil) in rhizosphere soil of tomato plants.

Treatments	Seedling stage			
	Seed inoculation		Seed + root inoculated seedlings	
Stage I	Control	*F. oxysporum*	Control I	*F. oxysporum*
I	202.6	198.6	283.3	250.7
II	273.6	240.7	314.5	290.5
III	282.4	260.3	321.4	293.6
IV	285.8	271.6	332.6	322.3
Stage II	**Flowering stage**			
I	275.8	253.2	412.5	302.6
II	325.4	305.4	445.3	415.7
III	373.5	343.4	461.6	432.4
IV	395.5	367.6	472.4	445.3
Stage III	Fruiting stage			
I	240.6	204.2	291.4	257.3
II	282.8	263.2	325.4	296.5
III	296.4	274.3	331.6	302.4
IV	298.5	281.1	334.3	352.4

The same footnotes of Table (2).

soil. These results are in agreement with Gupta *et al.* (1995) who reported that the population of diazotrophs (azotobacters and azospirilla) colonizing roots were marginally greater in the seed + root - inoculated seedings as compared to the seed-inoculated ones and also, reduced the disease incidence by *F. oxysporum* pathogen. Azotobacters densities gradually decreased after flowering stage. The lowest counts of azotobacters were recorded with inoculation with both *Azospirillum brasilense* and *F. oxysporum* in seed inoculation only at seedling stage.

Generally, data in Table (3) clearly emphasized that seed + root - inoculated seedings gave considerably increased bacterial population than seed inoculation only. These results due to that the seed + root-inoculation of seedlings by non-symsiotic N_2- fixers are effective inoculants, due to their ability to establish themselves on tomato root surface in sufficient numbers and stimulate the seedling growth. Moreover, *Azotobacter* spp. and *Azospirillum* spp. act as a plant growth-promotors (Kloepper, 1992), which reduced the disease incidencey of damping-off.

2. Changes in azospirilla densities during various plant growth stages:

Data in Table (4) indicate that the densities of azospirilla in rhizosphere soil increased in all treatments compared to control. Application of seed + root-inoculation by a mixture of *A. brasilense* and *A. chroococcum* gave the highest densities of azospirilla different growth stages. Data showed that the population of azospirilla gradually increased during seedling stage till flowering stage then, gradually decreased thereafter. All treatments with seed + root- inoculation increased azospirilla counts compared to seed inoculation only. The same trends were noticed in all plant growth stages. Results indicated that the mixed biofertilization of seed or seed + roots and single biofertilizer of tomato gave higher magnitudes of rhizosphere bacterial densities.

Also, Noguera and Smits (1982) suggested that root exudates of infected tomato stimulate soil microflora reproduction and account for the greater number of colonies. Results also showed that the application of seed + root-inoculated seedlings increased *Azospirillum* spp in rhizosphere soil at different growth stages with all treatments. Interactions between treatments indicate that the biofertilization with mixed bacteria proved to be better than individual *A. brasilense* or *A. chroococcum* in increasing rhizosphere bacteria.

Effect of seed inoculation and seed + root-inoculation of seedlings on changes of dehydrogenase activity during growth stages:

Data in Table (5) show that the dehydrogenase activity as an indication of microbial activity differed between plant growth stages and varied between inoculation treatments. The dehydrogenase activity increased in all treatments over control. The combined inoculation by non-symbiotic N_2-fixers increased dehydrogenase activity more than individual inoculation of either seeds or seeds + root-inoculation at all growth stages.

The highest values of dehydrogenase activity were observed in control without infested pathogen at flowering stage. This result could be attributed to the high activity of rhizobacteria in root zone of plants and rich exudates of roots with carbohydrates and amino acids. These results are in agreement with those reported by Kloepper (1992). Data in Table (5) clearly indicate that non-symbiotic N_2-fixers of both seed and seed + root-inoculation. The inoculation of seed + root (seedlings) by diazotrophas increased dehydrogenase activity better than inoculation of seed only (Gupta *et al.*, 1995). It is worthy to notice that dehydrogenase activity values increased gradually from seedling stage till giving higher values at flowering stage and then, decreased at fruiting stage.

Table (4): Effect of seed and seedlings biofertilization by non-symbiotic N_2-fixers on the azotobacters densities (x 10^4 cell/g dry soil) in rhizosphere soil of tomato plants.

Treatments	Seedling stage			
	Seed inoculation		Seed + root inoculated seedlings	
Stage I	Control	*F. oxysporum*	Control I	*F. oxysporum*
I	260.5	240.3	294.4	275.5
II	480.8	470.4	510.6	493.3
III	340.7	280.2	442.8	385.4
IV	402.4	295.6	520.7	450.8
Stage II	**Flowering stage**			
I	460.2	420.6	510.8	468.2
II	760.2	788.6	820.3	70.6
III	640.3	560.5	710.8	650.5
IV	792.5	590.3	485.6	810.6
Stage III	**Fruiting stage**			
I	320.5	280.3	380.4	330.5
II	520.5	480.2	640.2	580.3
III	430.3	355.4	560.5	495.2
IV	550.4	495.5	670.2	610.5

The same footnotes of Table (2).

Table (5): Effect of seed and seed + root seedling biofertilization on the changes of dehydrogenase activity (μl H/g dry soil/ 24hr) in rhizosphere soil of tomato plants.

Treatments	Seedling stage			
	Seed inoculation		Seed + root inoculated seedlings	
Stage I	Control	*F. oxysporum*	Control I	*F. oxysporum*
I	65	140	181	163
II	316	281	357	309
III	293	265	310	297
IV	367	298	364	331
Stage II	**Flowering stage**			
I	217	210	253	225
II	491	471	510	502
III	433	454	470	451
IV	483	477	537	514
Stage III	Fruiting stage			
I	215	206	250	231
II	476	384	478	452
III	375	343	421	396
IV	409	395	477	431

The same footnotes of Table (2).

Effect of seed inoculation and seed + root-inoculated seedlings on percentage of pre and post-emergence damping-off and plants survival:

Data presented in Table (6) show that inoculation by biofertilizer of non-symbiotic N_2-fixers significantly decreased pre and post emergence damping-off and gave a significant increase in survived plants over control. Application of seed + root-inoculation by non-symbiotic N_2-fixers significantly increased plant emergence and gave a significant decrease in pre and post

emergence damping-off percentage than treating seeds only by the N_2-fixing biofertilizer. Furuya *et al.* (1997) suggested that inoculation of tomato roots with N_2- fixers at the time of transplanting in causal pathogen of damping-off infested soil increased the percentage of seedling survival. Also, soaking the roots of tomato seedlings in bacterial suspension resulted in the highest suppression and gave protection against with diseases, suggesting that mechanisms, such as induced resistance and infection sites competition were involved in suppression of the disease. Either single inoculation by *A. brasilense* or *A.chroococcum* gave significant increases of plant emergence and plant survival, but mixed inoculation was superior over them in both of seed inoculation and seed + root-inoculated seedlings. The best treatment significantly reduced pre and post emergence damping- off was resulted in the case of mixed inoculation with seed + root-inoculation. Also, a mixture of diazotrophs gave significant increases of survival of tomato plant.

Table (6): Effect of seed and seedlings biofertilization by asymbiotic N_2-fixers on percentage of pre and post emergence damping-off and survived plants.

Inoculation	Seed inoculation						Seed + root inoculation					
Damping-off	Pre-emergence %		Post-emergence %		Survived plants %		Pre-emergence %		Post-emergence %		Survived plants %	
Treatment	Control	*F. oxysporum*	Control	*F. oxysporum*	Control	*F. oxysporum*	Control	*F. oxysporum.*	Control I	*F. oxysporum.*	Control I	*F. oxysporum*
I	2.00	40.00	14.60	22.80	83.40	37.20	0.00	6.00	10.20	20.50	84.80	73.50
II	2.00	10.00	2.60	8.80	95.40	81.20	0.00	1.50	2.50	4.40	97.50	94.10
III	2.00	20.00	2.40	9.60	45.60	70.90	0.00	1.60	2.20	7.60	97.80	90.80
IV	1.00	5.00	1.00	5.20	98.0	89.80	0.00	1.40	0.00	4.60	100.0	94.0

The same footnotes of Table (2)

L.S.D. at 5% **Treatments (T) = 0.004**
Inoculation (I) = 0.887
I x T = 0.010

Effect of seed inoculation and seed + root inoculated seedlings on the percentage of N, P and K in tomato plants at flowering stage:

Data in Table (7) clearly indicate that N, P and K percentage in tomato plant shoots appreciably increased in treatments than that resulted in control treatment (without inoculation). Recorded data revealed that the highest percentage of nitrogen, phosphorus and potassium content in shoots was obtained with combined inoculation by *Azospirillum* + *Azotobacter* in the case of seed + root-inoculated seedlings. Also, biofertilization by *Azotobacter* gave a considerable increase of nitrogen, phosphorus and potassium percentage over control in both of seed inoculation or seed-root-inoculated seedlings treatments. This result are in agreement with Mohandas (1987) who reported that the inoculation by *Azotobacter* significantly increased phosphorus content and yield of tomato plants. Data in Table (7) also showed that the single and mixed biofertilizers gave considerable increased of N, P

and K percentage in tomato plant shoots. Increasing of N, P and K percentage consequently increased plant growth. These results can be related to the role of non-symbiotic N-biofertilizer in the production of phytohormones improving the availability and acquisition of nutrients or by both (Jagnow *et al.,* 1991). It is obvious from data that severity of *Fusarium oxysporum* damping-off and wilt decreased with mixed of *Azospirillum* and *Azotobacter* and due to the disease incidence reduction of damping-off (see Table, 6). The hormonal exudates of the biofertilizers can modify root growth, morphology and physiology, resulting in more absorption of N and P. Monib *et al.* (1990) reported that the inoculation of tomato plants with *Azotobacter* and *Azospirillum,* led to higher N and P content than control.

Table (7): Effect of seed and seedling biofertilization by asymbiotic N_2-fixers on the percentage of NPK in tomato shoots at flowering stage.

Treatments	flowering stage											
	Seed inoculation						Seed + Root inoculated Seedlings					
	Control			*F. oxysporum*			Control I			*F. oxysporum*		
	%			%			%			%		
	N	P	K	N	P	K	N	P	K	N	P	K
I	3.32	0.13	4.02	3.15	0.09	3.97	3.46	0.15	4.19	3.42	0.13	4.08
II	3.50	0.19	4.50	3.50	0.17	4.22	3.60	0.21	4.72	3.58	0.18	4.62
III	3.42	0.17	4.44	3.35	0.16	4.15	3.72	0.18	4.63	3.53	0.15	4.53
IV	3.87	0.21	4.66	3.82	0.18	4.40	4.06	0.25	4.76	3.96	0.22	4.72

The same footnotes of Table(2)

Effect of seed inoculation and seed + root-inoculated seedlings on physical characteristics of tomato fruits and plant height:

It is obvious from data presented in Table (8) that, in general, inoculation by N_2-fixers *(Azotobacter* and *Azospirillum)* significantly increased plant height, fruit fresh weight, fruit diameter and fruit length. The seed + root-inoculated seedlings gave significant increase of physical characteristics and plant shoot height higher than those recorded in the case of single seed inoculation with non-symbiotic N_2-fixers in all treatments. The mixture of *Azotobacter* and *Azospirillum* was more pronounced to give a significant increase of plant height, fruit fresh weight, fruit length and fruit diameter (fruits quality) for both of seed inoculation and seed-root-incoulated seedlings, while mixed inoculation of seed + root inoculation of seedlings significantly increased plant height and fruits quality more than seed inoculation. These results are in agreement with those obtained by Barakat and Gabr (1998) who suggested that mixed biofertilization by *Azotobacter* and *Azospirillum* together considerably improved tomato plant growth, fruits yield and increased plant shoot height. It is worthy to mention that significant increase

Table (8)

of plant height and fruits quality due to significant increase of disease incidence reduction of damping-off and wilt by *Fusarium* pathogen in all treatments and significantly decreased *Fusarium* severity (see Table 6). This result may attribute to the effects of non-symbiotic N_2-fixers of seed inoculation or seed + root inoculated seedlings. These results are in agreement with those obtained by Gubta *et al.* (1995).

Effect of seed inoculation and seed + root - inoculated seedlings on percentage of phenoles and indoles in tomato fruits:

Data presented in Table (9) revealed that inoculation of tomato seeds and tomato seedlings markedly decreased phenoles and indoles percentage of tomato fruits. The mixed inoculation with *Azotobacter* and *Azospiriluum* of seeds and seedlings gave more pronounced decrease of phenoles and indoles percentage than *Azospirillum* or *Azotobacter* separately. Also, seed + root-inoculated seedlings gave a considerable decrease of phenoles and indoles percentage in tomato fruits more than seed inoculation treatment.

It is also obvious that phenoles and indoles percentage increased in *Fusarium* infested treatments more than those detected in other treatments particularly, in seed inoculation treatment. It is worthy to mention that the increase of phenoles and indoles percentage in tomato fruits considered, as disease incidence indicator of *Fusarium* severity of damping-off and wilt, whereas seed inoculation or seedling inoculation appreciably reduced disease incidence. Reduction can be due to the decrease of phenoles and indoles percentage in tomato fruits. These results are in agreement with Abo-Arkoub (1994) who reported that phenoles and indoles compounds are increased in tomato plants infected by *F. oxysporum* as a plant mechanical reaction, i.e. disease incidence increased phenoles and indoles compounds as a plant natural defense.

Table (9): Effect of seed and seedling biofertilization by non-symbiotic N_2-fixers on the percentage of phenoles and indoles in tomato fruits.

Treatments	Total Phenoles and indoles %							
	Seed inoculation				Seed + root-inoculation (seedlings)			
	Control		*F. oxysporum*		Control I		*F. oxysporum*	
	Phenoles	indoles	Phenoles	Indoles	Phenoles	Indoles	Phenoles	Indol
I	0.752	0.484	0.885	0.637	0.692	0.475	0.830	0.622
II	0.727	0.471	0.826	0.586	0.704	0.460	0.807	0.564
III	0.708	0.446	0.780	0.624	0.691	0.429	0.762	0.609
IV	0.688	0.420	0.772	0.522	0.672	0.408	0.754	0.507

The same footnotes of Table(2)

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تأثير التلقيح الحيوى لبذور وشتلات الطماطم بواسطة البكتريا المثبتة للنتروجين لاتكافليا على نمو النبات وشدة مرضية فطر الفيوزاريوم

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قسم النبات الزراعى (ميكروبيولوجيا زراعية) كلية الزراعة بمشتهر ، فرع بنها ـ جامعة الزقازيق ـ مصر

فى هذا البحث تم دراسة تأثير التلقيح الحيوى بالبكتريا المثبتة لنتروجين الهواء الجوى *Azospirillum brasilense* وبكتريا *Azotobacter chroococcum* لكل من بذور الطماطم وجذور شتلات الطماطم وذلك فى وجود الفطر الممرض *Fusarium oxysporum* المسبب لمرض موت البادرات والذبول فى الطماطم.

وقد أظهرت النتائج الآتي:

وجد أن التلقيح الحيوى للشتلات (بذرة + جذور) أعطى أعلى معدل من نشاط إنزيم الدهيدروجنيز وأعلى أعداد من البكتريا المثبتة للنتروجين لا تكافلى وكذلك أعطى أعلى معدل من صفات الثمار الطبيعية (الوزن – الطول – القطر) كما وجد أن تلقيح الشتلات يؤدى إلى خفض نسبة الإصابة بالفيوزاريوم (موت البادرات + ذبول).

أدى استخدام التلقيح المشترك بين كل من *Azotobacter* + *Azospirillum* إلى زيادة معدل النشاط الميكروبى فى التربة والقياسات النباتية وخفض نسبة الإصابة بفطر الفيوزاريوم الممرض وذلك عن استخدام التلقيح الفردى بنوع واحد من البكتريا المثبتة لنتروجين الهواء الجوي لاتكافليا سواء كان ذلك فى حالة تلقيح البذور فقط أو تلقيح الشتلات (البذرة + الجذر).

Table (8): Effect of seed and seedlings biofertilization by non- symbiotic N_2-fixers on physical characteristics of tomato fruits and plant height.

Parameters / Treatments	Seed inoculation								Seed + root inoculated seedlings							
	Control				*F. oxysporum*				Control				*F. oxysporum*			
	Fruit fresh weight (g)	Fruit length (cm)	Fruit Diameter (cm)	Plant height (cm)	Fruit fresh weight (g)	Fruit length (cm)	Fruit Diameter (cm)	Plant height (cm)	Fruit fresh weight (g)	Fruit length (cm)	Fruit Diameter (cm)	Plant height (cm)	Fruit fresh weight (g)	Fruit length (cm)	Fruit Diameter (cm)	Plant height (cm)
I	48.40	3.92	5.52	44.70	42.80	3.86	5.43	42.50	53.50	3.98	5.64	49.60	50.30	3.91	5.62	45.70
II	68.80	4.02	5.84	52.40	63.60	3.96	5.72	48.30	74.40	4.10	6.06	58.20	73.3	4.03	6.03	57.30
III	61.50	3.95	5.71	50.40	58.40	3.85	5.63	44.70	65.50	4.06	5.98	55.80	62.7	4.00	5.82	53.10
IV	75.40	4.15	5.95	58.20	67.40	4.05	5.84	54.50	78.70	4.22	6.12	62.10	78.10	4.12	6.00	58.70

The same footnotes of Table (2)

L.S.D. at 5% **Treatments (T) = 6.035** **F. Diameter = 0.016**

Plant height = 5.021 **Fresh weight = 5.017**

Tx I = 4.081 **Inoculation (I) = 26.07** **Fruit length = 0.931**