APPLICATION OF BIOFERTILIZATION AND BIOLOGICAL CONTROL FOR TOMATO PRODUCTION

ABSTRACT

Field experiment was carried out at El-Bagour, Minofia governorate during 2003 and 2004 seasons. This experiment aimed to study the effect of antagonistic strains (*Trichoderma harzianum, Bacillus subtilis* and *Streptomyces aureofaciens* in combination with *Azotobacter chroococcum* on growth and yield of tomato. Obtained results showed that dual inoculation with either *A. chroococcum + B. subtilis* or *A. chroococcum + T. harzianum* showed lower percentage of disease rate and disease severity of tomato plants. The highest values of were observed in the treatment of inoculation with *A. chroococcum* combined with *B. subtilis + T. harzianum*.

Also, tomato inoculation with *A. chroococcum* combined with *B. subtilis + T. harzianum* gave the highest records of growth characters, yield and yield components of tomato. In view of the obtained results, it can be concluded that the dinitrogen fixers and bicontrol agents can be used for improving the quantity and quality of tomato production .

Key words: Tomato, biofertilization, biological control, damping-off and root rot, dehydrogenase and nitrogenase.

INTRODUCTION

Tomato (*Lycopersicom esculentum* Mill) is one of the most important vegetable crops in Arab Republic of Egypt. The cultivated area of tomato in 2006 growing season reached 190160 feddans in old and newly reclaimed lands, which produced 4,173,845.5 million tons. The A. R. E. government is pressing hard to increase the production of tomato to face the increasing demand of the populations and to increase the exportation.

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 *R. solani* and *S. rolfsii* attack the roots and stem base of tomato **(Wokocha 1990; Ristaino *et al.*, 1991).**

Fusarium tomato wilting is considered as one of the most deleterious diseases which attack tomato seedlings either in the nurseries or in fields after transplanting and this disease causing great of losses **(Niknejad *et al.*, 2000).** The excessive use of broad spectrum or persistent chemicals may result in soil contamination, fungicidal resistance or other harmful effects **(Maloy, 1993).**

Fungicides are still the primary means to control fungal pathogens, but their use is becoming more and more controversial. Many investigations have indicated potentially undesirable environmental effects on humans, plants and other beneficial organisms. In addition, upsetting the biological balance by toxic chemicals can lead to sever outbreak of diseases as well as the appearance of new pathogenic strains. Therefore, the need for more research into nonchemical methods of crop production seems to be justified.

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Biological control is usually more enduring with no toxic residue in nature's food chains, safe for application and cheaper in cost. Biological control lends itself as a suitable alternative of chemical**.** It relies on the potency of beneficial antagonistic microorganisms to colonize the roots and displace the pathogenic microorganisms. Now it could be targeted the researches into the biological control of microbial pathogens are receive the increasing of attention.

Khalifa (1991) tested *T. harzianum* as a biological control agent against *F. oxysporum* f.sp. *lycopersici.* The organism *suppressed* the growth of *F. oxysporum* f.sp. *lycopersici. T. harzianum* decreased the population of *F. oxysporum f.sp. lycopersici* in rhizosphere, while the population of *T. harzianum* increased up to the 4^{th} week after transplanting.

Ghonim (1999) reported that treatment of tomato seeds with *B. subtilis* reduced tomato wilting disease severity caused by *F. oxysporum f.sp. lycopersic*. *B. subtilis* application improved some growth parameters such as fresh and dry weights of shoots and roots, plants height, number of flowers, root depth and number of branches.

Biofertilizers are of particular important because beneficial microorganisms such as *Azotobacter* spp, *Azospirillum* spp, *Bacillus polymyxa* which were used. These microorganisms successfully increased the yield and improved the quality of many crops when applied. **Aguilar *et al.* (1996)** studied the effect of tomato seedling inoculation by *Azotobacter chroococcum* and *A. vinelandii.* Best results of tomato growth characters were obtained with *A.chroococcum* compared to *A. vinelandii*.

The present investigation was conducted to study the following:

1- Effect of certain bio-control agents (fungi and bacteria) on controlling of root- rot and wilting diseases of tomato.

2- Effect of simultaneous biofertilization and biological control on the incidence of root rots and wilting diseases of tomato plants as well as growth performance and productivity.

MATERIALS AND METHODS

This experiment was carried out under field conditions at El Bagour, Minofia governorate during 2003 and 2004 seasons. Efficient antagonistic strains (*T.harzianum*-I, *B. subtilis*-I and *S.aureofaciens*) in combination with (*A. chroococcum*) were used to evaluate their ability to protect tomato plants (cv. Strain B) against natural infection with soil-borne pathogens of root-rot, damping-off and wilting diseases which mainly caused by *R.solani ,S. rolfsii* and *F. oxysporum* f.sp. *lycopersici*.

Growth parameters, yield and yield components of tomato plants were estimated. Chemical control by vitavax-thiram treatment and chemical fertilization was used as control during the two successive seasons. Mechanical and chemical analyses of used soil are presented in Table (1).

Table (1): Mechanical and chemical analyses of used soil.

Chemical properties				Soil particle size ditribution				
Total N (%)	**$CaCo_3$ (%)**	**P^H**	**EC (dsm^{-1})**	**Textural class**	**Clay (%)**	**Silt (%)**	**Sand (%)**	
0.18	1.81	8.1	4.27		61.9	10.8	27.3	**2003**
0.12	1.92	7.8	3.49		64.7	9.9	25.4	**2004**

Experimental design

Treatments were distributed in a randomized complete block design with three replicates. The experimental area was 21 m^2 (6 m x 3.5 m). Plants were transplanted at 35 cm apart. Every plot consists of six rows; 3.5 m length and 1m width.

This experiment included the following treatments:

1 Uninoculated and unfertilized (control-1)

2 Chemical fertilization and chemical control (control- 2)

3 Azotobacter chroococcum

4 Streptomyces aureofaciens

5 *Bacillus subtilis*

6 *Trichoderma harzianum*

7 *A.chroococcum + S.aureofaciens*

8 *A.chroococcum + B.subtilis*

9 *A.chroococcum + T. harzianum*

10 *A. chroococcum + S.aureofaciens+ B.subtilis*

11 A. chroococcum + S.aureofaciens+ T.harzianum

12 *A. chroococcum + B.subtilis + T.harzianum*

A. chroococcum strain UF5 was provided from the Unit of Biofertilization, Fac. of Agric. Ain Shams Univ., While, *T.harzianum, S.aureofaciens* and *B. subtilis* were isolated and identified in our previous study.

Inocula preparation

For preparation of *A. chroococcum* inoculum, modified Ashby's medium (**Abdel- Malek and Ishac, 1968**) was inoculated with *A. chroococcum* UF5, and then incubated at 30°C for 7 days.

For preparation of *S. aureofaciens* inoculum, starch nitrate medium (**Waksman and Lechevalier, 1961**) was inoculated with *S. aureofaciens*, and then incubated at 35°C for 7 days.

For preparation of *B. subtilis* inoculum, nutrient broth medium **(Cross *et al.* 1968)** was inoculated with *B. subtilis*, then incubated at 25°C for 2-3 days.

For preparation of *T.harzianum* inoculum, gliotoxin fermentation medium **(Abdel-Moity and Shatla, 1981)** was inoculated with *T. harzianum*, then incubated at 28°C for 7 days.

Treatment of transplants

Except for control and bio-control agent treatments, tomato transplants were washed with water and air dried, then transplants (7 days-old) were inoculated by dipping the root system in cell suspension of *A. chroococcum* ($9x10^7$ c.f.u./ml) for 60 minutes before transplanting. Sucrose solution (30%) was added as an adhesive agent prior to inoculation. Also, in chemical control treatment the transplants of tomato were immersed in the fungicide (Vitavax-thiram 0.15%) for one hour before transplanting.

Regarding the bio-control agent treatments transplants were inoculated by dipping in 3 days-old cell suspension of *B.subtilis* ($2.5x10^8$ c.f.u./ml) for 60 minutes before transplanting.

Regarding the *T. harzianum* treatment, tomatos were inoculated by dipping the root system in 7 days-old cell suspension of *T. harzianum* ($2 x10^7$ spores /ml) for 60 minutes before transplanting.

Regarding the *S. aureofaciens* treatment, tomato were dipped in 7 days-old cell suspension of *S. aureofaciens* ($7x10^6$ spores/ml) for 60 minutes before transplanting.

Cultivation process

Except for control and biological control agents treatments, all plots were supplemented with a half dose of inorganic nitrogen fertilizer (75 kg N/feddan) as ammonium sulphate in three equal doses i.e. at vegetative, flowering and setting stages.

For uninoculated treatments soil was fertilized with the recommended dose of nitrogen of 150 kg N/feddan as ammonium sulphate applied in three equal doses i.e. at vegetative, flowering and setting stages.

Except for control (1) treatment (uninoculated and unfertilized), all plots were supplemented with potassium sulphate at a rate of 150 kg potassium sulphate (48% K_2O) in three equal doses as mentioned before.

A control (1) treatment was prepared where the soil was left without fertilization and transplants were soaked in N-deficient medium instead of *Azotobacter* inoculum. Another control was also prepared where transplants were kept without inoculation, but the soil was fertilized with recommended dose of NPK and chemical control by Vitavax-thiram (transplants soaking).

Transplanting was performed on 30 March and 1st April in 2003 and 2004 seasons, respectively.

Diseases assessment

Disease severity and disease rate of root-rot and wilting diseases after 45 days of transplanting were estimated according to **Osullivan and kavanagh (1991).**

Microbiological determinations.

Dehydrogenase activity was assayed according to **Thalmann (1967).** Nitrogenase activity (N_2-ase), was measured by using the acetylene reduction technique given by **Diloworth (1970)**.

Growth characters

Plant height, number of branches, fresh and dry weights of the plants were determined at flowering stage.

Yield and yield components

Number of fruits/plant, individual plant yield, weight of fruits and total yield /feddan were estimated

Chemical analysis

Phenolic compounds were determined using colorimetric method according to **Snell and Snell (1953)**. The total amino acids were determined in ethanolic extract according to the method described by **Rosein (1957).** Total soluble solids (T.S.S.) vitamin C and titratable acidity were assayed according to (**A.O.A.C, 1980**).

Statistical analysis

Data collected were subjected to the statistical analysis according to the standard methods recommended by **Gomez and Gomez (1984)** using the computer program **(Costat).** The differences between the mean values of various treatments were compared by Duncan's multiple range test **(Duncan, 1955)**

RESULTS AND DISCUSSION

Effect of biofertilization and biological control agents on disease rate and disease severity.

Data in **Table (2)** reveal that disease rate and disease severity of root-rot and wilting of tomato plants were the highest in case of untreated soil (control-1). Whereas, the lowest percentage was observed in the treatment of fungicide application (control-2).

Except control-2 (fungicide application), *A. chroococcum* in combination with *B. subtilis* + *T. harzianum* significantly decreased the disease rate and disease severity of root-rot and wilting diseases of tomato plants compared to other biocontrol agents treatments.

This result could be attributed to the synergistic effect in case of dual inoculation. These results are in harmony with those reported by **Sanhita *et al.* (1995)** and **Cal *et al.* (2004)** who found that the combination of *A. chroococcum* and biocontrol agents (*T. harzianum* and *B. subtilis*) significantly decreased disease severity in comparison with the individual ones. The mechanism of *Trichoderma* and *Bacillus* action on pathogens is by attacking and binding to the pathogenic organisms by sugar linkage and begins to secrete extracellular protease and / or lipase.

Dual inoculation with either *A. chroococcum* + *B. subtilis* or *A. chroococcum* + *T. harzianum* showed lower percentage of disease rate and disease severity than the application each of them individually.

Also**, Niknejad *et al.* (2000)** found that *T. harzianum* application significantly decreased damping-off and root-rot diseases and increased percentage of survived plants.

Table (2): Effect of biofertilization and biological control on disease rate and disease severity of tomato.

	Disease rate (%)				Disease severity (%)			
	Root-rot		Wilting		Root-rot		Wilting	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Control-1 (untreated)	70.0 **a**	58.3 **a**	60.4**a**	46.3 **a**	42.0**a**	35.2 **a**	33.3 **a**	27.5**a**
Control-2	24.1 **i**	17.9 **h**	19.4**j**	13.2 **i**	15.4**g**	8.93 **h**	7.67 **h**	3.7 **j**
***Azotobacter chroococcum* (AC)**	53.3**b**	46.0 **b**	50.3**b**	39.3 **b**	39.3**b**	29.0 **b**	25.5 **b**	22.6**b**
***Streptomyces aureofaciens* (SA)**	52.4**b**	41.8 **c**	48.0**b**	36.7**bc**	36.5 **c**	27.3 **b**	23.1 **c**	20.0 **c**
***Bacillus subtilis*-I (BS)**	48.2 **c**	39.3**cd**	44.4**c**	36.2 **c**	35.0 **c**	24.7 **c**	20.7 **d**	17.1**d**
***Trichoderma harzianum*-I (TH)**	44.5 **d**	35.0 **e**	35.3 **f**	29.0 **e**	31.7**d**	22.4 **d**	18.0 **e**	14.6**e**
AC + SA	47.4 **c**	38.5 **d**	41.8 **d**	31.8 **d**	35.6**c**	24.5 **c**	19.9 **d**	16.9 **d**
AC + BS	43.8 **d**	32.6 **ef**	38.9 **e**	27.13**e**	29.8**d**	20.8 **d**	16.5 **e**	12.7 **f**
AC + TH	38.8 **e**	27.1 **g**	31.9 **g**	20.1 **g**	25.9 **e**	18.6 **e**	14.7 **f**	10.9 **g**
AC + SA + BS	36.0 **f**	30.5 **f**	27.7 **h**	23.3 **f**	24.6 **e**	15.9 **f**	13.1 **f**	8.7 **h**
AC + SA + TH	31.1 **g**	24.6 **g**	24.6 **i**	18.1**gh**	20.4 **f**	13.3 **g**	11.3 **g**	5.5 **i**
AC + BS + TH	26.6 **h**	20.4 **h**	20.0 **j**	15.9 **h**	16.6**g**	10.7 **h**	8.7 **h**	4.5 **j**

Control-2: Chemical fertilization (NPK) + fungicide application.

S_2 : The second season S_1 : The first season

Moreover**, Abd El-Wahab (2004)** and **Getha *et al*. (2005)** reported that *T.harzianum, B. subtillus, Gliocladium virens* and S.*griseovirdis* were effective antagonists against *F. oxysporum f.sp. lycopersici, R. solani* and S. *rolfsii*.

Effect of biofertilization and biological control on dehydrogenase and nitrogenase activity in the soil.

Data presented in Table (3) show that the (control-2) treatment exhibited the lowest values of dehydrogenase (DHA) and N_2-ase activity compared with other treatments. Such results indicated that the vitavax when reach to the soil change in quantitative aspects of several microorganisms and disturb the microbial equilibrium.

Dual inoculation of tomato transplants with *A. chroococcum* combined with *S. aureofaciens, B. subtilis* or *T. harzisanum* showed higher values of DHA and N_2-ase activity in tomato rhizosphere than individual inoculation. This result could be attributed to the synergistic effect in case of dual inoculation.

Table (3): Effect of biofertilization and biological control on dehydrogenase and nitrogenase activity.

	Dehydrogenase activity µgTPF/g dry soil/day				N_2-ase activity (nmoles C_2H_4/gdry soil/ hr.)			
	Vegetative stage		Flowering stage		Vegetative stage		Flowering stage	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Control-1 (untreated)	31.5 **i**	36.5 **j**	145.8 **k**	151.6 **k**	5.2 **j**	6.2 **d**	17.9 **j**	21.9 **k**
Control-2	26.0 **j**	31.3 **k**	142.3 **j**	138.1 **j**	13.1 **g**	7.3 **d**	13.1 **k**	16.1 **i**
***Azotobacter chroococcum* (AC)**	43.2 **g**	47.2 **h**	180.1 **g**	185.4 **h**	18.6 **f**	25.1 **abc**	38.8 **h**	53.3 **g**
***Streptomyces aureofaciens* (SA)**	40.5 **h**	42.2 **i**	164.5 **h**	174.1 **i**	6.3 **i**	8.9 **d**	24.7 **i**	34.3 **j**
***Bacillus subtilis*-I (BS)**	49.0 **f**	51.7 **g**	161.2 **i**	172.7 **i**	7.4 **h**	9.8 **cd**	31.3 **i**	42.1 **i**
***Trichoderma harzianum*-I (TH)**	56.7 **e**	58.0 **f**	189.0 **f**	193.7 **g**	13.7 **g**	17.9 **bcd**	36.4 **g**	47.2 **h**
AC + SA	60.1 **d**	72.5 **d**	221.5 **d**	231.6 **e**	20.1 **e**	27.4 **abc**	46.4 **e**	62.9 **f**
AC + BS	61.3 **d**	62.9 **e**	196.9 **e**	206.8 **f**	23. 1 **d**	26.1 **abc**	50.3 **f**	66.9 **e**
AC + TH	68.7 **c**	72.5 **d**	268.3 **c**	260.9 **d**	22. 9 **d**	33.7 **ab**	52.0 **d**	69.2 **d**
AC + SA + BS	74.5 **b**	82.9 **c**	270.1 **c**	281.2 **c**	25.4 **c**	31.5 **ab**	55.7 **c**	73.8 **b**
AC + SA + TH	84.6 **a**	96.0 **b**	308.8 **b**	320.6 **b**	27.6 **b**	37.9 **a**	68.4 **b**	94.2 **a**
AC + BS + TH	86.8 **a**	96.7 **a**	351.9 **a**	368.7 **a**	29.1 **a**	40.1 **a**	92.9 **a**	102.4 **a**

It is worthy to mention that tomato transplants inoculation with *A. chroococcum* in combination with *S.aureofaciens + B. subtilis*,*S. aureofaciens + T. harzianum* or *B. subtilis + T. harzianum* gave higher values of DHA and N_2-ase activity compared to dual inoculation..

The highest values of DHA activity were observed in case of inoculation with *A. chroococcum* combined with *B. subtilis + T.harzianum*. This was true at vegetative and flowering stages.

In general, the DHA and N_2-ase activity in various treatments were higher at flowering stage than vegetative one.

The higher activity of DHA and N_2-ase at flowering stage is likely be due to the difference in multiplication rate of different soil microorganisms which usually be maximum during flowering stage. Such differences could be attributed to the qualitative and quantitative changes in the nature of root exudates during different growth stages **(Abdel-Jawad, 1998).**

These findings are in agreement with **Ravikumar *et al.* (2004)** who found that non symbiotic N_2-fixers such as *A. chroococcum* and *Azospirillum* sp increased the nitrogenase and dehydrogenase activity over non-inoculated control.

Also, **Song (1990)** and **Kennedy *et al.* (2004)** reported that inorganic N-fertilizers application decreased the dehydrogenase and nitrogenase activity compared to biofertilization with associative diazotrohps.

Effect of biofertilization and biological control on growth characters of tomato plants.

Data in **Table (4)** reveal that growth parameters, i.e. plant height, number of branches, fresh and dry weight of tomato plants were the lowest in untreated soil (control-1).

Except number of branches, growth characters of tomato plants were significantly increased with the combined inoculation of *A.* chroococcum and the biocontrol agents compared to tomato transplants inoculation with *A. chroococcum, S. aureofaciens, Bacillus subtilis* and *T. harzianum* individually. *A. chroococcum* inoculation in combination with *S. aureofaciens, B. subtilis* or *T. harzianum* exhibited higher growth performance compared to individual inoculation.

Tomato transplants inoculation with *A. chroococcum* combined with either *Streptomyces aureofaciens + B. subtilis*, *S. aureofaciens + T.harzianum* or *B. subtilis + T.harzianum* gave higher records of growth characters in comparison with dual inoculation by these microorganisms.

The highest records of growth parameters were observed in the treatment of tomato inoculation with *A. chroococcum* in combination with *B. subtilis + T.harzianum*.

These results are in harmony with those reported by **Barakat and Gabr (1998)** who found that inoculation of tomato transplants with *A.chroococcum*, *Azospirillum* sp and *B. polymyxa* as single or mixed biofertilizers significantly increased the growth characters of tomato.

Table (4): Effect of biofertilization and biological control on growth characters of tomato.

	Plant height (cm)		No. of branches per plant		Fresh weight g / plant		Dry weight g / plant	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Control-1 (untreated)	39.2 **j**	43.3 **h**	3.3 **d**	3.7 **e**	101.0**k**	122.3**k**	14.4 **k**	18.0 **k**
Control-2	63.7 **d**	68.5 **e**	4.7 **abc**	5.0 **ad**	361.3**c**	483.0**c**	62.8 **c**	66.4 **c**
***Azotobacter chroococcum* (AC)**	47.1 **h**	51.6 **g**	4.0 **c**	4.3 **cde**	121.3**j**	141.7**j**	17.8 **j**	24.5 **j**
***Streptomyces aureofaciens* (SA)**	42.3 **i**	49.5 **g**	4.7 **abc**	4.0 **de**	127.0**ij**	181.7**i**	20.3 **ij**	31.5 **i**
***Bacillus subtilis*-I (BS)**	51.1 **g**	55.8 **f**	4.0 **c**	4.7 **be**	141.7**i**	236.7**h**	23.3 **i**	39.8 **h**
***Trichoderma harzianum*-I (TH)**	54.5 **f**	59.1 **f**	5.0 **abc**	5.3 **abc**	217.7**g**	311.0**f**	33.1 **g**	47.0 **g**
AC + SA	58.7 **e**	58.6 **f**	4.0 **c**	4.3 **cde**	188.0**h**	276.0**g**	28.0 **h**	42.4 **h**
AC + BS	60.7 **e**	72.5 **d**	4.3 **bc**	5.0 **ad**	254.3**f**	325.7**f**	39.1 **f**	51.4 **f**
AC + TH	68.6 **c**	76.8 **c**	5.7 **a**	5.3 **abc**	295.7**e**	345.7**e**	46.5 **e**	55.4 **e**
AC + SA + BS	70.**7 bc**	80.0 **bc**	5.0 **abc**	5.0 **ad**	316.7**d**	383.7**d**	53.9 **d**	62.4 **d**
AC + SA + TH	72.7 **b**	82.1 **b**	5.3 **ab**	5. 7 **ab**	419.7**b**	523.0**b**	68.6 **b**	76.7 **b**
AC + BS + TH	75.6 **a**	88.3 **a**	5.7 **a**	6.0 **a**	529.0**a**	658.3**a**	80.1 **a**	87.7 **a**

It is worthy to mention that the increase of plant growth could be attributed to the role of both microorganisms present in dual inoculum. These has been increasing evidence that *Azotobacte*r besides its role as N_2 – fixer, it produces phytohormones such as cytokinin, gibbrilic acids, vitamins and P- solubilizer. These factors often affect root development and morphology resulting in greater root surface area that facilitating the absorption o nutrients and minerals. In addition the protection afforded by biocontrol agents of *Trichoderma* and *Bacillus* against pathogens. **Sanhita *et al.* (1995), Aguilar *et al.* (1996)** and **Kennedy *et al.* (2004)** found that inoculation of tomato roots with *A. chroococcum, B. subtilis* and *Ps. fluorescense* significantly increased plant growth parameters and increased the total dry weight.

Also, **Niknejad *et al.* (2000)** and **Tsahouridou and Thanassoulopoulos (2002)** found that using the antagonist *T. harzianum* increased plant growth characters of tomato.

Effect of biofertilization and biological control on yield and yield components of tomato plants.

Data in Table (5) indicated that yield components of tomato plants, i.e. number of fruits, weight of one fruit, fruits yield/plant as well as fruits yield/fed. were the lowest values in untreated soil (control-1).

The fungicide application and chemical fertilization significantly increased yield and yield components of tomato plants.Tomato plants inoculation with *A. chroococcum*, *S. aureofaciens*, *B. subtilis* and *T. harzianum* individually showed remarkable increases in yield and yield components, compared to untreated (control-1).

Dual inoculation significantly increased yield and yield components of tomato plants compared to individual inoculatio.

Tomato inoculation with *A. chroococcum* combined with either *S. aureofaciens* + *B. subtilis*, *S. aureofaciens* + *T. harzianum* or *B. subtilis* + *T. harzianum* gave higher records of yield and yield components in comparison with dual inoculation by these microorganisms.

The highest values of yield and yield components of tomato plants were observed in the treatment of inoculation with *A. chroococcum* in combination with *B. subtilis* + *T. harzianum*. This is likely be due to the synergistic effect between the diazotroph and biocontrol agents.

These results are in agreement with **Martinez *et al.* (1994)** who found that inoculation with *A. chroococcum*, *A. lipoferum* + NPK resulted earlier flowering, increasing of weights average and numbers of tomato fruits and increased yields than those grown with nitrogen application alone in the field trials. The beneficial effects of azotobacters are related not only to their N_2 -fixing proficiency but also due to their ability to produce antibacterial and antifungal compounds, growth regulators and siderophores.

Table (5): Effect of biofertilization and biological control on yield and yield components of tomato.

	Number of fruits/plant		Weight of one fruit (g)		Fruits yield/plant (kg)		Fruits yield (ton/fed)	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Control-1 (untreated)	10.1 **g**	10.5 **h**	94.5 **g**	98.4 **i**	0.96 **i**	1.0 **i**	8.8 **j**	10.7 **i**
Control-2	16.9 **ab**	18.1 **ab**	109.7 **ab**	117.3 **b**	1.85 **ab**	2.1 **ab**	18.4 **a b**	22.4 **ab**
***Azotobacter chroococcum* (AC)**	10.3 **g**	11.0 **gh**	98.3 **f**	104.5 **h**	1.01 **i**	1.2 **hi**	10.1 **i**	11.3 **i**
***Streptomyces aureofaciens* (SA)**	10.9 **g**	12.1 **g**	100.4 **ef**	106.3**gh**	1.09 **hi**	1.3 **h**	10.6 **i**	12.5 **hi**
***Bacillus subtilis*-I (BS)**	11.3 **fg**	13.6 **f**	101.8 **de**	107.4**fg**	1.15 **gh**	1.5 **g**	11.0 **hi**	14.0 **gh**
***Trichoderma harzianum*-I (TH)**	12.4 **def**	15.2 **de**	105.6 **bc**	109.8**ef**	1.31 **ef**	1.7 **ef**	12.7 **fg**	16.1 **ef**
AC + SA	12.2 **ef**	14.0 **ef**	104.3 **cd**	108.7**fg**	1.27 **fg**	1.5 **fg**	12.0 **gh**	14.9 **fg**
AC + BS	12.6 **de**	15.5 **cde**	107.0**abc**	112.1**de**	1.34 **ef**	1.7 **e**	13.6 **f**	17.6 **de**
AC + TH	13.5 **d**	16.1 **cd**	107.0**abc**	111.8**de**	1.44 **de**	1.8 **de**	14.9 **e**	17.3 **de**
AC + SA + BS	14.9 **c**	16.8 **bc**	105.9 **bc**	113.5**cd**	1.57 **cd**	1.9 **cd**	15.9 **d**	19.1 **cd**
AC + SA + TH	15.6 **bc**	17.7 **ab**	107.5 **ab**	115.7**bc**	1.68 **bc**	2.1 **bc**	17.1 **c**	21.0 **bc**
AC + BS + TH	18.1 **a**	18.9 **a**	111..0 **a**	119.7 **a**	1.94 **a**	2.2 **a**	19.8 **a**	23.4 **a**

Also, **Barakat and Gabr (1998)** indicated that the application of N fertilizer combined with inoculation by*Azotobacter* sp, *Azospirillum* sp and *Klebsiella* sp alones as single biofertilizers or together increased number of fruits/ plant and the total yield / fed. of tomato plants. While, **Fang and Zhang (1990) and Niknejad *et al.* (2000)** reported that application of the selected antagonists (*B. subtilis*, *Pseudomonas* sp, *T. harzianum*) significantly increased number of fruits per plant, weight of fruits and total yield of tomato fruits. **Kennedy *et al.* (2004)** found that the application of biofertilizer (*Azospirillum*, *Azotobacter* and Bacillus sp) significantly increased tomato fruits and total yield /fed. compared with control.

Effect of biofertilization and biological control on phenols and amino acid of tomato shoots

Data in Table (6) reveal that untreated soil (control-1) gave the lowest values of total phenols in shoots of tomato plants.

Fungicide application and chemical fertilization remarkably increased the total phenols content in tomato shoots compared to tomato inoculated with *Azotobacter chroococcum* or biocontrol agents either individually or together.

Dual inoculation with *A. chroococcum* and biocontrol agents significantly increased the total phenol content in comparison with individual inoculation.

Table (6): Phenols and total free amino acids (mg / g fresh weight) in tomato plants

	Phenoles mg / g fresh weight							
	Total		Free		Conjugated			
	S1	S2	S1	S2	S1	S2	S1	S2
Control-1 (untreated)	8.53 **l**	9.59 **l**	4.15 **h**	4.65 **i**	4.38 **l**	4.94 **l**	2.59 **a**	2.90 **a**
Control-2	12.52 **b**	14.84 **b**	5.36 **b**	6.34 **b**	7.16 **d**	8.50 **d**	1.59 **i**	1.88 **h**
***Azotobacter chroococcum* (AC)**	9.600 **k**	10.83 **k**	3.18 **i**	3.58 **j**	6.20 **g**	7.25 **g**	2.23 **d**	2.51 **d**
***Streptomyces aureofaciens* (SA)**	9.700 **j**	10.96 **j**	4.58 **e**	5.17 **g**	5.12 **k**	5.79 **k**	2.43 **b**	2.74 **b**
***Bacillus subtilis*-I (BS)**	10.12 **h**	11.59 **h**	2.37 **k**	2.71 **l**	7.75 **c**	8.88 **c**	2.36 **c**	2.69 **c**
***Trichoderma harzianum*-I (TH)**	10.31 **g**	11.88 **g**	4.64 **d**	5.34 **e**	5.67 **i**	6.54 **i**	1.89 **e**	2.17 **e**
AC + SA	9.92 **i**	11.23 **i**	4.54 **f**	5.13 **h**	5.38 **j**	6.10 **j**	1.75 **g**	1.98 **g**
AC + BS	10.38 **f**	12.12 **f**	4.48 **g**	5.22 **f**	5.90 **h**	6.90 **h**	1.81 **f**	2.11 **f**
AC + TH	11.93 **d**	14.07 **d**	5.36 **b**	6.31 **c**	6.57 **f**	7.76 **f**	1.79 **f**	2.11 **f**
AC + SA + BS	11.71 **e**	13.71 **e**	2.50 **j**	2.92 **k**	9.21 **a**	10.79 **a**	1.69 **h**	1.98 **g**
AC + SA + TH	12.25 **c**	14.48 **c**	5.30 **c**	6.25 **d**	6.95 **e**	8.23 **e**	1.47 **j**	1.73 **i**
AC + BS + TH	13.75 **a**	16.36 **a**	5.62 **a**	6.69 **a**	8.13 **b**	9.67 **b**	1.15 **k**	1.37 **j**

The highest records of total phenols content were observed in the treatment of inoculation with *A. chroococcum* and *B. subtilis + T. harzianum.* This result was observed in the two growing seasons.

The total phenol content of tomato shoots was higher during second season than the first one. This may be due to the changes in climatic conditions between the two growing seasons.

These results are in harmony with **Abo-ElIil *et al.* (1998, a)** and **Ibrahim (2000)** who found that positive correlation between level of phenols and root-rot and wilting infection caused by *S. rolfsii*, *R. solani* and *Fusarium* spp in tomato. Many of these compounds exhibit antifungal properties. Therefore, phenols might play an important role in disease resistance.

Concerning the total amino acids content in shoots of tomato, data in Table (6) indicate that untreated soil (control-1) gave the highest values. Fungicide application significantly decreased amino acids content.

Moreover, dual inoculation with *A. chroococcum* and any biocontrol agent significantly decreased total amino acids in tomato shoots. *A. chroococcum* inoculation with either S. *aureofaciens + B. subtilis, S. aureofaciens + T. harzianum* or *B. subtilis + T. harzianum* gave lower values of amino acids than dual inoculation of tomato plants.

The lowest records of amino acids content were observed in the treatment of inoculation with *A. chroococcum* in combination with *B. subtilis + T. harzianum.* This trend of results was obtained in the two growing seasons.

These results are in harmony with **Awad (1990) and Aguilar *et al.* (1996)** who reported that free amino acids play an important role in deciding whether a host could resist a fungus invasion or not. The increasing of free amino acids content was noticed in roots, stems and leaves of the susceptible cultivars, while it was only found

in roots and stems of resistant ones. Inoculation of highly resistant cultivar's by *F. oxysporum f.sp. lycopersici* decreased the total amino acids in roots. Meanwhile, total amino acids increased in stems and leaves. Also, **Khalifa (1991)** and **Ibrahim (2000)** found that total free amino acids content increased in infected tomato plants than in the healthy plants.

Effect of biofertilization and biological control on titratable acidity, vitamin C and total soluble solids.

Data presented in **Table (7)** indicated that untreated soil (control-1) gave the lowest values of titratable acidity (T.S.S.), vitamin C and total soluble solids in tomato fruits.

Dual inoculation with *A. chroococcum* and biocontrol agents significantly increased abovementioned parameters compared to individual inoculation.

Table 7. Effect of biofertilization and biological control on titratable acidity, vitamin C and TSS of tomato fruits.

	Titratable acidity ($mg/100cm^3$)/ juice		Vitamin C ($mg/100cm^3$)/ juice		Total soluble solids (%)	
	S_1	S_2	S_1	S_2	S_1	S_2
Control-1(untreated)	538.4 **l**	542.8 **h**	38.1 **h**	36.1 **j**	4.300 **e**	4.835 **f**
Control-2	591.2 **c**	592.3 **b**	38.8 **gh**	39.3 **i**	4.655 **d**	5.340 **c**
Azotobacter chroococcum	547.0 **j**	554.2 **f**	39.4 **g**	40.5 **h**	4.625 **d**	5.185**cd**
***Streptomyces aureofaciens* (SA)**	578.2 **e**	576.1 **c**	43.2 **e**	41.3 **g**	5.475 **b**	5.645 **b**
***Bacillus subtilis*-I (BS)**	542.0 **k**	549.2 **g**	43.8 **e**	42.9 **e**	4.595 **d**	5.100**de**
***Trichoderma harzianum*-I (TH)**	563.2 **h**	543.8 **h**	47.8 **b**	45.1 **c**	5.030 **c**	5.320 **c**
AC + SA	593.6 **b**	597.2 **a**	39.1 **gh**	43.1 **e**	4.605 **d**	5.100**de**
AC + BS	559.0 **i**	564.4 **d**	42.1 **f**	42.0 **f**	4.625 **d**	5.035**de**
AC + TH	583.7 **d**	591.1 **b**	46.7 **c**	44.2 **d**	4.310 **e**	4.970**ef**
AC + SA + BS	571.1 **f**	576.5 **c**	45.3 **d**	47.1 **a**	4.585 **d**	5.180**cd**
AC + SA + TH	569.3 **g**	558.0 **e**	49.2 **a**	46.0 **b**	4.625 **d**	5.545 **b**
AC + BS + TH	601.7 **a**	598.2 **a**	49.7 **a**	47.1 **a**	5.610 **a**	5.810 **a**

The highest records of titratable acidity, vitamin C and total soluble solids in tomato fruits were observed in the treatment of inoculation with *A. chroococcum* combined with *B. subtilis + T.harzianum*.

These results are in harmony with **George *et al.* (2004)** who found that ascorbic acid change according to maturity of the fruits. Also, they found that fruit chemical contents i.e. vitamin C, titratable acidity and total soluble solids were affected by using biofertilizers, biological control agents and fungicides either

individually or in combination. Antioxidants i.e. vitamin C and titratable acidity were increased in fruits of tomato by biofertilization and decreasing of NPK fertilization level.

Results of the present study provide sufficient evidence to the recommended the use of the mixture of antifungal strains of *Streptomyces aureofaciens*, *Bacillus subtilis* and *Trichoderma harzianum* in combination with dinitrogen fixers of *Azotobacter chroococcum* is successful biocontrol agent against soil borne pathogens of root-rot and wilting diseases of tomato.

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استخدام التسميد الحيوى والمقاومة الحيوية فى إنتاج الطماطم

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-1قسم النبات الزراعى –كلية الزراعة –جامعة بنها

-2معهد بحوث أمراض النبات-مركز البحوث الزراعية

تم إجراء تجربة حقلية بمركز الباجور– محافظة المنوفية خلال موسمى 2003، 2004لدراسة إمكانية استخدام التسميد الحيوى بميكروب *A. chroococcum* والمقاومة الحيوية بميكروبات *T. harzianum, B. subtilis and S. aureofaciens* فى إنتاج الطماطم ولقد أوضحت نتائج هذه الدراسة ما يلى

١. التلقيح المزدوج بميكروبات *A. chroococcum + B. subtilis* أو *A. chroococcum + T. harzianum* أدى إلى نقص معنوى فى نسبة إصابة جذور الطماطم بفطريات أعفان الجذور.

٢. أعلى نشاط لإنزيمى الديهيدروجينيز والنيتروجينيز لوحظ عند تلقيح شتلات الطماطم بميكروب *A. chroococcum* مختلطاً بميكروبى *B. subtilis + T. harzianum*.

٣. أيضاً أوضحت النتائج أن تلقيح شتلات الطماطم بميكروب *A. chroococcum* مختلطاً بميكروبى *B. subtilis + T. harzianum* أدى إلى الحصول على أعلى صفات نمو ومحصول لنباتات الطماطم ، وفى ضوء هذه النتائج يمكن أن يوصى بالتوسع فى استخدام اللقاحات الحيوية سواء المثبتة لأزوت الهواء الجوى أو تلك التى تستخدم فى مقاومة أمراض أعفان الجذور فى الطماطم وذلك لتقليل استخدام الأسمدة الكيماوية والمبيدات الفطرية مما يترتب عليه الحفاظ على صحة الإنسان وحماية البيئة من التلوث.