Egypt. J.Microbiol. **46**, pp.193- 211(2011)

Growth and Yield Responses of Tomato (*Lycopersicon esculentum*) Grown under Soilless Cultivation to Application of *Azospirillum*, *Azotobacter* and Nitrogen

Naziha M. Hassanein[#], U.A. El–Behairy[*], M.H. El–Kattan[**] and Dina A. Shafik[**]

*Microbiology Department, Faculty of Science[*] , [*]Horticulthure Department, Faculty of Agricuture, Ain Shams University and **Central Laboratory for Agricultural Climate, Agricultural Research Center, Cairo,Egypt.*

THIS STUDY was undertaken to investigate the possibility of using *Azospirillum brasilense* and *Azotobacter chroococcum* applied with inorganic nitrogen to enhance tomato (*Lycopersicon esculentum*, Mill) production in soilless systems.The effect of inoculation with *A. brasilense* and / or *Azoto. chroococcum* with the application of 75 % (of the recommended dose) of N_2 on certain tomato growth parameters (shoot height, number of leaves, and fresh and dry weights of plants) after 30, 60 and 90 days of transplanting were determined. In addition, total yield, mean fruit weight and number of fruits per plant were evaluated in plants inoculated with the two bacteria (separately or in combination) with 75 % N_2, and plants inoculated with 75 % N_2 (control 1) and 100 % N_2 (control 2). Plants inoculated with a mixed inoculum of *A. brasilense* and *Azoto. chroococcum* with 75 % N_2 gave the highest values of the growth parameters tested while plants inoculated with 75 % N_2 (control 1) gave the lowest. The same treatment also resulted in the highest tomato yield, mean weight and number of fruits per plant followed by plants treated with *A. brasilense* and 75 % N_2. The responses to the tested biofertilizers on nitrogen, phosphorous and potassium (NPK) uptake, population of diazotrophic bacteria and nitrogenase and dehydrogenase activity of the tested bacteria on the rhizosphere of tomato showed that the mixed inoculum of *A.brasilense* and *Azoto. chroococcum* with 75 % N_2 gave the highest NPK uptake levels after 30, 60 and 90 days of transplanting. The same treatment gave the highest population of diazotrophic bacteria and dehydrogenase and nitrogenase activity of the tested bacteria compared with the plants inoculated with a single organism or controls. In contrast, control 1 gave the least values among all treatments. This study shows that inoculation of tomato plants with *Azospirillum brasilense* and *Azotobacter chroccocum* have a significantly beneficial effect on tomato yield under soilless cultivation.

Keywords: *Azospirillum brasilense*, *Azotobacter chroccocum*, Nitrogen biofertilizer, Inoculation, Tomato, Yield, Dehydrogenase and Nitrogenase enzymes, Soilless.

#Correspondence: Naziha M. Hassanein, Department of Microbiology, Faculty of Science, Ain Shams University, 11566 El-Khalifa El-Mamoun Street, Abbassia, Cairo, Egypt. E-mail: Naziha50@yahoo.com

Tomatoes are considered to be a component of healthy diets as they contribute to well-balanced nutrition, providing minerals (potassium, magnesium, calcium, iron, zinc, etc.), vitamins (A, B_1, B_2, C, E, etc.), dietary fiber (pectin), citric acid, etc. In addition, they also contain in abundance the red pigment, lycopene (Dorais *et al*., 2008).

Soilless culture involves the cultivation of crops in nutrient solutions or media without soil or is the production of crops isolated from the soil either with or without a solid substrate, with their total water and nutrient requirements supplied by the production system. In addition, soilless culture also improves food security and more specifically in small-scale urban horticulture. This is especially so where there is the lack of fertile soils, or space as well as the need to produce safe vegetables to avoid health hazards (Hunger, 1979).

The need for soilless culture is expected to increase rapidly as a result of the current ban on the use of methyl bromide for soil disinfestations. Currently available alternatives to soil fumigation are not considered to be efficient. The limited supply of water resources and organic manures and in addition the rapid increases in population are also the major factors that have drawn attention towards the use of soilless culture world-wide (Baudoin *et al*., 1990).

Plant production in developed and certain developing countries has increased due to the use of high yielding cultivars and enhanced consumption of inorganic fertilizers, especially the nitrogenous ones (Barakat & Gaber, 1998).

The progressive increases in the cost of synthetic fertilizers have prompted the farmers to look for alternative sources of fertilizers which may lower the cost of production while maintaining the fertility status of the soil. Use of microbial inoculants to supplement a part of nitrogen requirements has attained immense importance. Application of *Azospirillum* and *Azotobacter* inoculants especially for vegetable crops has attracted much attention, as they not only fix atmospheric nitrogen but also produce growth promoting and antifungal substances and ultimately increase the total yield per unit area (Sharma, 2002).

Relatively little attention has been paid to the effect of biofertilizers such as the non – symbiotic nitrogen fixing bacteria, on vegetable crops. Saber & Gomma (1993) indicated that application of one third (1/3) the recommended dose of nitrogen, phosphorous and potassium (NPK) rate and inoculation with a mixed biofertilizer containing bacteria of the genera *Azotobacter* and *Azospirillum* together increased plant dry weight, nitrogen and phosphorous uptake of tomato by 42, 19 and 14.5 % over the recommended NPK rate, respectively. Shahaby *et al*. (1993) noticed that inoculation of tomato seedlings with biofetilizes containing diazotrophs *Azospirillum* and *Azotobacter*

significantly increased fruit yield. Also, Omar & El – Kattan (2002) found that biofertilization of vegetables enhanced yield of both sweet pepper and cucumber.

The importance of *Azospirillum* as an active nitrogen fixer was reported by many investigators (*e.g*. Döbereiner & Day, 1976 and El-Tayeb, 2000). There are reports of positive plant responses to *Azotobacter* and /or *Azospirillum* inoculation on plant fresh weight, shoot height, dry weight and protein yield (El - Demerdash *et al*., 1993 and El - Tayeb, 2000).

Mixed biofertilizer application gave better results than dual or single biofertilizer application (Amer *et al.*, 2003). The results of Alfonso *et al*. (2005), Alfonso & Leyva Galan (2006) and Kumar *et al*. (2007) showed that the combined use of inorganic fertilizers and biofertilizers increase the growth, development and yield of tomato plants. Sakthivel *et al.* (2009) also found that the highest fruit yield of tomato plants was recorded when tomato plants were inoculated with the triple combination of *Pseudomonas fluorescens*, *Azotobacter chroococcum* and *Azospirillum brasilense.*

This study was undertaken to investigate the effectiveness of *Azospirillum brasilense* , *Azotobacter chroccocum* inoculation and mineral nitrogen (N_2) singly or in combination on: (i) Growth and yield of tomato, (ii) Nitrogen, phosphorous and potassium (NPK) uptake, (iii) Population of diazotrophic bacteria and (iv) Dehydrogenase and nitrogenase activity of the tested biofertilizers on the rhizosphere of tomato.

Materials and Methods

Organisms and growth conditions

The study was conducted at the experimental site of Central Laboratory for Agricultural Climate (CLAC), Agricultural Research Centre (ARC), Dokki, Giza Governorate, in a plastic house, under soilless conditions, during the season of 2005/ 2006.

Two strains of bacteria were tested, *Azospirillum brasilense* and *Azotobacter chroococcum*, and their combinations. These biofertilizers were obtained from Bio-fertilization Unit, Faculty of Agriculture, Ain Shams University. Tomato (*Lycopersicon esculentum,* Mill.) cv. Castle Rock was used and seedlings were transplanted in the greenhouse in October 2005).

Inoculation technique

Soil application technique was carried out using liquid culture of Döbereiner media (Döbereiner, 1978) for *A. brasilense* at a rate of 20 ml / plant and Ashby media (Abdel-Malek & Ishac, 1968) for *Azoto. chroococcum* (1 ml contains

$16x10^3$ cell / ml for *Azospirillum* and 0.12 x 10^3 cell / ml for *Azotobacter*). This application was repeated three times during the season.

Experimental layout

The experimental design consisted of complete randomized blocks with three replicates for each treatment, where each replicate contained 4 plants. The soilless culture system used plastic pots of 25 cm in diameter (8 L capacity). They were placed in a gully made from black on white polyethylene sheet (200 microns thickness) with a slope 1% for collecting the nutrient solution. The used drainage system was a closed system. Nutrient solution was collected and returned to a catchment tank by polyvinyl chloride (PVC) pipe (1.25 cm in diameter). The nutrient solution was circulated by a submersible pump and delivered to each pot via a dripper. The pots were filled with a mixture of perlite and peat-moss substrates 1:1 v/v which are inorganic substrates (8 L per pot). The physical properties of perlite and peat - moss are illustrated in Tables 1 and 2, respectively.

TABLE 1. Physical properties of perlite .

Particle size	0.5 mm
Bulk density	89.2
% Pore space	90.1
% Air space	32.0
% Easily available water	24.6

TABLE 2. Physical properties of peat – moss .

Apparent density gm / cm^3	0.07
% Total pore space	94.3
% Air content	32.6
% Easily available water	26
% Buffering capacity	6.2
% Non available water	28.9

Nutrient solution

The used nutrient solution was adapted from Cooper solution depending on the analysis of the local water as described by El-Behairy (1994). Concentrations of elements in the nutrient solution are illustrated in Table 3. The nutrient solution contained 75 % of the recommended dose of mineral nitrogen and desired initial concentration of the nutrient solution was maintained by adding tap water to the stock solutions. The solution volume was adjusted twice a week. Electrical conductivity (EC) was maintained between 2.0 - 2.5 m. $mhos^{-1}$ and pH was maintained between 5.5 - 6.5 throughout the period of the experiment. Digital EC meter was used to adjust the EC to the required level. The irrigation system was connected to a digital timer in order to circulate the nutrient solution for 15 min every 2 hr during day time and for 15 min 2 times during night.

TABLE 3. Composition of the nutrient solution .

Element	Concentration (ppm)
Nitrogen	250
Phosphorus	35
Potassium	350
Calcium	180
Magnesium	50
Iron	3
Manganese	1
Copper	0.1
Zinc	0.06
Boron	0.1
Molybdenum	0.01

Preparation of nitrogen fixing inocula and treatments

Cell suspension of *A. brasilense* and *Azoto. chroococcum* was obtained by inoculating separately semi - solid malate medium and modified Ashby's medium, respectively for 15 d at 28± 2°C. Cell suspensions of *Azospirillum and Azotobacter* strains (containing about 10^3 cells ml^{-1}) were used as N_2 - fixers inocula, and five treatments were carried out as follows:

1. Substrate + *A. brasilense* + 75 % N_2
2. ″ + *Azoto. chroococcum* + 75 % N_2
3. ″ + *A. brasilense* + *Azoto. chroococcum* + 75 % N_2
4. ″ + 75 % N_2 (control 1).
5. ″ + 100 % N_2 (control 2).

Measured parameters

Growth parameters, total yield and NPK percentage

Plant height, number of leaves per plant, fresh and dry weights of shoots and roots were recorded after 30, 60 and 90 days of transplanting. Total yield was recorded at the end of cultivation season (3 months).

Nitrogen (N), phosphorus (P), potassium (K) were determined in leaves after 30, 60 and 90 days of transplanting. Samples were dried at 70 Cº for 72 hr according to ADAS / MAFF (1987). Dried leaves were digested in sulphuric acid and hydrogen peroxide according to the method described by Allen (1974). Total nitrogen was determined by Kjeldahl method (FAO, 1980), phosphorus was determined using a spectrophotometer according to Watanabe & Olsen (1965), and potassium was determined photometrically using a flame photometer described by Chapman & Pratt (1961).

Microbiological parameters

The number of A. *brasilense* and *Azoto. chroococuum* were determined by the most probable number technique (MPN) using Döbereiner medium and Ashby medium after incubation for 2 weeks at 37^{o}C, respectively.

Dehydrogenase activity

Dehydrogenase activity in the rhizosphere region was assayed according to Thalmann (1967). Two grams of substrate were transferred to a 20 ml test tube and 2 ml of 0.5 % 2,3,5-triphenyl tetrazolium chloride (TTC) dissolved in 0.1 M tris buffer were added. The tris buffer solution was prepared by dissolving 12.114 g of hydroxyl methyl aminomethane in 313 ml HCl 0.2 N and made up to one liter, its pH was 7.8. This volume of TTC solution was sufficient to cover the soil surface. Tubes were stoppered and incubated at 30 ^{o}C in a dark place for 24 hr. Afterwards, 10 ml pure acetone was added, and tubes were thoroughly shaken and left in the dark for 2 hr with intermittent mixing for the extraction of 2,3,5-triphenyl formazan (TPF). After filtration through Whatman No. 1 filter paper, the color density of the filterate was measured at 485 nm using a spectro-colourmeter (Spectronic 20 D). The concentration of formazan was calculated and presented in µg TPF / g dry substrate / day using a conversion factor obtained from a standard curve. Analysis in duplicator and blank determinations for the chemicals used were carried out.

Nitrogenase activity

In liquid culture

A. brasilense and *Azoto. chroococuum* were activated in Döbereiner medium and Ashby medium, respectively and incubated for 2 weeks at 37^{o}C. One ml of the activated culture was transferred to a test tube filled with 5 ml Mdlica (ML) medium and incubated at 37^{o}C for 48 hr and the cotton plugs were then replaced by rubber stopper. A suitable volume of acetylene was then injected into the tubes using plastic syringe to give acetylene concentration equals to 10 % of the gas phase in the tubes and incubated at 37^{o}C. After 48 hr incubation, 0.1 ml samples were withdrawn from the gas phase of the tubes and injected in gas chromatography (PYE unicam). The temperature of the column was 100^{o}C and nitrogen was used as a carrier gas at a flux rate of 120 ml / min (Mdlica *et al.*, 1985). The amount of produced ethylene was calculated according to the equation:

$$\mu \text{ Mole } C_2H_4 \text{ / ml / hr} = \frac{\text{GLC read x } 10^3 \text{ x volume of air}}{\text{Volume of media x 22.4 x incubation period (48 hr)}}$$

In substrate samples

Nitrogenase activity of substrate samples was determined after 30, 60 and 90 days after cultivation by acetylene reduction method ($C_2H_2 \rightarrow C_2H_4$) according to Dart *et al.* (1972).

Preparation of samples

To determine the potential N_2 - ase (C_2H_2) activity, 100 gm of substrate samples were put into 420 ml serum bottles, then amended with 3 ml of 33 % freshly prepared glucose solution to give a final concentration of 1 % glucose in the substrate, and mixed thoroughly. Moisture content of the substrate mixture was also determined.

Assessment technique

Bottles containing substrate were sealed with tide rubber stopper fitted with a silicon rubber seal. A suitable volume of acetylene was then injected into the bottles using plastic syringe to let acetylene concentration equals to 10 % of the gas phase of the bottles. The bottles were incubated at $30^{O}C$ for 24 hr. After incubation , 2 ml gas samples were withdrawn and assayed for ethylene concentration using a PYE unicam gas liquid chromatography fitted with dual flame ionization detectors and a stainless steel column (150 cm long) fitted with 10 % $Na_3 PO_4$ on Spherosil XOB 075, 100 - 120 mesh (prepared by Perkmen Elmer Company Bodensee werke. F. R. germany), using nitrogen as a carrier gas at a flow rate of 30 ml / min, hydrogen and air for flame ionization detector were used at rates of 30 ml and 300 ml / min, respectively. The detector temperature was $100^{o}C$, where the column and injector port temperatures were $35^{o}C$ and $60^{o}C$ respectively. The retention times of ethylene and acetylene were 4 and 5 min, respectively.

The standard curve of the pure ethylene was made by injecting 1200 µL C_2H_4 into a 1200 ml serum bottle (concentration 100 ppm), of which serial dilutions were made. Out of each dilution 2 ml gas samples were withdrawn and injected into the gas chromatography. A linear relation between ethylene concentration and ethylene peak heights was obtained. To calculate the amount of ethylene detected in the gas samples, the peak heights were measured in cm and converted to the equivalent µL C_2H_4 using the standard's factors. The µL C_2H_4 values of the samples were then converted to µmole C_2H_4 by dividing through the volume of the molecular weight of gases (22.4). The results were then presented as µL C_2H_4 / weight / hr. A conversion factor of 3 was used to obtain the equivalent values of N-fixed. Three replicates were run for each treatment of which 3 injections were made and the average values of the nine injections were calculated.

Results

Effect of biofertilizers and inorganic nitrogen fertilization on certain growth parameters of tomato plants

The effect of biofertilization with *A. brasilense* and / or *Azoto. chroococcum* with 75 % N_2 on plant growth parameters (shoot height (cm), number of leaves / plant, and fresh and dry weights of shoot and root (g), after 30, 60 and 90 days of transplanting were determined. Also, total yield / plant (kg), mean fruit weight (g / plant) and number of fruits / plant were measured in plants inoculated with

biofertilizers (separately or in combination) with 75 % N_2 , and plants inoculated with 75 % N_2 (control 1) and 100 % N_2 (control 2) without bacterial inoculation.

Data recorded in Table 4 indicated that plants inoculated with a mixed inoculum of *Azospirillum* and *Azotobacter* and 75 % N_2 gave the highest shoot heights values and measured 80.6, 89.3 and 93.3 cm after 30, 60 and 90 days of transplanting, respectively, followed by plants inoculated with *Azospirillum* and 75 % N_2 and measured 76.3, 79.6 and 82.3 cm after the same intervals, respectively. Results also showed that plants treated only with 75 % N_2 (control 1) gave the lowest values.

TABLE 4. Effect of biofertilizers and inorganic nitrogen fertilization on certain growth parameters of tomato plants.

Treatment	Days after transplanting											
	30 days				60 days				90 days			
	SH	NL	FW	DW	SH	NL	FW	DW	SH	NL	FW	DW
A. + 75 % N_2	76.3	36.0	501.0	94.2	79.6	64.0	522.6	139.6	82.3	87.0	842.3	205.7
Azoto. + 75 % N_2	71.0	35.0	402.0	82.4	77.0	63.0	438.5	131.5	78.0.	82.0	789.3	196.7
A. + *Azoto.* + 75 % N_2	80.6	42.0	522.7	94.7	89.3	69.0	540.5	145.6	93.3	112.0	900.5	215.8
75 % N_2 (control 1)	69.0	32.6	391.7	67.5	70.6	46.6	395.8	113.9	71.0	52.3	624.7	174.4
100 % N_2 (control 2)	70.6	33.6	395.2	77.6	74.0	49.0	398.6	126.9	76.0	61.3	763.0	178.4
LSD at 5 %	8.6	5.6	67.3	15.2	11.9	18.9	73.0	21.2	2.0	41.4	137.0	33.6

Note: SH = Shoot height (cm).
NL = Number of leaves / plant.
FW = Fresh weight of shoot and root (g).
DW = Dry weight of shoot and root (g)

Table 4 also shows that plants inoculated with the mixed inoculum of *Azospirillum* and *Azotobacter* with 75 % N_2 gave the highest numbers of leaves / plant after 30, 60 and 90 days of transplanting, with values 42.0, 69.0 and 112.0, respectively followed by the plants inoculated with *Azospirillum* with 75 % N_2 and gave values of 36.0, 64.0 and 87.0 after the same periods of transplanting, respectively. Plants treated with 100 % N_2 (control 2) gave less values and the lowest values among all treatments were recorded with plants with 75 % N_2 (control 1). It is obviously clear that the bacterial inoculation (separately or in combination) gave significant results compared to the controls.

Inoculation with a mixed inoculum of *Azospirillum* and *Azotobacter* with 75 % N_2 progressively stimulated the plant fresh weight to reach its maximum value (900.5 g / plant) after 90 days of transplanting, followed by plants inoculated

with *Azospirillum* and 75 % N_2 (842.3 g / plant) while plants treated with 75 % N_2 (control 1) gave the lowest value (624.7 g / plant). In contrast, the mixed inoculum of *Azospirillum* and *Azotobacter* with 75 % N_2 gave the highest dry weight values and measured 94.7, 145.6 and 215.8 g / plant, after 30, 60 and 90 days of transplanting, respectively, followed by inoculation with *Azospirillum* and 75 % N_2 , and gave 94.2, 139.6 and 205.7 g / plant after the same intervals, respectively. On the other hand, plants treated only with 75 % N_2 (control 1), gave the lowest dry weight values (Table 4).

Effect of biofertilizers and inorganic nitrogen fertilization on total yield, mean fruit weight and number of fruits of tomato plants

Data in Table 5 showed that plants inoculated with the mixed culture of *Azospirillum* and *Azotobacter* with 75 % N_2 gave the highest tomato yield per plant (11.8 kg), mean fruit weight per plant (982.4 g) and 15 fruits per plant, followed by the plants inoculated with *Azospirillum* with 75 % N_2, while plants treated with 75 % N_2 (control 1) gave the lowest values (4.6 kg, 390.6 g and 5 fruits, respectively).

TABLE 5. Effect of biofertilizers and inorganic nitrogen fertilization on total yield, mean fruit weight and number of fruits of tomato plants.

Treatment	Total yield (kg / treatment)	Mean fresh fruit weight (gm / plant)	No. of fruits/ plant
A. + 75 % N_2	11.5	962.9	14
Azoto. + 75 % N_2	8.8	733.4	11
A. + *Azoto.*+ 75 % N_2	11.8	982.4	15
75 % N_2 (control 1)	4.6	390.6	5
100 % N_2 (control 2)	7.9	658.9	9
LSD at 5 %	2.1	181.1	1

Effect of biofertilizers and inorganic nitrogen fertilization on nitrogen, phosphorous and potassium (NPK) uptake of tomato plants

Data in Table 6 indicates that plants inoculated with a mixed inoculum of *Azospirillum* and *Azotobacter* with 75 % N_2 gave the highest levels of (NPK) uptake. For N, the percentages were 1.6 %, 1.1 % and 1.0 %; for P, the percentages were 1.3 %, 0.7 % and 0.8 % and for K the percentages were 4.5 %, 2.6 % and 2.4 % after 30, 60 and 90 days of transplanting, respectively, followed by plants inoculated with *Azospirillum* with 75 % N_2, and gave percentages of 1.4 %, 0.8 %, and 1.0 % for N; 1.0 %, 0.7 %, and 0.8 % for P, and 4.4 %, 2.2 % and 2.4 % for K after the same intervals of transplanting, respectively. On the

other hand, plants inoculated with 75 % N_2 (control 1) and 100 % N_2 (control 2) gave the lowest percentages of NPK uptake.

TABLE 6. Effect of biofertilizers and inorganic nitrogen fertilization on nitrogen, phosphorous and potassium (NPK) uptake of tomato plants.

Treatment	Days after transplanting								
	N %			P %			K%		
	30 days	60 days	90 days	30 days	60 days	90 days	30 days	60 days	90 days
A.+ 75 % N_2	1.4	0.8	1.0	1.0	0.7	0.8	4.4	2.2	2.4
Azoto. + 75 % N_2	1.3	0.9	0.8	0.8	0.7	0.5	3.4	2.3	2.0
A.+ *Azoto.*+ 75 % N_2	1.6	1.1	1.0	1.3	0.7	0.8	4.5	2.6	2.4
75 % N_2 (control 1)	1.0	0.8	0.7	0.4	0.5	0.4	4.1	2.2	1.6
100 % N_2 (control 2)	1.0	0.9	0.7	0.4	0.5	0.5	3.2	1.5	1.8
LSD at 5 %	0.1	0.1	0.1	0.4	0.1	0.2	0.1	0.3	0.5

Effect of biofertilizers and inorganic nitrogen fertilization on the population of diazotrophic bacteria in the rhizosphere of tomato plants

Data in Table 7 obviously indicated that the population of *A. brasilense* and *Azoto. chroococcum* increased in the rhizosphere of plants inoculated with biofertilizers compared with the non - inoculated ones. The rhizosphere of plants inoculated with the mixed inoculum of *Azospirillum* and *Azotobacter* with 75 % N_2 recorded the highest population of diazotrophic bacteria. The numbers increased considerably after 0, 30 and 60 days of transplanting, followed by a sharp drop. It contained 5.2 , 5.7 , 6.2 and 5.9 cfu / g dry rhizosphere substrate for *Azospirillum* after 0, 30, 60 and 90 days of inoculation , respectively. It also, contained 3.5, 5.5, 5.6 and 4.9 cfu / g dry rhizosphere substrate for *Azotobacter* after the same intervals of inoculation, respectively.

Effect of biofertilizers and inorganic nitrogen fertilization on the dehydrogenase and nitrogenase activity of the tested bacteria

The dehydrogenase activity in rhizosphere substrate was estimated as an indication of the respiratory activity of roots and substrate microorganisms. Data in Table 8 showed that the enzymatic activities in the inoculated treatments were higher than the non-inoculated controls. This effect was reflected on tomato yield.

TABLE 7. Effect of biofertilizers and inorganic nitrogen fertilization on the population of diazotrophic bacteria in the rhizosphere of tomato plants.

Treatment	Days after transplanting							
	0 days		30 days		60 days		90 days	
	TC		TC		TC		TC	
	A.	Azoto.	A.	Azoto.	A.	Azoto.	A.	Azoto.
A.+ 75 % N_2	5.2	0	5.9	0	6.7	0	6	0
Azoto. + 75 % N_2	0	4.7	0	5.6	0	6	0	5.8
Azoto. + *A.*+ 75 % N_2	5.2	3.5	5.7	5.5	6.2	5.6	5.9	4.9
75% N_2 (control 1)	0	0	0	0	0	0	0	0
100 % N_2 (control 2)	0	0	0	0	0	0	0	0

Note: TC = Total count of diazotrophic bacteria (Log N^o g^{-1} rhizosphere soil).

TABLE 8.Effect of biofertilizers and inorganic nitrogen fertilization on enzymatic activities of the tested bacteria in the rhizosphere of tomato plants.

Treatment	Days after transplanting					
	30 days		60 days		90 days	
	Dehydro-genase	Nitro-genase	Dehydro-genase	Nitro-genase	Dehydro-genase	Nitro-genase
A.+ 75 % N_2	129.4	20.3	173.0	188.1	164.6	155.0
Azoto. + 75 % N_2	158.0	15.3	179.4	100.0	127.2	99.0
A. + *Azoto.* + 75 % N_2	189.5	210.0	292.6	192.0	199.0	160.0
75 % N_2 (control 1)	10.8	0	21.3	0	22.4	0
100 % N_2 (control 2)	15.1	0	26.5	0	28.7	0

Results showed that plants inoculated with *Azospirillum* and *Azotobacter* with 75 % N_2 gave the highest respiratory activity than the ability of each bacterium individually, and gave values of 189.5, 292.6 and 199.0 µg TPF / g dry substrate / day, after 30, 60 and 90 days of transplanting, respectively, and this ability affect the tomato yield significantly. The corresponding value of the nitrogenase activities, which is responsible for the nitrogen fixation was also higher in the inoculated treatments than the non - inoculated controls and gave values of 210 , 192 and 160 nmole C_2 H_4 / g dry substrate / hr after the same intervals of transplanting, respectively (Table 8).

Discussion

Great effort was made to get the benefits of the biological activities in soil, particularly, those concerned with nitrogen fixation. Previous reports have shown that inoculation with associative N_2 - fixers have the potential to improve plant growth and increase yield of vegetable crops not only due to their high N_2 - fixation activity, but also by the ability of these bacteria to produce antibiotics, growth promoting substances (phytohormones), siderophores and the ability to solubilize phosphate (Sudhakar *et al.,* 2000 and Joo *et al.,* 2004). Such beneficial associations have been reported for species of *Azospirillum* and *Azotobacter.* (Hernandez & Chailloux, 2004 and Satesh Kumar & Sharma, 2007).

Results of this study indicated that inoculation of tomato crop with the associative diazotrophs *A. brasilense* and *Azoto. chroococcum* or their mixtures increased plant growth, N - content and yield of tomato cultivars over the uninoculated plants. These increases in plant growth and yield of tomato crop might be attributed to available nitrogen supplemented by the inoculated organisms due to their high N_2 – fixing ability and probable production of plant growth promoting substances.

These results are in accordance with those of Pandey & Kumar (1989) who concluded that inoculation of *Azotobacter* without application of nitrogen, phosphorus and potassium can increase tomato yield per unit area. Also, Panwar & Ompal (2000) and Siddiqui & Mahmoud (2003) found that inoculation of tomato with *Azotobacter chroococcum* improved tomato growth.

Amer *et al.* (2003) reported that bio - fertilizer application significantly improved the vegetative growth, total fruit yield, flesh thickness, total soluble solids and vitamin C contents of the tomato. Mixed bio -fertilizer application gave better results than application of single organisms. In their work the combined application of mineral fertilizers and bio-fertilizers further increased the vegetative growth, total fruit yield and fruit quality.

Results of the current study showed that plants inoculated with the bio - fertilizers individually or in combination significantly gave higher values of mineral uptake. Plants inoculated with the mixed inoculum gave better responses than plants inoculated with each strain of bacteria applied singly. The beneficial effect of bacterial inoculation on N content may be attributed to the growth promoting effect of the free living N_2 – fixers used.

These results concur with those obtained by Shabaev *et al*. (1990) and Saad *et al.* (1999) who observed that plants inoculated with *Azospirillum* produced similar or higher root yield, vigorous vegetative growth, and higher N content in the roots and leaves than plants given the recommended rate of N fertilizer.

The positive effect of inoculating non - legumes with *A. brasilense* and *Azoto. chroococcum* at low rates of mineral N-fertilization on crop yield has been reported by Martinez - Toledo *et al.* (1988).

Barakat & Gaber (1998) reported that inoculation of tomato plants with single or mixed bio - fertilizer of *Azospirillum* sp.,and *Azotobacter* sp.,in addition to N applied at a rate of up to 100 kg N fed^{-1} resulted in higher N contents. Shalaby (2001) found that nitrogen and phosphorus contents of shoots of tomato plants inoculated with a mixture of *Glomus mosseae* and *Azospirillum lipoferum* were far greater than those of shoots of plants inoculated with each microorganism individually.

The results in the present study revealed that bacterial inoculation significantly increased total diazotrophic bacterial count compared with the controls. This increase may be related to the increase in the total counts of the other microorganisms which inhabit the rhizosphere. These rhizosphere microorganisms may help in the release of nutrients rendering them available to be taken up by the plant resulting in increases in the growth and yield of the plant. This observation is in agreement with those obtained by Swedrzyńska & Sawicka (2001) and Verma *et al.* (2001) who found that inoculation of wheat and maize plants with *Azospirillum* and *Azotobacter* increased total bacterial count and activities. Also, Shalaby (2001) recorded that coupling organisms, *Glomus mosseae* and *Azospirillum lipoferum* significantly increased bacterial, actinomycete and *Azospirillum* counts in the rhizosphere of tomato plants.

Alfonso *et al.* (2005) proposed that inoculation of tomato plants with *Azospirillum* and *Azotobact*er caused a positive effect on seedling growth as well as on plant nutritional status, the yield being higher compared to the control plants. A high microbial population level was recorded in the rhizosphere of the inoculated plants.

Alfonso & Leyva Galan (2006) stated that inoculation of *A. brasilense* under different nitrogen dosages greatly influence growth, development, yield and colonization in tomato. Also, results of Raut *et al.* (2006) showed that soil microbial count was higher after harvest of tomato crop as compared to initial soil samples and related this to the treatment of tomato plant rhizosphere with 30 tonnes farm yard manure + 5 kg *Azospirillum* and not to the activity of the root exudates of the plants.

El- Shanshoury (2008) found that single or dual inoculation of wheat seedlings with, *A. brasilense*, *Azoto. chroococcum* or *Streptomyces mutabilis* in sterilized soil resulted in significant stimulation of their population in the rhizosphere, compared with the initial values at inoculation.

Inoculation of wheat plant with a mixture of *Azoto. chroococcum*, *Bacillus megatherium*, *Pseudomonas fluorescence*, *Streptomyces fulvissimus*, *Aspergillus*

candidus, *Lactobacillus lactis* and *Sacchromyces cervesiae* significantly increased total microbial counts, CO_2 evolution, *Azotobacter*, phosphate dissolving bacteria (PDB), fungi and actinomycete counts. Wheat growth criteria (shoot height, root length, shoot fresh and dry weights, root fresh and dry weights, chlorophyll content, number of leaves), yield parameters, mineral content (NPK) of wheat in rhizosphere and in plant were increased by inoculation (Abd El-Ghany *et al.,* 2010).

The bacterial inoculation in our study had a positive effect on the dehydrogenase activity of *A.brasilense* and *A.chroococcum*, when compared with the control. Dehydrogenase activity results in the release of carbon dioxide in the rhizosphere, which causes the formation of carbonic acid leading to a decrease in pH value. This process increases nutrient availability and uptake in the rhizosphere. This, in turn, supports higher plant growth and crop yield (Omar & Ismail, 2002).

A. brasilense and *Azoto. chroococcum* are also well known for their ability to fix the atmospheric nitrogen via the activity of the nitrogenase enzyme. The results of this study revealed that the inoculation of tomato plants with these bacterial strains increased the nitrogenase activity in the rhizosphere of the inoculated plants when compared with the non-inoculated ones. The nitrogenase activity may be attributed to the effect of exudation of carbon compounds that have special importance in the growth of nitrogen fixing microorganisms. These bacteria produce growth regulating substances mainly indole acetic acid, gibberellins and cytokine-like substance, which may improve plant productivity by hormonal stimulation besides nitrogen fixation (Tien *et al.,* 1979). The beneficial effect of *Azotobacter* and *Azospirillum* are related not only to their nitrogen fixing proficiency but also to their ability to produce antibacterial and antifungal compounds, growth regulators and siderophores (Pandey & Kumar 1989).

Döbereiner & Day (1976) found that both higher plant growth and yield which were observed in *Azospirillum* - inoculated subtropical grasses were primarily attributable to the biological nitrogen fixation exerted by the bacteria. The process is performed by a nitrogenase complex, and occurs when the availability of N compounds and oxygen tension are low (Steenhoudt & Van der Leyden, 2000). Also, Shalaby (2001) proposed that coupling of *Glomus mosseae* and *Azospirillum lipoferum* significantly increased nitrogenase activity in the rhizosphere of the tomato plants.

Finally, the results of the present results match those of Abou-Aly (2005) who found that inoculation of tomato plants with *Azospirillum* alone or with a phosphate dissolver enhanced activities of dehydrogenase and nitrogenase enzymes, in comparison to the control.

References

Abd El-Ghany, B. F., Arafa, R. A. A. M., El - Rahmany, T. A. and El-Shazly, M. M. (2010) Effect of some soil microorganisms on soil properties and wheat production under North Sinai conditions. *Journal of Applied Sciences Research*, **4** (5), 559 - 579.

Abd El - Malek, Y. and Ishac, Y. Z. (1968) Evaluation of methods used in counting *Azotobacter*. *Journal of Appllied Bacteriology*, **31**, 26.

Abou - Aly, H. E. (2005) Stimulatory effect of some yeast applications on response of tomato plants to inoculation with biofertilizers. *Annals of Agricultural Science, Moshtohor*, **43**, 2, 595 - 609.

ADAS / MAFF (1987) "*The Analysis of Agricultural Material Book*", 3*rd* Eedition HMSO. London. 427 p.

Alfonso, E. T. and Leyva Galan, A. (2006) Agrobiological evaluation of coinoculation mycorrhiza - rhizobacteria in tomato. *Agronomia Costarricense*, **30**, 1, 65-73.

Alfonso, E. T., Leyva, A. and Hernandez, A. (2005) Beneficial microorganisms as efficient biofertilisers for tomato crops *Lycopersicon esculentum*, Mill). *Revista Colombiana de Biotecnologia*, **7**, (2) 47 - 54.

Allen, S.E. (1974) "*Chemical Analysis of Ecological Materials*". Black-well, Oxford, 565 p.

Amer, A. H., El-Shemi, I. Z. and Zayed, G. A. (2003) Response of tomato plants grown in newly reclaimed sandy soils to bio - and mineral fertilization. *Annals of Agricultural Science, Moshtohor*, **41** (2), 925 -938.

Barakat, M. A. S. and Gaber, S.M. (1998) Effect of different biofertilizer types and nitrogen fertilizer levels on tomato plants. *Alexandria Journal of Agricultural Reserches*, **43** (1), 149 - 160.

Baudoin, W. O., Winsor, G. W. and Schwarz, M. (1990) Soilless culture for horticulture crop production. Food and Agriculture Organization of the United Nation Roma (FAO). Lands man's books hop ltd Buckenttil; Bromyard and Herefords. Technical Paper : p 188**.**

Chapman, H. D. and Pratt, **P. F**. **(1961)** "*Methods of Analysis for Plant and Water*". Calif., Univ. USA.

Dart, P. J., Day, J. M. and Harris, D. (1972) Assay of nitrogenase by acetylene reduction. *From Technical Report* No. 149 from FAO / IAEA. Use of Isotopes for Study of Fertilizer Utilization by legume Crops. pp. 85-100.

Döbereiner, J. (1978) Influence of environmental factors on the occurrence of *Azospirillum lipoferum* in soil and roots. *Ecol. Bull Stokholm*, **26**, 342 - 352.

Döbereiner, J. and Day, J. M. (1976) Associative symbioses in tropical grasses In: Characterization of microorganisms and dinitrogen fixing sites. Newton, W. E. and Nyman, C.J. (Ed.). *Proc. of the First Inter. Symp. on Nitrogen Fixaton* .Vol. 2. Washington State. Univ. Press. Pullman, pp. 301 - 333.

Dorais, M., Ehret, D. L. and Papadopoulos, A. P. (2008) Tomato (*Solanum lycopersicum*) health components: from the seed to the consumer. *Phytochemistry Reviews*, **7** (2), 231-250.

El-Behairy, U.A. (1994) The effect of levels of phosphorus and zinc in the nutrient solution on macro and micronutrients uptake and translocation in cucumber (*Cucumus sativus* L.) grown by the nutrient film technique. *Ph. D. Thesis*, London University.

El-Demerdash, M. E., Gamal, Rawia F., Mashhoor, W. A. and Nasr, Soher A. (1993) Response of wheat plants to inoculation with high or low PHB cells of associative N_2 - fixers. *Proc. VIII. Conf. Microbiol*. Cairo, Egypt, pp. 67 - 78.

El-Shanshoury, A. R. (2008) Interactions of *Azotobacter chroococcum*, *Azospirillum brasilense* and *Streptomyces mutabilis*, in relation to their effect on wheat development. *Journal of Agronomy and Crop Science*, **175** (2), 119 - 127.

El - Tayeb, M. A. (2000) Physiological studies on halotoleant *Azospirillum* in improving cereals productivity under desert soil conditions. *Ph. D. Thesis*, Fac. Agic., Ain Shams Univ.

FAO (1980) Soil and plant analysis. *Soils Bulletin*, **38** (2), 250.

Hernandez, M. I. and Chailloux, M. (2004) Arbuscular mycorrhizas and rhizobacteria as an alternative to mineral nutrition of tomato. *Cultivos Tropicales*, **25** (2), 5 - 12.

Hunger, B. C. (1979) Hydroponic fundamentals. In Commercial applications of hydroponics'. *Agriculture Note Series No. 12*, Dept. of Agriculture, Victoria, Australia. Paper No. 1, 4 pp.

Joo, G. J. , Kim, Y. M., Lee, I. J. and Rhee, I. K. (2004) Growth promotion of red pepper plug seedlings and the production of gibberellins by *Bacillus cereus*, *Bacillus macroides* and *Bacillus pumilus*. *Biotechnology Letters*, **26**, 487 - 491.

Kumar, A., Kumar, J. and Ram, B. (2007) Effect of inorganic and bio-fertilizers on growth, yield and quality of tomato (*Lycopersicon esculentum* Mill.). *Indian. J. Agric*. **77** (5).

Martinez - Toledo, M. V., de la Rubia, T., Moreno, J. and Gonzalez- Lopez, J. (1988) Root exudates of *Zea mays* and production of auxins, gibberellins and cytokinins by *Azotobacter chroococcum*. *Plant and Soil*, **110**, 149 - 152.

Mdlica, M. L., Elsas, V., Elisa, J. D. and Penido, G. (1985) An improved method to detect acetylene reducing activity in *Bacillus* strains. *J. Microbiol. Meth*. **3**, 147 - 157.

Omar, M. N. A. and El - Kattan, M. H. (2002) Utilization of microbial inoculants for the enhancement of some vegetables yield under protected agriculture system. *Acta Horticulturae No. 608*. *Proceedings of the International Symposium on the Horizons of Using Organic Matter and Substrates in Horticulture*. pp. 133 - 139.

Omar, M. N. A. and Ismail, H. (2002) Complementation of diazotrophs and yeast as plant growth promoting agents for wheat plants. *Egypt. J. Agric. Res*. **80** (1), 29 - 40.

Pandey, A. and Kumar, S. (1989) Potential of azotobacters and azospirilla as biofertilizers for upland agriculture. *J. Sci. Indust. Res*. **48** (3) , 134 - 144.

Panwar, J. D. S. and Ompal, S. (2000) Response of *Azospirillum* and *Bacillus* on growth and yield of wheat under field conditions. *Indian. J. Plant Physiology*, **5** (1), 108 - 110.

Raut, R. L., Rawat, A. K. and Baghel, S. S. (2006) Soil microbial population and tomato yield as influenced by plant nutrient sources. *International Journal of Agricultural Sciences*, **2** (1), 42 - 43.

Saad, M. S., Ali Sabuddin, A. S., Yunus, A. G. and Shamsuddin, Z. H. (1999) Effects of *Azospirillum* inoculation on sweet potato grown on sandy tin-tailing soil. *Commun. Soil Sci. Plant Anal*. **30** (11&12), 1583 - 1592.

Saber, M. S. M. and Gomma, A. M. H. (1993) Associative action of a multi-strain biofertilizer on tomato plants grown in a newly reclaimed soil. 6th *Inter. Symp. on Nitrogen Fixation with non – legumes*, Ismailia, Egypt, 6–10 Sept. pp, 495 – 497.

Sakthivel, U., Mahalakshmi, S. and Karthikeyan, B. (2009) Studies on isolation and characterization and its effect of seed inoculation of PGPR. *Journal of Pathology*, **1**(1), 33 - 39.

Satesh Kumar and Sharma, S. K. (2007) Effect of integrated nutrient management strategies in tomato production. *Indian Journal of Horticulture*, **64** (1), 96 - 97.

Shabaev, V. P., Smolin, V. Yu., Burlutskaya, G. R. and Umarov, N. M. (1990) Nitrogen balance in a soil cropped with non-leguminous plants inoculated with nitrogen - fixing microorganisms. *Agrokemia-es-Talajan*, **39**, 377-381. *Proceeding of the Tenth International Symbosium on Soil Biology*, Keszthely, Hungary, 27 - 31 August, 1989.

Shahaby, A. F., Amin, G. and Khalafalla, G. M. (1993) Response of rice and tomato seedlings to inoculation with diazotrophs and their culture filtrates. 6th *Inter. Symp. on Nitrogen Fixation with Non – legumes*, Ismailia, Egypt, 6–10 Sept. pp. 375 – 376.

Shalaby, A. M. (2001) Response of rhizospheric microflora and growth of tomato plants to the interaction between arbuscular mycorrhizal fungus *Glomus mosseae* and *Azospirillum lipoferum Egyptian Journal of Microbiology*, **36** (4) , 363 - 376.

Sharma, S. K. (2002) Effect of *Azospirillum*, *Azotobacter* and nitrogen on growth and yield of cabbage (*Brassica oleracea* var *capitata). Indian Journal of Agricultural Sciences*, **72** (9), 555 - 7.

Siddiqui, Z. A. and Mahmoud, I. (2003) Effect of plant straws and plant growth promoting bacteria on the reproduction of *Meloidogyne incognita* and growth of tomato. *Biological Agriculture & Horticulture*, **21**(1), 53 - 62.

Steenhoudt, O. and Van der Leyden, J. (2000) *Azospirillum* a free - living nitrogen fixing bacterium closely associated with gasses genetic, biochemical and ecological aspects. *FEMS Microbiology Reviews*, **24**, 487 – 506.

Sudhakar, P., Chattopadhyay, G. N., Gangwar, S. K. and Ghosh, J. K. (2000) Effect of *Azotobacter* biofertilizer with inorganic nitrogen on leaf yield and quality of mulberry (*Morus alba*). *Tropical Sci*. **40** (2), 75 - 82.

Swedrzyńska, D. and Sawicka, A. (2001) Effect of inoculation on population numbers of *Azospirillum* bacteria under winter wheat, oat and maize. *Polish Journal of Environmental Studies*, **10** (1), 21 - 25.

Tien, T. M., Gaskins, M. H. and Hubbel, D. H. (1979) Plant growth substances by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.) *Applied Environmental Microbiology*, **37**, 1016-1024.

Thalmann, A. (1967) Uberdie microbiello Aktivitat undihr Beziehung Zu Fruchtbartkeits merkmalen einiger Acherboden uter besonderer Berucksi chtigung der Dehydrogenase akativitat (TTC reduction). Biss. Gieben. *Ph. D. Thesis*.w. Germany.

Verma, S., Kumar, V., Narula, N. and Merbach, W. (2001) Studies on *in vitro* production of antimicrobial substances by *Azotobacter chroococcum* isolates / mutants. *Zetschrift – Fur –Pflanzenkrankheiten –und - Pflanzenscutz*, **108** (2), 152 - 165.

Watanabe, F. S. and Olsen, S. R. (1965) Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. *Soil Sci. Soc. Amer. Proc.* **29,** 677 - 678.

(*Received* 27/1/2011;
accepted 20/4/2011)

إستجابة نمو ومحصول نبات الطماطم المزروع تحت ظروف الزراعة بدون تربة للمعالجة ببكتريا *Azotobater, Azospirillum* والنيتروجين

نزيهه محمد حسنين ، اسامة أحمد البحيرى *، مصطفى حمدى القطان و دينا عادل شفيق**
قسم الميكروبيولوجى – كلية العلوم ،*قسم البساتين – كلية الزراعة – جامعة عين شمس و ** المعمل المركزى للمناخ الزراعى – مركز البحوث الزراعية – القاهرة– مصر .

أجريت هذه الدراسة لمعرفة إمكانية أستخدام بكتريا *الأزوسبيريلم برازيلنس* و بكتريا *الأزوتوبكتر كروكوكم* مع النيتروجين الغير عضوي لتحسين إنتاجية الطماطم فى نظم الزراعة بدون تربة. و قد تم تحديد تأثير التلقيح *بالأزوسبيريلم* و *الأزوتوبكتر* مع ٧٥٪ نيتروجين على بعض قياسات النمو فى نباتات الطماطم (إرتفاع المجموع الخضرى، عدد الأوراق، الوزن الطازج والجاف لنبات الطماطم) بعد ٣٠ ، ٦٠ و ٩٠ يوم من الزراعة. و أيضا قد تم تعيين المحصول الكلى، متوسط وزن الثمرة و عدد الثمار فى النبات الواحد للنباتات الملقحة بالأسمدة الحيوية (منفردة أومجتمعة) بنسبة ٧٥ ٪ نيتروجين (كنترول ١) وأيضا للنباتات الملقحة بنسبة ١٠٠ ٪ نيتروجين (كنترول ٢) . وقد أوضحت النتائج إن النباتات الملقحة بخليط من *الازوسبيريلم برازيلينس* و *الازوتوبكتر كروكوكم* مع ٧٥ ٪ نيتروجين قد أعطت أعلى قيم لقياسات النمو المختبرة بينما أعطت النباتات الملقحة بنسبة ٧٥ ٪ نيتروجين (كنترول ١) أقل القيم . و قد أعطت نفس المعاملة أيضا أعلى نسبة من المحصول الكلى من الطماطم ، متوسط وزن الثمرة وعدد الثمار فى النبات الواحد و يليها النباتات المعاملة *بالأزوسبيريلم* مع ٧٥ ٪ نيتروجين . و قد أظهرت دراسة تأثيرالمعاملة بالأسمدة الحيوية المختبرة على إمتصاص النبات للنيتروجين والفوسفور والبوتاسيوم و عدد البكتريا و نشاط النيتروجينيز والديهيدروجينيز للبكتريا المختبرة فى المنطقة الجذر محيطية لنبات الطماطم أن التلقيح بمخلوط من *الأزوسبيريلم* و *الأزوتوبكتر* مع ٧٥ ٪ نيتروجين قد أعطى أعلى نسبة من إمتصاص النبات للنيتروجين و الفوسفورو البوتاسيوم بعد ٣٠، ٦٠ و ٩٠ يوم من الزراعة .وقد أعطت نفس المعاملة أعلى عدد من البكتريا وأعلى نشاط للنيتروجينيز والديهيدروجينيز للبكتريا المختبرة مقارنة بالنباتات الملقحة بلقاح منفرد أو الكنترول . و من ناحية أخرى ، قد أعطى كنترول ١ أقل قيم لكل المعاملات المختبرة . لذلك فإن تلقيح نباتات الطماطم *بالأزوسبيريلم برازيلنس* و *الأزوتوبكتر كروكوكم* له أثر نافع على محصول الطماطم تحت الزراعة بدون تربة.