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RESPONSE OF SWEET PEPPER TO INOCULATION WITH *Azotobacter* - AZIDE RESISTANT MUTANTS

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ABSTRACT

Two varieties of sweet pepper (Marconi and California wander) inoculated with Azide resistant mutants induced in *Azotobacter* . Three *Azotobacter* strains used in this study were treated with three concentrations of sodium azide as follows, 25, 36 and 46 µg / ml. Most biofertilizer inoculants showed significant increase in growth parameters (root and shoot dry weight at 40 days plant-old , number of branches and plant height) above uninoculated plants in field and pots experiments. Whereas, four inoculates (Az^r_1, Az^r_2, SMR230 and Az^r_5) induced significant increase in the number of branches per plant above the full dose in pots experiment. All biofertilizer inoculants (except for three inoculants; Az^r_1 , Az^r_2 and Az^r_3) appeared significant increased in root dry weight with CW variety above the full dose in field experiment. Whereas, only three inoculants (SMR230, Az^r_4 and Az^r_6) appeared the same trend with M Variety. The interaction between varieties and biofertilization revealed significant effect on root dry weight at 40 days plant-old on field experiments and number of branches in pots and field experiments. All *Azotobacter* strains and their mutants induced significant increase in yield components (number and weight of fruits per plant) above uninoculated plants in pots and field experiments. However, three inoculants (ATCC 132, SMR230 and Az^r_6) appeared significant increase in the number of fruits per plant above the full dose in the field experiment. Most of *Azotobacter* inoculants appeared significant increase above uninoculated plants in shoot biochemical traits (total Chlorophyll , nitrogen percentage at 40 days plant-old and nitrogen percentage at the end of season) among the plants grown in the field and pots. One inoculant ATcc132 appeared significant increase in total chlorophyll concentration with CW variety above the full dose in field experiment. Most inoculants appeared significant increase in nitrogen concentration at the end of season with M variety above the full dose in pots experiment whereas three inoculants (Atcc132, Az^r_3 and Az^r_5) appeared the same effect with CW variety above the full dose in pots experiment. The interaction between pepper varieties and biofertilizeras inoculants revealed significant effect on chlorophyll a concentration in pots experiment and in total chlorophyll in field , as well as, in nitrogen percentage at the end of season in pots and field experiments.
Keywords : *Azotobacter* , mutants , sodium azide , sweet pepper .

INTRODUCTION

Pepper (*Capsicum annuum* L.) belongs to the solanaceae family. The main quality parameters for Capsicum varieties are color and pungency (Govindarajan *et al*., 1987) . However, current research is also focusing on the flavor as an important parameter for the quality of fresh fruits and vegetables. Capsicum fruits can be used in food industry as colorants and spices, in the pharmaceutical and cosmetic industries in the form of a powder (paprika) or concentrated extract (oleoresin). As a medicinal plant, the *Capsicum* species has been used as a carminative, digestive irritant, stomachic, stimulant and tonic.

Nitrogen-fixing bacteria are some of the more useful organisms on earth because they can contribute to the growth of organisms through their conversion of N_2 into compounds that plants can use. These compounds are useful for the production of proteins and hormones that plants can use in their metabolism. Therefore, these kinds of bacteria are essential for the nitrogen cycle on earth.

Azotobacter is gram negative, free living aerobic nitrogen fixing organism belonging to family Azotobacteriaceae. Among the several species, *Azotobacter chroococcum* happens to be the dominant inhabitant of the rhizosphere. There have been many reports on the beneficial effects of *Azotobacter chroococcum* on growth and yield of various agriculturally important crops. It benefits plants in multiple ways, which includes ; ability to produce ammonia, vitamins and growth substances that enhance seed germination;. production of indole acetic acid and other auxins such as gibberllins and cytokinins which enhance root growth and aid in nutrient absorption (Verma *et al*., 2001). inhibition of phytopathogenic fungi through antifungal substances (Sharma and Chahal, 1987). Azides are potent metabolic inhibitors affecting the activities of a variety of oxidative enzymes, notably those involved in the electron transport system of respiration. There is ample information on the toxicological properties of sodium and potassium azides on humans (Toxline, 2001). Na and azide when added to soils release HN3 which is converted to NH_4^+ and to nitrate through the action of nitrifying bacteria. (Parochetti and Warren, 1970). Also, azide resistance genes express independent of *nif* genes and are dependent on *fix* genes (Kashyap and Narula 1990) . This work aimed to induce sodium-azide resistant mutants to be tested for improved the growth and yield components of sweet pepper varieties **.**

MATERIALS AND METHODS

Genetic materials :

a. Bacterial strains and growth conditions :

The phenotypic properties of different *Azotobacter* strains are shown in Table 1, together with references of their origin. These strains were kindly provided from Microbiology Dept., Giza, Egypt and IAM culture collection, Univ. of Tokyo, Japan; Agric. Res. Center.

b. Plant varieties

Two Pepper *(Capsicum annuum L.)* varieties were used in this study which was as follows :

1- California wander (CW) : It was the most popular bell type , the fruit shape is blocky ,the color immature fruit is glossy green , fruit flash-thicknesses thick and strong against virus.

2 -Marconi (M): The fruit shape is conical, the color fruit is red , fruit flash – thickness is moderate thick and good quality . These varieties were kindly provided from Vegetable Research Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt through Dr. Hammed saied, Prof. of Vegetable breeding , Agric. Res. Center .

Table 1: Bacterial strains used in this study .

Strains	Source or reference	Designation	Azide conc. µg/ml	Azide resistant mutants+	Azide resistant mutants selected
Azotobacter beijerinkii	Micro. Lab., Water ,Soil and Environmental Res. Institute, Agric. Res. Center, Giza, Egypt.	ATCC 132	46	Az^r_1 Az^r_2 Az^r_3 Az^r_4 Az^r_5	Az^r_1 Az^r_2
Azotobacter vinelandii	Micro. Lab., Water ,Soil and Environmental Res. Institute, Agric. Res. Center, Giza, Egypt.	SMR230	46	Az^r_1 Az^r_2 Az^r_3 Az^r_4 Az^r_5	Az^r_3 Az^r_4
Azotobacter chroococum	IAM Culture Collection, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Yayoi, Bunnkyo- ku. U20032 Japan .	IAM	46	Az^r_1 Az^r_2 Az^r_3 Az^r_4 Az^r_5	Az^r_5 Az^r_6

+ Azide resistant mutants (AZ^r) were isolated from the concentration of 46 µg/ml .
Media : *Azotobacter* strains and their mutants were grown in Ashby's mannitol agar medium according to Levine and Schoenlein (1930) .

Methodology:

Induction and isolation of sodium azide resistant mutants (AZ^r):

Azotobacter strains were growing in Ashby broth mudium under shaking condition at 30º C for three days. To obtain AZ^R mutants, 10^9 cells of *Azotobacter* strains were plated on Ashby medium containing 25, 36 and 46 µg / ml sodium azide. After one week , single colonies were picked up and sub cultured on slant agar medium. Resistant colonies were purified on the same medium containing higher concentration of sodium azide ;46, 48,50,52 and 56 µ g/ml . The colonies were not grown on the concentration above 46µg/ml . Sodium azide resistant mutants selected as shown in Table (1) were used to inoculate pepper varieties.

Pots experiment

This experiment was performed in split plot design, with three replicates . Inoculants including three wild type strains and 6 azide resistant mutants were tested for their effects on plant growth and biochemical traits. Soil in pots consists of bottoms and fermoklit 1:1 without any source of fertilization. Seeds were sown without inoculation, after 10 days of transplanting, plants were inoculated with biofertilizer inoculants, as well as, 50% of recommended dose of N fertilization. Plants were thinned to two plants / pot . Soil water content was observed daily and keeping soil at 80% field moisture capacity. After 40 days of transplanting, chlorophyll concentration in leaves was measured .At the same time , plants were collected to air dried and then transferred for oven dried at 65 Cº for 48 hours. Shoot and root dry matter (DW) was recorded . This experiment was conducted according to Shrestha and Ladha , (1998) .

Field experiment :

Dry seeds of two pepper (*Capsicum annuum L*.) varieties were grown independently in separated pots for 45 days, after this period the plants were transplanted to the field. Plants were grown in split plot design consisted of three replicates. The rows in each plot measuring 5.0 m in length and distance maintained 30 cm between plants. The plants were inoculated after one week from transplanting with *Azotobacter* suspension (10^9 cells/ml) for four times with the rate of 5 ml/plant, as shown in Table 2 . The plants were fertilized with recommended dose of phosphorus and potassium, as well as , with 50% of nitrogen recommended dose among all treatments. On the other hand, there was a treatment fertilized with the recommended dose of nitrogen, phosphorus and potassium. Control plants were not inoculated with nitrogen but inoculated with recommended dose of phosphorus and potassium. Several traits including morphological, physiological, chemical and yield components were measured to asses plant response to inoculation with Az^r mutants .Culture in mid-log phase growing in ashby medium were used for inoculation (Johnson and Curl, 1972). The plants were watered to the field capacity with water as needed until harvest. The plants were fertilized with phosphorus at the rate of 150 kg. / feddan . Biofertilization was done as shown in Table 2 , based on plant density equal present 13000 plant / feddan.

Table 2: Biochemical fertilization of pepper varieties .

Time of inoculation from transplanting in the field	Chemical fertilization (N recommended dose / plant) (control)	Biofertilization with 50 % N / plant
After 15-20 day	13.9g ammonium sulphate + 9g potassium sulphate	6.95g ammonium sulphate + Potassium sulphate same in control + bacterial inoculant
After 45-50 day	17.89 ammonium sulphate + 11g potassium sulphate	8.945g ammonium sulphate + potassium sulphate as the same in control + bacterial inoculant
After70-75 day	8.69 g ammonium sulphate	4.35 g ammonium sulphate + bacterial inoculant
After 90 day	5.3 g ammonium sulphate	2.65 g ammonium sulphate + bacterial inoculant

Definition and traits studied :

A. Vegetative traits :

1. Shoot and root dry weight :

Different plant parts (shoots and roots) at 40 days plant old from transplanting were oven dried at 70^{o}C until reached to a constant weight and then turned immediately to weight.

2- Plant height (cm.) :

This trait was measured when the plants became to blooming at harvest time by centimeters from the first leaf to the apex.

3. **Yield:**

The number of fruits per plant were counted and weighted in kg. (Zaied *et al*. 2006) .

b. Chemical traits:

Photosynthetic pigments: Chlorophyll (a, b , chl and Carotene per g tissue) were extracted from wheat leaves using 80% methanol. The pigments were determined spectrophotometrically according to the Lichtenthaler and Wellburn (1983) .

II -Nitrogen determination: It was determined according to APHA, (1992). Color photo metrically was measured as absorbance using a Spectrophotometer. Samples were reading at 425 nm for 1-cm light path. Calibration curve was prepared according to APHA (1992) using the linear regression equation as follows:

$$x = \frac{y - a}{b}$$

Where ; y = Optical density at 530 nm, x = Concentration of nitrogen, b = Regression = 0.14, a = Absorbance at 425 nm when the concentration of N equal zero = 0.01

Ascorbic acid determination :

Ascorbic acid other wise known as vitamin C is an anti scorbutic . It is present in pepper and in all fresh vegetables and fruits. Ascorbic acid reduce the 2,6- dichlorophenol indophenol dye to a colorless leucobase . The ascorbic acid gets oxidized to dehydro ascorbic acid . Through the dye is a blue colored compound , the end point is the appearance of pink color .The dye is pink colored in acid medium . Oxalic acid is used as titrating medium . Pipette out 5 ml of the working standard solution into a 100 ml conical flask . Added 10 ml of 4 % oxalic acid and titrate against the dye (V1 ml) . End point is the appearance of pink color persists for a few minutes . The amount of the dye consumed in equivalent to the amount of ascorbic acid (V2 ml) . The amount of ascorbic acid (mg / 100 ml fruit juice) was calculated from the following formula :

$$\frac{V\,2\text{ ml} \times V\,1\text{ ml}}{\text{Volume of the sample}} \times 100$$

This methodology was used as described by Ranganna (1979) .

Fruit quality:

Total soluble solids (TSS): This trait was measured using a hand refractometer according to A.O.A.C., (1990).

Experimental design and statistical analysis:

Field and pots experiments used in this study was designed in split-plot design. Pepper varieties (Marconi and Calfornia wonder) was the main plots arranged in a completely random. However, biofertilization was assigned to subplots within each main plot. Data were subjected to the analysis of variance according to Snedecor and Cochran (1955). Least significant difference (L.S.D.) was used to compare between means if the F-test was significant.

RESULTS AND DISCUSSION

Effect of *Azotobacter* strains and their mutants on growth traits of pepper

Root colonization is one of the most important steps in the interaction of bacteria and host plants, however, plant growth promoting rhizobacteria (PGPR) including biological control agents were reported to be beneficial to plants (Verma *et al.* 2001).

Data summarized in Table 3, illustrated that most biofertilizer inoculants showed significant increase in growth parameters above uninoculated plants . These results agreed with Abraham and Money (l994) , who found that *Azotobacter chroococcum* TG1 strain isolated from the soils of Laccadive Islands increased yields in crops like rice, tuber, tapioca and pepper and vegetative growth in *Acacia mangium,* a fast growing timber yielding legume tree. In addition, Sena and Das (1998) found that protein and curcumin content of Turmeric were increased when the plants were inoculated with both *Azotobacter* and *Azospirillum*. This indicated that biofertilizer has been used as an economic and sustainable input for increasing the productivity of a variety of crops, viz. vegetables (Pandey and Kumar 1989) .

Table 3: Effect of biofertilizer on growth parameters at 40 day plant- old .

Biofertilizers	Root DW† (g/plant)		Shoot DW(g/plant)		Plant height (Cm)		Number of branches	
	Pots	Field	Pots	Field	Pots	Field	Pots	Field
Uninoculated	1.30	1.50	8.0	11.8	48.5	51.32	4.83	6.50
Full dose	3.08	2.46	11.4	17	53.6	60.8	8.0	9.8
ATCC 132	3.54	4.29	10.7	18.3	51.8	57.2	9.5	10.5
AZ^r_1	2.78	2.22	10.2	19.1	52.1	57.6	10.3	10.8
AZ^r_2	2.95	2.39	12.8	17.4	49.3	56.9	10.5	11.2
SMR 230	2.51	3.35	10.7	19.3	53.3	55.0	11.0	10.0
AZ^r_3	2.43	2.94	9.8	17.0	51.5	59.0	7.5	8.8
AZ^r_4	3.42	3.27	11.3	17.1	50.8	56.8	6.5	8.3
IAM	2.94	3.20	12.5	20.1	50.6	57.5	8.7	9.3
AZ^r_5	2.50	2.94	12.1	16.2	51.8	57.47	10.0	10.2
AZ^r_6	3.15	3.51	11.8	18.9	54.1	58.4	8.8	10.3
F-Test	**	**	**	**	*	*	**	**
LSD 5%	0.89	0.46	2.15	3.08	2.79	4.27	2.13	1.06
1%	1.19	0.62	2.88	4.12	3.73	5.71	1.59	1.41

†= Dry weight., *, ** = significant at 0.05 and 0.01 probability levels, respectively.

On the other hand, all biofertilizer inoculants except for four inoculants (Az^r_1 , Az^r_2, Az^r_3 and Az^r_5) appeared significant increase in root dry weight at 40 days plant–old above the plants fertilized with N recommended dose in field experiment, as well as, only one inoculant (IAM) induced significant increase in shoot dry weight above the full dose of N in pots experiment . Whereas, four inoculates (Az^r_1 , Az^r_2 , SMR230 and Az^r_5) induced significant increase in branches number per plant above the full dose in pots experiment. This agreed with Pandey and kumar (1989), who found that inoculation of *Azotobacter* to without application of nitrogen, phosphorus and potassium had increased the yield per unit area. However, Mehrotra and Lehri (1971) achieved that successful proliferation of *Azotobacter* in association with synthetic fertilizers and yield increases up to 50 per cent in cabbage and 62 per cent in brinjal by the application of *Azotobacter*, however they observed that these increases extremely depend upon the fertility status of the soil and the type of strain used. *Azotobacter* has long been used in Russia to inoculate seeds or roots of crop plants and increase in yields (Mishustin and Naumora 1962) . Jackson *et al.* (1964) found accelerated growth of tomato stem with inoculation of *Azotobacters*.

Data presented in Table 4 showed the effect of the interaction between pepper varieties and biofertilizer inoculants on growth traits. It was achieved that most *Azotobacter* inoculants appeared significant increase in root dry weight and branches number of two pepper varieties (except for; Az^r_1 and Az^r_4 with CW) above uninoculated plants.

Table 4: Effect of interaction between Azotobacter strains and pepper varieties on growth parameters.

Biofertilizers	Root DW (g/plant)				Shoot DW(g/plant)			
	Pots		Field		Pots		Field	
	M	CW	M	CW	M	CW	M	CW
Uninoculated	1.5	1.1	1.2	1.8	8.2	7.9	12.1	11.5
Full dose	3.2	3.0	2.5	2.5	11.0	11.7	16.7	17.3
ATCC 132	3.7	3.4	2.8	4.0	11.7	9.6	20.2	16.3
AZ^r_1	3.2	2.4	2.3	2.2	10.2	10.3	20.2	18.0
AZ^r_2	2.6	3.3	2.0	2.7	10.8	14.8	17.5	17.2
SMR 230	2.8	2.2	3.3	3.4	10.6	10.7	19.6	19.0
AZ^r_3	2.7	2.1	2.5	3.4	11.2	8.5	18.6	15.4
AZ^r_4	4.1	2.7	3.7	2.8	11.3	11.2	16.1	18.1
IAM	3.2	2.7	2.9	3.5	12.0	13.1	18.0	22.1
AZ^r_5	2.5	2.5	2.6	3.3	11.3	12.9	16.2	16.2
AZ^r_6	2.3	4.0	3.1	3.9	12.1	11.6	19.3	18.5
F-Test	NS	NS	**	**	NS	NS	NS	NS
LSD 0.05			0.7	0.7				
0.01			0.9	0.9				

Table 4: Continued.

Biofertilizers	Plant height (Cm)				Number of branches			
	Pots		Field		Pots		Field	
	M	CW	M	CW	M	CW	M	CW
Uninoculated	61.1	45.4	61.1	41.5	5.3	4.3	7.3	5.7
Full dose	60.6	46.6	71.2	50.4	9.3	6.7	11.7	8.0
ATCC 132	60.2	43.3	66.9	47.5	5.0	8.3	7.3	9.3
AZ^r_1	60.6	43.7	66.7	48.4	10.0	10.7	12.3	9.3
AZ^r_2	56.9	41.4	65.0	48.9	13.3	7.7	13.3	9.0
SMR 230	62	44.6	63.3	46.6	11.7	10.3	11.3	8.7
AZ^r_3	59.2	43.8	67.8	50.3	7.7	7.3	9.0	8.7
AZ^r_4	58.9	42.8	62.6	51.1	8.0	5.0	10.0	6.7
IAM	57.7	43.6	63.7	51.3	10.3	7.0	10.7	8.0
AZ^r_5	60.6	42.9	62.2	52.8	10.7	9.3	11.7	8.7
AZ^r_6	62.2	46.0	67.4	49.3	9.7	8.0	11.3	9.3
F-Test	NS	NS	NS	NS	*	*	**	**
LSD 0.05					2.25	2.25	1.5	1.5
0.01					3.01	3.01	2.0	2.0

NS,*, ** = Insignificant and significant at 0.05 and 0.01 probability levels, respectively.

However, all biofertilizer inoculants, except for; three inoculants Az^r_1 , Az^r_2 and Az^r_3, appeared significant increase in root dry weight of CW variety above the full dose in field experiments. Whereas, only three inoculants (SMR230, Az^r_4 and Az^r_6) appeared the same trend with M Variety. These results agreed with Umar *et al.* (2009) , who found that biofertilizers like *Azotobacter* fix atmospheric nitrogen and enhances the production of various field crops. Plant growth-promoting rhizobacteria (PGPR) are beneficial native soil bacteria that colonize plant roots and result in increased plant growth (Kloepper, 1994) . *Azotobacter chroococcum* is an important PGPR (plant growth promoting rhizobacteria) producing compounds needed for plant growth (Rajaee *et al* 2007) .

Data summarized in Table 5 showed that pepper varieties was significantly affected on root dry weight and number of branches in field experiment. Whereas, plant height was significantly affected by pepper varieties among the pots and field experiments. Furthermore, biofertilizater inoculants appeared the same effect on root dry weight , plant height and number of branches in field experiment. However, plant height and number of branches in pots experiment were significantly affected by biofertilizer inoculants . Although, the interaction between varieties and biofertilization revealed significant effect on root dry weight and number of branches in field experiment. These results agreed with Naruala *et al.* (2007), who found that increased in total dry weight of the plants increased in all the *Azotobacter chroococcum* inoculation and nutrient treatments if compared to the control . Whereas, Anantha *et al.* (2007) found that plant height and leaves per plant were significantly increased in the *Azotobacter* inoculated treatments compared to uninoculated plants. The increased growth might be attributed to nitrogen fixation, improving the absorption of nutrient by pepper plants and production of growth hormones by *Azotobacter chroococcum* inoculants*.* Awasthi *et al.* (1996) observed increased growth of peach seedlings when inoculated with *Azotobacter* and *Glomus fasciculatum.* The enhanced growth was attributed to continuous production of growth substances by *Azotobacter* spp. and its interaction with G. *fasciculatum* for better root colonization, which increased the ability of nutrients absorbed by the plants. (Awasthi *et al.* 1996) .

Table 5: Mean squares obtained from split plot analysis for different growth parameters of two pepper varieties affected by biofertilization.

S.V	D.F	Root DW†		Shoot DW		Plant height		Number of branches	
		Pots	Field	Pots	Field	Pots	Field	Pots	Field
Main plot	5								
Rep.	2	0.15	0.11	8.27	31.4	4.02	136.5	0.28	0.97
Varieties	1	0.82	1.19*	0.49	3.24	423**	4408*	66	114.7*
Error -A	2	0.22	0.02	6.64	11.7	4.45	58.23	5.31	1.27
Sub plot									
Biofertilizers	10	2.23	3.37**	11.2	29.8	13.1*	34.64**	21.1**	10.6**
Varieties x Bio.	10	0.98	0.55**	4.86	7.62	2.25	20.20	4.3*	1.72*
Error -B	40	0.58	0.16	3.41	6.98	5.70	13.39	1.85	0.82

***, ** = significant at 0.05 and 0.01 probability levels, respectively. †= Dry weight**

Effects of *Azotobacter* strains and their mutants, on yield components of pepper varieties.

Data summarized in Table 6 showed the effect of biofertilizers on yield components (number of fruits and the weight of fruits per plant) among pots and field experiments . Data appeared that there was a significant differences between inoculants in yield components. These results are in harmony with Sharma and Vasudeva (2005), who found that azide resistant mutants, when used as biofertilizers, showed increased plant height, early flowering, more yield, and high biomass and total nitrogen content. They also increased, in cotton genotypes, the indole acetic acid production and ammonia excretion due to high nitrogenase activity. Rodelas *et al.* (1999) reported that *Azotobacter* increased the yield of sugar beet, carrot and cabbage as much as 10%. These bacteria also help to preserve the health of the plant by controlling the pathogenic agent indirectly as that growth improvement and crop yield (Mrkovacki and Milic, 2001). However, Shahaby (1981) found that tomato plants inoculated with *Azospirillium* and *Azotobacter* increased dry matter by 44% and 55.1%, respectively during the summer season. On the other hand, three inoculants ; ATCC 132, SMR230 and Az^r_6 appeared significant increase in number of fruits per plant above the full dose in field experiment . These results agreed with Prasad and Prasad (2004) , who found positive effect of *Azotobacter* on the yield and height of Brassica plant. In addition, Martinez *et al.* (1993) reported that soil inoculation with *Azotobacter* increased tomato seed germination by 33 – 46 percent , shortened the period between sowing and transplanting by 5-7 days, increased the yield by 38-60 percent . Whereas , El-Akabawy *et al. (* 2000) mentioned that cotton seed yield increased significantly through the use of biofertilizer nitrobien. On the other hand, Sreeramlu and Srikantalah (2003) found that the yields of Banana varieties (yalakki and robusta) was improved when inoculated with *Azotobacter chroococcum* in southern parts of Karnataka .

Table 6: Effect of *Azotobacter* azide resistant mutants on yield components

Biofertilizer inoculants		Number of fruits / plant		Fruit weight (Kg)	
		Pots	Field	Pots	Field
Un inoculated		10.66	12.50	1.012	1.10
Full dose		14.83	16.83	1.218	1.54
ATCC 132		15.16	19.00	1.207	1.46
AZ^r_1		15.16	17.83	1.150	1.40
AZ^r_2		15.50	17.00	1.222	1.33
SMR 230		14.50	18.33	1.172	1.46
AZ^r_3		15.50	17.66	1.175	1.44
AZ^r_4		14.83	17.00	1.153	1.39
IAM		14.16	18.00	1.156	1.44
AZ^r_5		14.33	17.83	1.197	1.43
AZ^r_6		14.66	18.50	1.182	1.44
F test		**	**	**	**
LS.D	0.05	1.393	1.43	0.07	0.14
0.01		1.863	1.91	0.09	0.19

**** = significant at 0.01 probability level.**

Data presented in Table 7 did not show any significant differences between inoculants in yield components (number of fruits and fruit weight) in response to the interaction between pepper varieties and biofertilizer inoculants . These results disagreed with Galindo *et al.* (1996) , who found that the interaction between the plants and microorganisms can be beneficial, neutral or detrimental . Whereas, these results agreed with Jensen (1987) , who found that inoculation of pea plants with *Rhizobium leguminosarum* did not gave significant influence in the dry matter production of two pea cultivars used .

Table 7: Mean of different yield components resulted from the interaction between pepper varieties and biofertilizer inoculants.

Biofertilizers	Number of fruits				Fruit weight (Kg)			
	Pots		Field		Pots		Field	
	M	CW	M	CW	M	CW	M	CW
Uninoculated	11.33	10	15	10	1.023	1.002	1.240	0.963
Full dose	16.33	13.33	16.83	18	1.283	1.153	1.6505	1.485
ATCC 132	17.33	13.00	21.33	16.66	1.259	1.154	1.689	1.282
AZ^r_1	15.66	14.66	20.66	15	1.159	1.141	1.471	1.198
AZ^r_2	16.00	15.00	19.66	14.33	1.293	1.151	1.530	1.273
SMR 230	16.00	13.00	21.00	15.66	1.222	1.122	1.67	1.240
AZ^r_3	16.33	14.66	20.66	14.66	1.257	1.094	1.611	1.263
AZ^r_4	16.00	13.66	19.66	14.33	1.200	1.107	1.525	1.256
IAM	15.66	12.66	21.33	14.66	1.231	1.080	1.620	1.259
AZ^r_5	16.00	13.33	19.33	16.33	1.256	1.139	1.580	1.288
AZ^r_6	16.00	12.66	21.00	16.00	1.255	1.108	1.619	1.263
F test	NS	N.S	NS	NS	NS	NS	NS	NS

NS = Insignificant .

As shown form the results summarized in Table 8 that varieties appeared significant effect on yield components (number of fruits and fruit weight) of plants grown in the field. These results agreed with Adetula and Olakojo (2006), who found that pepper accessions were significantly different (p<0.01) for growth habit, stem pubescence, leaf pubescence and inflorescence position. Fruit characteristics such as fruit position, calyx shape margin, fruit color at maturity, fruit length, width and weight at pedicel were equally highly significant (p<0.01). Adamu *et al.* (1994) also reported sufficient genetic variation in local chilies which warrant selection and hybridization among this species for development of superior genotypes. Furthermore, biofertilization appeared significant effect on all yield components among pots and field experiments. These results agreed with Karthikeyan *et al* (2007) who found that the maximum germination percentage (70%) was recorded in *Azotobacter* treatment followed by *Azospirillum* (66%). The native isolates of *Azotobacter* and *Azospirillum* significantly increased the germination rate in *C. roseus* which was 70 % against 35 % recorded by untreated control. The vital seedling parameters such as germination percentage and vigor index were improved. *Azotobacter* treatment influenced maximum of 50 % germination. Yield increases in rice due to inoculation of *Azospirillum* and *Azotobacter* are reported to be in the 5 – 60 % range (Balandreau 2002) . However, Yanni and El- Fattah (1999) found that rice yields in field trials increased by 0.4 – 0.9 t/ha (7 – 20 % increase) due to *Azotobacter* application. In addition, Van Berkum and Bohlool (1980) reported that diazotrophic bacteria in the rhizosphere utilize the products of nitrogen fixation for their own growth but do not release it while they are alive, the other reason could be the production of phytohormone in the rhizosphere by the microorganisms (Zimmer *et al.* 1988). Biari *et al* (2008) found that maize treated with PGPR(s) significantly increased plant height , shoot and seed dry weight, ear dry weight and length and number of seeds per row. Plants nutrient uptake of N, P, K, Fe, Zn, Mn and Cu were also significantly influenced by application of PGPR(s) . This indicated that some PGPR inoculants have the potential to increase plant growth, yield and nutrients uptake. Also *P. agglomerans* and *A. chroococcum* were able to produce phytohormones in pure culture (Kumar and Narula 1999).

Table 8: Mean squares of different yield parameters from split plot analysis.

S.V.	D.F	Fruits number		Weight of fruits	
		Pot	Field	Pot	Field
Main plot	5				
Rep.	2	20.924	11.227	0.002	237.14
Varieties	1	96.970	402.56**	0.192	1.534**
Error - A	2	20.924	0.742	0.016	0.006
Sub plot					
Biofertilizers	10	10.782**	18.015**	0.019**	0.077**
Varieties x Bio.	10	1.703	2.361	0.003	0.010
Error - B	40	1.424	1.502	0.003	0.015

**** = significant at 0.01 probability level, respectively**

Effect of *Azotobacter* strains and their mutants on biochemical traits of pepper.

Data presented in Table 9 showed that there was a significant differences between inoculants in biochemical traits (total Chlorophyll , nitrogen percentage at 40 days plant-old and nitrogen% at end of season) of shoots in the field and pots experiment, however, all *Azotobacter* inoculants appeared significant increase in chlorophyll a and chlorophyll b above uninoculated plants in field experiment, whereas most inoculants appeared the same trend in pots experiment. On the other hand, most inoculants appeared significant increase in nitrogen percentage at the end of season above the full dose among field and pots experiments. These results agreed with Sena and Das (1998), who found increased protein and curcumin content of Turmeric when inoculated both *Azotobacter* and *Azospirillum* **.** The application of biofertilizer was found to be beneficial for the growth of nutmeg seedlings such as an effect has been reported earlier in black pepper.

As shown from the results presented in Table10 most *Azotobacter* inoculants and their azide resistant mutants induced significant increase in chlorophyll a in pots experiments and total chlorophyll concentration in field experiment with M variety above uninoculated plants, whereas , all inoculants (except for Az^{r}_{6}) appeared the same effect in nitrogen concentration at the end of season with M variety in pots and field experiments. All inoculants (except for $Az^r{}_2$) appeared significant increase in nitrogen concentration at the end of season with CW variety above uninoculated plants in pots and field experiments .

Table 9: Means of shoot biochemical traits affected by biofertilizers.

Biofertilizers	Chlorophyll a (mg/ml)		Chlorophyll b (mg/ml)		Total chlorophyll (mg/ml)		Nitrogen% at 40 days		Nitrogen% at end of season	
	Pots	Field	Pots	Field	Pots	Field	Pots	Field	Pots	Field
Uninoculated	0.172	1.269	0.526	0.955	0.260	0.923	0.159	0.411	0.407	0.454
Full dose	0.818	2.099	0.806	2.106	0.648	1.813	1.119	1.154	1.102	1.264
ATCC 132	0.612	2.215	0.678	2.373	0.732	2.135	1.906	1.941	1.720	1.715
$AZ^r{}_1$	0.601	2.091	0.640	2.335	0.717	1.563	1.819	1.525	1.679	1.668
$AZ^r{}_2$	0.355	2.069	1.018	2.538	0.867	1.563	1.718	2.063	1.536	1.311
SMR 230	0.521	2.382	0.834	2.464	0.949	1.813	1.362	1.791	1.578	1.184
$AZ^r{}_3$	0.741	1.997	0.888	1.811	0.818	1.635	1.697	2.359	1.929	2.155
$AZ^r{}_4$	0.732	2.723	0.705	2.131	1.031	1.885	1.628	2.809	1.573	1.831
IAM	0.623	2.225	0.915	2.190	0.792	1.800	1.627	2.369	1.593	1.338
$AZ^r{}_5$	0.330	2.318	0.885	1.849	0.805	1.377	2.285	2.024	2.051	2.259
$AZ^r{}_6$	0.460	1.820	0.799	1.882	0.817	1.477	2.531	1.804	1.248	1.441
F-Test	**	**	**	**	**	**	**	**	**	**
LSD 5%	0.3	0.54	0.22	0.58	0.27	0.34	0.99	0.63	0.56	0.53
1%	0.41	0.72	0.30	0.78	0.36	0.46	1.33	0.84	0.74	0.71

**** = significant at 0.01 probability level, respectively**

On the other hand, one inoculant ATCC132 appeared significant increase in total chlorophyll concentration with CW variety above the full dose in field experiment, whereas, most inoculants appeared significant increase in nitrogen concentration at the end of season with M variety above the full dose in pots experiment. However, three inoculants (Atcc132, $Az^r{}_3$ and $Az^r{}_5$) appeared the same effect with CW variety above the full dose in pots experiment. These results agreed with Sattar *et al.* (2007) , who found that all three inoculants were similar and significantly higher amounts of total N uptake over the uninoculated control due to inoculations, as well as, *Azotobacter* showed the potential to save 20 kg N/ha with additional yield of 1.24 t/ha. Total N uptake increased significantly due to N fertilization up to 100 kg N/ha. The increases in total N uptake due to inoculations and N rates were due to increased grain and straw yields. Narula *et al* (1979) found that the increase in nitrogen percentage was observed mostly with *A. chroococcum* and *P. agglomerans* in the absence of minerals. In addition, the utilization of biological nitrogen fixation (BNF) technology can decrease the use of urea-N, prevent the depletion of soil organic matter and reduce environmental pollution to a considerable extent (Kennedy *et al.* 2004). Yield increases in rice due to inoculation with *Azospirillum* and *Azotobacter* are reported to be in the 5–60% range (Balandreau 2002). Ridge and Rovira (1968) conducted extensive field and pot trials in Australia with wheat inoculated with *Azotobacter, Bacillus* and *Clostridium.* , they found that of 71 field comparisons of grain yield, inoculation with *Azotobacter* resulted in 28 increases greater than 5%, they concluded that inoculation also advanced the head emergence when N fertilizer was applied.

Table 10: Effect of interaction between biofertilizers inoculants and pepper varieties on shoot biochemichal traits.

Biofertilizers	Chlorophyll a				Chlorophyll b				Total chlorophyll			
	Pots		Field		Pots		Field		Pots		Field	
	M	CW	M	CW	M	CW	M	CW	M	CW	M	CW
Uninoculated	0.213	0.131	1.304	1.233	0.923	0.128	0.471	1.440	0.300	0.219	0.930	0.917
Full dose	1.329	0.307	2.166	2.032	1.280	0.332	1.840	2.371	0.806	0.490	1.473	1.717
ATCC 132	0.999	0.226	2.499	1.932	1.110	0.247	2.326	2.419	1.050	0.414	1.743	2.527
AZ^r_1	0.933	0.268	1.957	2.225	1.063	0.217	2.024	2.647	1.060	0.374	1.663	1.463
AZ^r_2	0.415	0.295	2.063	2.074	1.433	0.604	2.170	2.906	0.867	0.867	1.890	1.237
SMR 230	0.773	0.269	2.275	2.489	1.097	0.572	2.166	2.762	1.058	0.841	1.670	1.957
AZ^r_3	1.171	0.311	2.186	1.807	1.257	0.518	1.627	1.995	0.974	0.662	1.843	1.427
AZ^r_4	1.014	0.450	2.527	2.918	1.137	0.273	1.689	2.574	1.338	0.724	1.880	1.890
IAM	0.949	0.298	2.111	2.339	1.430	0.400	1.557	2.823	0.975	0.610	1.883	1.717
AZ^r_5	0.377	0.284	2.406	2.230	1.317	0.454	1.498	2.201	0.967	0.644	1.457	1.297
AZ^r_6	0.716	0.203	2.174	1.467	1.097	0.501	1.914	1.849	0.987	0.647	1.330	1.623
F-Test	*	*	NS	NS	NS	NS	NS	NS	NS	NS	*	*
LSD 5%	0.4	0.4									0.48	0.48
1%	0.5	0.5									0.65	0.65

NS,* = Insignificant and significant at 0.05 probability level .

Table 10: Continued.

Biofertilizers	Nitrogen% at 40 days				Nitrogen% at end of season			
	Pots		Field		Pots		Field	
	M	CW	M	CW	M	CW	M	CW
Uninoculated	0.135	0.182	0.36	0.456	0.44	0.367	0.541	0.367
Full dose	1.050	1.189	1.15	1.154	1.16	1.038	1.490	1.038
ATCC 132	2.462	1.351	1.55	2.323	1.55	1.883	1.547	1.883
AZ^r_1	1.756	1.883	1.45	1.594	2.19	1.163	2.172	1.163
AZ^r_2	2.230	1.205	1.91	2.207	2.13	0.935	1.686	0.935
SMR 230	1.953	0.772	2.12	1.455	2.36	0.786	1.582	0.786
AZ^r_3	2.101	1.293	2.25	2.462	1.79	2.068	2.242	2.068
AZ^r_4	2.439	0.818	2.55	3.064	1.37	1.767	1.895	1.767
IAM	1.845	1.409	2.46	2.277	2.01	1.175	1.501	1.175
AZ^r_5	2.416	2.155	2.42	1.620	2.02	2.080	2.439	2.080
AZ^r_6	2.381	2.682	1.81	1.794	1.13	1.358	1.524	1.358
F-Test	NS	NS	NS	NS	**	**	**	**
LSD 5%					0.79	0.79	0.75	0.75
1%					1.06	1.06	1.00	1.00

NS,= Insignificant and significant at 0.01 probability level, respectively.**

Certain microorganisms found in the rhizosphere are known to improve soil fertility and consequently plant health and growth. These microorganisms supply nutrients to plants by degrading organic matter, convert atmospheric nitrogen into a useable form, protect plants from disease and stimulate plant growth directly through the production of phytopromoting compounds. Some studies suggesting that stimulation of root growth by plant growth hormones – producing bacteria is the major mechanism involved Kucey (1988).

Data presented in Table 11 demonstrated that pepper varieties were significantly affected on biochemical traits of shoot (chlorophyll b, and nitrogen percentage at the end of season) among both pots and field experiments, as well as, varieties appeared the same trend on chlorophyll a, total chlorophyll and nitrogen percentage at 40 days plant - old. The same trend was also shown by biofertilizer inoculants in pots and field experiments, except for, chlorophyll a in field experiment. Whereas, the interaction between pepper varieties and biofertilizer inoculants revealed significant effect on chlorophyll a in pots experiment and on total chlorophyll in field experiment , as well as, on nitrogen percentage at the end of season in pots and field experiments . These results agreed with Damayani and Katerina (2008), who found that all rhizobacteria treated plants which showed better growth character, milder symptom expressions than control and increased peroxidase enzyme activities and ethylene but these depended on the species. It affected slightly the accumulation of TMV, however it suppressed the *Chili veinal mottle virus* (ChiVMV) accumulation .

Table 11: Mean squares obtained from split plot analysis for different biochemical parameters in shoots affected by biofertilization of two pepper varieties.

S.V.	DF	Chlorophyll a (mg/ml)		Chlorophyll b (mg/ml)		Total chlorophyll (mg/ml)		Nitrogen% at 40 days		Nitrogen% at end of season	
		Pots	Field	Pots	Field	Pots	Field	Pots	Field	Pots	Field
Main plot	5										
Rep.	2	0.02	0.44	0.01	0.68	0.03	0.08	2.16	0.52	0.28	0.02
Varieties	1	4.7**	0.12	10.8**	6.13**	2.06**	0.01	4.63*	0.01	2.89**	2.03*
Error A	2	0.03	0.43	0.02	0.06	0.08	0.02	0.21	0.14	0.02	0.05
Sub plot											
Biofertilizers	10	0.2**	0.79	0.12**	1.17**	0.24**	0.59**	2.32**	2.5**	0.92**	0. 8**
Varieties xBio.	10	0.15*	0.19	0.03	0.22	0.07	0.23*	0.64	0.32	0.53**	0.1**
Error B	40	0.07	0.21	0.03	0.25	0.05	0.08	0.73	0.29	0.14	0.25

***, ** = Significant at0.05 and 0.01 probability levels, respectively.**

Data summarized in Table 12 demonstrated that some biofertilizeres inoculants appeared significant increase in chlorophyll content (chl. A and total chl.) and carotene above uninoculated plants among the pots and field experiments. However, two inoculants (SMR 230 and Az^{r}_{4}) appeared the same effect on chlorophyll b of plants grown in field experiment. However, all biofertilizere inoculants induced significant increase in total soluble solids, vitamin c and anthocyanin above uninoculated plants in pots and field experiment.

These results agreed with Barea and Margaret (2008), who found that treating seedling roots of several plant species with cultures of *Azotobacter paspali* changed plant growth, development and significantly increased weight of leaves and roots; effects were probably caused by plant growth regulators which present in culture supernatant fluids contained indolyl-3-acetic acid, at least 3 gibberellins and 2 cytokinins . The added inoculum of *A. paspali* survived in plant rhizospheres for only a few weeks and no nitrogen was fixed in the root zone of young *Paspalum notatum* , the grass with which *A. paspali* is associated. In addition , Štajner *et al* (2004) found that pepper treated with rhizobacteria increased quantities of nitrogen, activities of antioxidant enzymes superoxide dismutase, (such as peroxidase and catalase) content of chlorophylls , carotenoids, soluble proteins and dry matter in leaves of sugar beet .

Table 12: Means of fruit biochemical traits affected by biofertilizers.

Biofertilizers	Chlorophyll a		Chlorophyll b		Total chlorophyll		Carotene	
	Pots	Field	Pots	Field	Pots	Field	Pots	Field
Un inoculated	0.082	0.046	0.074	0.313	0.156	0.358	0.074	0.094
Full dose	0.188	0.132	0.246	0.321	0.433	0.433	0.159	0.217
ATCC 132	0.149	0.092	0.177	0.370	0.326	0.463	0.167	0.312
AZ^r_1	0.198	0.150	0.197	0.385	0.396	0.535	0.233	0.350
AZ^r_2	0.180	0.248	0.342	0.362	0.546	0.560	0.319	0.223
SMR 230	0.214	0.168	0.367	0.545	0.581	0.713	0.606	0.235
AZ^r_3	0.218	0.201	0.243	0.271	0.461	0.471	0.274	0.276
AZ^r_4	0.322	0.106	0.188	0.647	0.510	0.753	0.175	0.268
IAM	0.223	0.144	0.272	0.490	0.495	0.634	0.382	0.310
AZ^r_5	0.283	0.448	0.253	0.228	0.535	0.675	0.352	0.271
AZ^r_6	0.224	0.230	0.234	0.356	0.458	0.586	0.286	0.425
F-Test	*	**	N.S	**	*	*	*	*
LSD 0.05	0.122	0.153		0.199	0.226	0.229	0.27	0.15
0.01	0.163	0.205		0.266	0.303	0.306	0.36	0.20

Table 12: Continued.

Biofertilizer	Total soluble solids%		Vitamin C ($mg100^{-1}$)		Anthocyanin	
	Pots	Field	Pots	Field	Pots	Field
Un inoculated	3.417	4.932	0.091	0.178	0.141	0.132
Full dose	4.500	5.800	0.554	0.705	0.244	0.235
ATCC 132	5.833	6.500	1.262	1.090	0.512	0.607
AZ^r_1	5.467	6.588	1.260	0.623	0.597	0.317
AZ^r_2	6.083	6.683	0.646	0.840	0.460	0.530
SMR 230	5.683	7.167	0.508	0.783	0.445	0.460
AZ^r_3	4.917	6.783	0.688	0.612	0.318	0.505
AZ^r_4	4.750	6.383	0.686	0.883	0.485	0.385
IAM	4.750	6.800	1.003	1.038	0.458	0.310
AZ^r_5	4.833	6.918	0.496	0.645	0.431	0.480
AZ^r_6	5.417	6.500	0.778	1.115	0.341	0.542
F test	**	**	**	**	**	**
L.S.D 0.05	1.15	0.946	0.082	0.16	0.10	0.09
0.01	0.859	0.707	0.061	0.12	0.13	0.12

NS,*, ** = Insignificant, significant at0.05 and 0.01 probability levels, respectively

Data presented in Table 13 showed the interaction between pepper varieties and biofertilizer inoculants on biochemical traits of pepper fruits. All biofertilizer inoculants (except for two inoculants ; ATcc132 and Az^r_1) appeared significant increase in total chlorophyll in CW variety above uninoculated plants in pots experiment. Whereas, most of biofertilizer inoculants appeared the same effect in vitamin C in field experiment . However, some inoculants appeared significant increase in vitamin C above the plants fertilized with N full dose with two varieties grown in pots and field experiment. However, all inoculants induced significant increase in anthocyanin concentration with two varieties grown in pots and field experiment. All biofertilizer inoculants appeared significant increase in anthocyanin concentration above the plants fertilized with N full dose with M variety grown in pots (except for Az^r_3). One inoculant (Az^r_5) appeared significant increase in chlorophyll a with CW variety above N full dose in field experiment , whereas , Az^r_4 appeared the same effect in chlorophyll b and total chlorophyll in CW variety grown in field experiment. These results are in agreement with Gopal *et al.* (2000), who reported increased N content in *Azotobacter* inoculated plants . Das *et al.* (1990) evaluated the mulberry yield after inoculation with *Azotobacter* and *Azospirillum* biofertilizers and found that *Azotobacter* inoculated plants had greater number of leaves, leaf area , plant height and leaf nitrogen content compared to *Azospirillum* inoculated plants . However, Vinutha (2005) reported increased growth , biomass, nitrogen, phosphorus, crude protein, soluble protein and phenol content in *Ocimum sanctum* and *Ocimum kilimandscharicum* inoculated with *Glomas fasiculatum, Azotobacter chroococcum* and *Aspergillus awamori* singly and in combinations.

Table 13: Effect of interaction between biofertilizer strains and pepper varieties on fruit biochemical traits.

Biofertiliz-ers	Chlorophyll a				Chlorophyll b				Total chlorophyll				Carotene			
	Pots		Field		Pots		Field		Pots		Field		Pots		Field	
	M	CW	M	CW	M	CW	M	CW	M	CW	M	CW	M	CW	M	CW
Uninocula-ted	0.07	0.09	0.05	0.04	0.06	0.09	0.13	0.50	0.13	0.18	0.18	0.54	0.08	0.07	0.10	0.09
Full dose	0.16	0.22	0.15	0.12	0.20	0.29	0.21	0.50	0.36	0.51	0.36	0.55	0.21	0.11	0.23	0.20
ATCC 132	0.10	0.20	0.12	0.06	0.16	0.19	0.27	0.58	0.26	0.39	0.39	0.54	0.17	0.17	0.33	0.29
AZ^r_1	0.22	0.17	0.23	0.07	0.19	0.20	0.40	0.65	0.42	0.37	0.56	0.55	0.21	0.25	0.28	0.42
AZ^r_2	0.10	0.31	0.20	0.30	0.08	0.60	0.09	0.64	0.13	0.96	0.29	0.83	0.41	0.23	0.22	0.23
SMR 230	0.16	0.27	0.12	0.22	0.16	0.57	0.37	0.74	0.32	0.84	0.47	0.96	0.54	0.67	0.23	0.24
AZ^r_3	0.17	0.26	0.16	0.24	0.19	0.30	0.20	0.57	0.36	0.56	0.36	0.58	0.34	0.21	0.33	0.22
AZ^r_4	0.19	0.45	0.13	0.08	0.10	0.27	0.24	1.05	0.30	0.72	0.37	1.14	0.14	0.21	0.29	0.25
IAM	0.15	0.30	0.18	0.11	0.23	0.31	0.25	0.73	0.38	0.61	0.43	0.84	0.24	0.52	0.38	0.24
AZ^r_5	0.29	0.28	0.19	0.70	0.17	0.34	0.30	0.56	0.46	0.61	0.49	0.86	0.52	0.19	0.25	0.29
AZ^r_6	0.22	0.23	0.20	0.26	0.17	0.30	0.34	0.66	0.39	0.53	0.55	0.63	0.27	0.31	0.52	0.33
F-Test	NS	NS	*	*	NS	NS	**	**	*	*	*	*	N.S	N.S	N.S	N.S
LSD 0.05			0.22	0.22			0.28	0.28	0.32	0.32	0.32	0.32				
0.01			0.29	0.2			0.38	0.38	0.43	0.43	0.43	0.43				

Table 13: Continued.

Biofertilizer	Total soluble solids %				Vitamin C ($mg100^{-1}$)				Anthocyanin			
	Pots		Field		Pots		Field		Pots		Field	
	M	CW	M	CW	M	CW	M	CW	M	CW	M	CW
Un inoculated	3.83	3.00	5.03	4.83	0.10	0.08	0.19	0.17	0.17	0.12	0.12	0.14
Full dose	4.50	4.50	5.90	5.70	0.96	0.14	1.11	0.30	0.26	0.22	0.22	0.25
ATCC 132	5.83	5.83	6.20	6.80	1.16	1.36	1.18	1.00	0.78	0.25	0.90	0.31
AZ^r_1	5.60	5.33	6.60	6.58	1.56	0.96	0.77	0.47	0.90	0.30	0.37	0.26
AZ^r_2	6.17	6.00	6.60	6.77	0.80	0.49	1.11	0.57	0.59	0.33	0.83	0.23
SMR 230	6.20	5.17	7.00	7.33	0.83	0.19	1.17	0.40	0.60	0.29	0.60	0.32
AZ^r_3	5.00	4.83	7.10	6.47	0.84	0.53	0.90	0.32	0.33	0.31	0.71	0.30
AZ^r_4	4.83	4.67	6.57	6.20	1.18	0.19	1.14	0.63	0.58	0.39	0.50	0.27
IAM	5.17	4.33	6.73	6.87	0.85	1.16	1.05	1.03	0.69	0.23	0.32	0.30
AZ^r_5	5.00	4.67	7.10	6.74	0.80	0.19	0.92	0.37	0.44	0.42	0.67	0.29
AZ^r_6	5.33	5.50	6.70	6.30	1.40	0.16	1.18	1.05	0.40	0.28	0.81	0.28
F - test	NS	NS	NS	NS	**	**	**	**	**	**	**	**
0.05					0.09	0.09	0.17	0.17	0.09	0.09	0.17	0.17
L.S.D 0.01					0.12	0.12	0.23	0.23	0.12	0.12	0.23	0.23

NS,*, ** = Insignificant, significant at0.05 and 0.01 probability levels, respectively.

Data summarized in Table 14 showed that pepper varieties were significantly affected on chlorophyll content (chlorophyll a , chlorophyll b and total chlorophyll**)** and carotene in plant grown in pots. Whereas, vitamin C and anthocyanin concentrations were significantly affected by pepper varieties among the plants grown in pots and field . The performance of pepper varieties vary significantly owing to difference in environmental conditions in addition to genetic differences. Different varieties perform based on its genotypic character and its exposure to environment (Sainamole Kurian *et al*., 2002). However, biofertilizers produced significant effect on all biochemical traits, except for , chlorophyll b in pots and total chlorophyll in field. In addition, the interaction between varieties and biofertilizer inoculants appeared the same effect on all biochemical traits , except for , on chlorophyll a and chlorophyll b in pots experiment , as well as , total soluble solids in plants grown in pots and the field. These results agreed with Mishustin and Naumora (1962), who demonstrated that bacterial fertilizers slightly improved yield of a wide range of crop plants , especially vegetable; however, yield increases have been reported up to 28.56, 18.25, 19.33 and 55 per cent in case of tomato, potato, cabbage and cucumber, respectively .

Table 14: Mean squares obtained from split plot analysis for fruit biochemical traits affected by biofertilization with two pepper varieties.

		Chlorophyll a		Chlorophyll b		Total chlorophyll		Carotene	
S.V	**D.F**	**Pots**	**Field**	**Pots**	**Field**	**Pots**	**Field**	**Pots**	**Field**
Main plot	5								
Rep.	2	0.03	0.029	0.11	0.13	0.17	0.06	0.173	0.055
Varieties	1	0.13**	0.028	0.42**	1.3	1.06**	1.64	1.06**	1.637
Error A	2	0.001	0.048	0.003	0.09	0.001	0.11	0.001	0.109
Sub plot									
Biofertilizers	10	0.02*	0.069**	0.04	0.09**	0.09*	0.09	0.086*	0.093*
Varieties x Bio.	10	0.02	0.046*	0.04	0.11**	0.09*	0.08*	0.091*	0.082*
Error B	40	0.01	0.017	0.02	0.03	0.04	0.04	0.038	0.038

Table 14: Continued.

		Total soluble solids		Vitamin C		Anthocyanin	
S.V	**D.F**	**Pots**	**Field**	**Pots**	**Field**	**Pots**	**Field**
Main plot	5						
Rep.	2	3.24	0.88	0.004	0.005	0.019	0.021
Varieties	1	1.80	0.12	3.4**	2.7**	0.92**	1.31**
Error A	2	0.56	1.35	0.006	0.005	0.002	0.003
Sub plot							
Biofertilizers	10	3.36**	2.26**	0.72**	0.44**	0.102**	0.13**
Varieties x Bio.	10	0.23	0.20	0.36**	0.12**	0.068**	0.09**
Error B	40	0.54	0.36	0.002	0.011	0.008	0.006

***, ** = Significant at 0.05 and 0.01 probability levels, respectively.**

In addition, Mehrotra and Lehri (1971) achieved successful proliferation of *Azotobacter* in association with synthetic fertilizers and yield increases up to 50 per cent in cabbage and 62 per cent in brinjal by the application of *Azotobacter*, however they observed that these increases extremely depend upon the fertility status of the soil and the type of strain used. However, Joe *et al* (2005) found that the growth of red pepper plants was enhanced by treatment with the rhizobacterium , *Bacillus cereus* MJ -1while , red pepper shoots showed a 1.38 - fold increase in fresh weight (Fw) and roots showed a 1.28-fold Fw gain. This because plant growth-promoting rhizobacterium (PGPR) has been reported to produce gibberellins (GAs) (Joe *et al*.2005) .

In conclusion, azide resistant mutants (Az^r) of *Azotobacter* have a beneficial effect on pepper productivity via significantly increase the growth and yield components above uninoculated plants and above the plants fertilized with recommended dose of nitrogen, as well as, improving biochemical traits of shoots and fruits .

REFERENCES

A.O.A.C. 1990. Official Methods Analysis. 13th ed. Association of Official Agricultural Chemists, Washington, DC.

Abraham, J. K. and N. S., Money, 1994, *Azotobacter chroococcum* TG1, A succesful biofertilizer. Paper presented at Micon 94 International and 35th Annual Conference of Association of Microbiologists of India. 9-12 November, 1994.

Adetula A.O. and S.A. Olakojo.2006. Genetic Characterization and Evaluation of Some Pepper Accessions *Capsicum frutescens* (L.): The Nigerian ‘Shombo’ Collections. American-Eurasian J. Agric. & Environ. Sci., 1 (3): 273-281.

Adamu, S.U., S.G. Ado, P.G. Eruotor and J.D. Olanrewaju, 1994. Variation in the fruit of pepper (*Capsicum* ssp.). Capsicum Newsletter, 7: 46.

APHA (American Public Health Association), American Water Work Association (AWWA) and Water Environmental Fedration (WEF). 1992. Standard Methods for the Examination of water and wastewater. The 18th Ed., American Public Health Association, washington.

Anantha Naik T. N. Earanna and C. K. Suresh. 2007. Influence of *Azotobacter chroococcum* Strains on Growth and Biomass of *Adathoda vasica* Nees. Karnataka J. Agric. Sci.,20(3), 613-615.

Awasihi, R.P., R.K. Godara, and N.S., Kaith.1996, Interaction effect of V A mycorrhizal and azotobacter inoculation on peach seedlings. Indian Journal of Horticulture, 53: 8-13.

Biari, A. A. Gholami and H.A. Rahmani. 2008. Growth Promotion and Enhanced Nutrient Uptake of Maize (*Zea mays*L.) by Application of Plant Growth Promoting Rhizobacteria in Arid Region of Iran. Journal of Biological Sciences. 8(6):1015-1020.

Balandreau J. 2002. The spermosphere model to select for plant growth promoting rhizobacteria. Pp. 55–63 in ‘Biofertilisers in action’, ed. by I.R. Kennedy and A.T.M.A. Choudhury. Rural Industries Research and Development Corporation: Canberra.

Barea J. M. and Margaret E. Brown.2008. Effects on Plant Growth Produced by *Azotobacter paspali* Related to Synthesis of Plant Growth Regulating Substances. J. of Appl. Microbiol. 37 (4) 583 – 593.

Bopaiah, B.M. and Khader, A. 1989. Effect of biofertilizers on growth of black pepper. Indian J, agric. Sci. 59: 682-83.

Das, P. K., Ghosh, A., R.S. Katiyar,and K., Sengupta. 1990. Response of irrigated mulberry to *azotobacter* and *azospirillum* biofertilizers under graded levels of nitrogen. *Journal of General Microbiology*, 31:255-251.

Damayani , T. A. and T. Katerina. 2008. protection of hot pepper against multiple infection of virusesby utilizing root colonizing bacteria. J. Issass . 14(1):92-100.

El-Akabawy, M.A.; Allam, S.M.M. and Monged, N.O. 2000. Some nutritional studies on cotton plant. Egypt. J. Appl. Sci. 15(7):34-43.

Galindo E. Fronteras en Biotecnologia y Bioingenieria. 1996. Ed. Sociedad Mexicana de Biotecnologia y Bioingenieria, A.C. P. 143.

Gopal, S., Somani, L. L., Totawat, R.L. and Singh, G., 2000, Effect of integrated nitrogen management on yield attributing characters and yield of wheat. *Crop Research,* 2:123- 127.

Govindarajan, V.S. 1987. CRC critical reviews in food science and nutrition. 24: 245-355.

Jackson, R. M., Brownm, . and K .Burlinghams. 1964. Similar effects on tomato plants of *Azotobacter* inoculation and application of gibberellins. Nature, Lond. 203, 85.

Jensen E.S. 1987. Inoculation of pea by application old *Rhizobium* in planting furrow. Plant and Soil, 97:63-70.

Johnson , L.F. and E.A. Curl. 1972. Methods for research on the Ecology of soil-borne plant pathogens. Burgess publishing Company, Minnessota.247P.

Joe GJ, Kim YM, Kim JT, Rhee IK, Kim JH, Lee IJ. 2005. Gibberellins-producing rhizobacteria increase endogenous gibberellins content and promote growth of red peppers. J. Microbiol. 43(6):510-505.

Karthikeyan, B., C.A. Jaleel, Gopir., M . Deiveekasundaram. 2007. Alterations in seedling vigour and antioxidant enzyme activities in *Catharanthus roseus* under seed priming with native diazotrophs. J Zhejiang Univ Sci B 8(7):453-457.

Kashyap, L.R. and Narula, R..1990. Expression of azide resistance is dependent on *fix* genes in *Rhizobium meliloti.* Proceedings of Indian National Science Academy *B* 56, 195–198.

Kennedy I.R., A.T.M.A. Choudhury and M.L. Kecskés. 2004. Non-symbiotic bacterial diazotrophs in cropfarming systems: can their potential for plant growth promotion be better exploited? Soil Biology andBiochemistry 36, 1229–1244.

Kloepper, J.W., 1994. Plant growth-promoting rhizobacteria. In: Okon, Y. (Ed.), Azospirillum/Plant Associations. CRC Press, Boca Raton, FL, pp. 137–166.

Kucey, R.M.N., 1988. Plant growth-altering effects of *Azospirillum brasilense* and *Bacillus* C-11-25 on two wheat cultivars. J. Appl. Bacteriol., 64: 187-196.

Kumar V., and Narula N.1999. Solubilization of inorganic phosphates by Azotobacter chroococcum mutants and their effect on seed emergence of wheat. Biol. Fertil. Soil, 28: 301–305.

Lichtenthaler, H.K. and A.R. Wellburn. 1983. Determination of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Biochem. Soc. Transactions 11:591-592.

Levine, M. and H. W. Schoenlein. 1930. A Compilation of Culture Media for the Cultivation of Microorganisms. The Williams and Wilkens Co., Baltimore.

Mrkovacki, N and V. Milic. 2001. Use of *Azotobacter chrooccocum* as potential useful in agricultureal allocation. Ann. Microbiol.,51:145-158.

Martinez, R.; B. Dibut and R. Gonzalez . 1993. Stimulation of tomato development and yield by inoculation of red ferrallitic with *Azotobacter chroococcum*

Mehrotra, C.L. and L.K. Lehri, 1971. Effect of *Azotobacter* inoculation on crop yields. Jurnal of the Indian Society of Soil Science. 19: 243-248..

Mishustin, EN and A.N. Naumora,1962. Bacterial fertilizers their effectiveness and mechanism of action. Microbiologiya. 31: 543-555.

Narula, N., R. Remus1, A. Deubel, A. Granse, S.S. Dudeja, R.K. Behl1, W. Merbach S. Ranganna,. 1979. Manual analysis of fruit and vegetable products. Tata Mc grow Hill publishing company limited, new Delhi, 634 p.

Pandey A. and S. Kumar .1989.Potential of *Azotobacter* and *Azospirillum* as biofertilizers for upland agriculture. A review. J. Sci. Industr. Res., 48: 134–144.

Parochetti, J. V. and Warren, G.F. 1970. Behavior of potassium azide in the soil. *Weed Science* 18, 555-560.

Prasad, M. and R. Prasad. 2004. Effect of mepiquat chloride , *Azotobacter* and nitrogen on cotton (*Gossypium hirsutum*) and their residual effect on succeeding transplanted Indianmustard Brassica juncea. Indian J. Agric. Sci., 74:273-275.

Rajaee S., H. A. Alikhani and F. Raiesi. 2007. Effect of Plant Growth Promoting Potentials of *Azotobacter chroococcum* Native Strains on Growth, Yield and Uptake of Nutrients in Wheat*.* J. Sci. & Technol. Agric. & Natur. Resour., Vol. 11, 41 : 297 .

Ranganna, S., 1979. Manual of Analysis of Fruits and Vegetables Products. Tata McGraw HillPublishing Co. Ltd. New Delhi, India

Ridge E H and A D Rovira. 1968 Microbial inoculation of wheat. Trans. 9th Int. Cong. Soft. Sci. 111,473-481.

Rodelas, B. J. Gonzalez-lopz, C. Pozo, V. Salmeron and M.V. Martinez-telod. 1999. Response of faba been (Vicia faba L.) to combined inoculation with *Azotobacter* and *Rhizobium leguminosarum* bv. *Viceae*, Al. Soil Ecol. 12:51-59.

Sattar M.A., M. F. Rahman, D. K. Das and Abu T.M.A. Choudhury.2007. Prospects of using *Azotobacter*, *Azospirillum* and cyanobacteria as supplements of urea nitrogen for rice production in Bangladesh.

Sena, M.K. and P.K., Das.1998. Influence of microbial inoculants on quality of turmeric. Indian Cocoa, Aracanut and Spices Journal, 21: 31-33.

Sainamole Kurian, P., Backiyarani, S., Josephrajkumar, A. and Murugan, M. 2002, Varietal evaluation of black pepper for yield, quality and anthracnose disease resistance in Idukki District, Kerala J. Spices Aromatic Crops, 11 : 122 – 124.

Sharma P.K. and Chahal V.P.S. 1987. Antagonistic effect of *Azotobacter* on some plant pathogenic fungi. J. Res. Punjab Agri.Univ., 24, 638-640.

Sharma P. and M. Vasudeva. 2005. Azide resistant mutants of *Acetobacter diazotrophicus* and *Azospirillum brasilense* increase yield and nitrogen content of cotton. Journal of Plant Interactions.1:145-149.

Shahaby, A.F.E. 1981. N2-fixing bacteria in the rhizosphere of certain plants. M.Sc. Thesis, Fac. Agric., Cairo Univ.

Shrestha R.K., Ladha J.K. 1998.Nitrate in groundwater and integration of nitrogen catch crop in rice–sweet pepper cropping system. Soil Sci. Soc. Am. J. 62:1610-1619.

Snedecor, G.W. and Cochran, W.G. 1955. Statistical Methods (7th ed.), Iowa State Univ. Press, Ames., Iowa, U.S.A.

Sreeramlu, K.R. and Srikantalah, M., 2003. Response of banana cultivars to biofertilizers. Paper presented at Microbes and Human Sustenance 44th Annual Conference of Association of Microbiologists of India, 12-14.

Toxline. 2001. Toxicology Literature Online Databank [http://toxnet.nlm.nih.gov].

Umar, I. V. K. Wall, R. Kher and M. Jamwal.2009. Effect of Fym, Urea and *Azotobacter* on Growth, Yield and Quality of Strawberry Cv. Chandler. Not. Bot. Hort. Agrobot. Cluj 37 (1), 139-143.

Van Berkum P. and B.B.Bohlool. 1980. Evaluation of nitrogen fixation by bacteria in association with roots of tropical grasses. Microbiol. Rev., 44: 491–517.

Verma S., Kumar V., Narula N. and Merbach W. 2001. Studies on in vitro production of antimicrobial substances by *Azotobacter chroococcum* isolates / mutants. Journal of Plant Diseases and Protection., 108, 1152- 1165.

Vinutha, T. 2005, Biochemical studies on *Ocimum* species inoculated with microbial inoculants. *M.Sc. (Agri.) thesis,* University of Agricultural Sciences, Bangalore, India.

Yanni Y.G. and F.K.A. El-Fattah 1999. Towards integrated biofertilization management with free living and associative dinitrogen fixers for enhancing rice performance in the Nile delta. Symbiosis 27, 319–331.

Zaied, N.S. , S.A.A. Khafagy and M.A Saleh. 2006. Effect of Nitrogen and Potassium Fertilization on Vegetative Growth, Fruit Set and Quality of Washington Navel Orange Trees. J. Appl. Sci.s Res., 2(11): 851-857.

Zimmer W., Roeben K. and H.Bothe .1988. An alternate explanation for plant growth promotion by bacteria of the genus Azospirillum. Planta, 176: 333–342.

إستجابة أصناف الفلفل الحلو للتلقيح بطفرات الأزوتوباكتر المقاومة للصوديوم أزيد

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استخدمت فى هذه الدراسة ثلاث سلالات من بكتيريا الأزوتوباكتر تمت معاملتها بثلاث تركيزات من الصوديوم أزايد هي ٢٥ ، ٣٦ ، ٤٧ ميكروجرام / مل بغرض استحداث طفرات مقاومة للصوديوم أزايد من الأزوتوباكتر ، وقد تم انتخاب عزلتين من كل تركيز لاستخدامهما فى تلقيح صنفين من الفلفل هما Marconi , California wander . أظهرت النتائج أن معظم المخصبات الحيوية أظهرت زيادة معنوية فى صفات نمو محصول الفلفل تحت الدراسة وهى : وزن الجموع الخضري والجذري عند عمر أربعين يوم من النقل ، عدد الأفرع للنبات ، طول النبات - بشكل يفوق النباتات الغير ملقحة على مستوى التجربتين الحقلية و تجربة الأصص . كما أحدثت كل اللقاحات ما عدا الطفرات التالية : z^r_1 , Az^r_2 , Az^r_3 - زيادة معنوية فى الوزن الجاف للمجموع الجذرى للصنف كاليفورنيا عند عمر أربعين يوم مقارنة بالنباتات التى عوملت بالجرعة الموصي بها . كما نتج عن اللقاحات الثلاث التالية : SMR230, Az^r_4 , Az^r_6 نفس التأثير مع الصنف ماركونى . هذا بالإضافة إلى أن معظم لقاحات الأزوتوباكتر و طافراتها أدت إلى إحداث زيادة معنوية فى تركيز كلوروفيل (a) على مستوى النباتات النامية في الأصص والتركيز الكلى للكلوروفيل للنباتات النامية فى الحقل مع الصنف ماركونى مقارنة بالنباتات الغير ملقحة ، بينما أدى التفاعل بين أصناف الفلفل و المخصبات الحيوية إلى إحداث تأثير معنوى على تركيز كلوروفيل (a) للنباتات النامية فى الأصص و التركيز الكلى للكلوروفيل للنباتات النامية فى الحقل بالإضافة الى تركيز النيتروجين فى نهاية الموسم للنباتات النامية فى الحقل .