

RESPONSE OF WHEAT (*Triticum aestivum* L.) TO DIFFERENT MINERAL NITROGEN LEVELS AND INOCULATION WITH N₂-FIXING BACTERIA

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ABSTRACT

Two field experiments were carried out on a sandy loam soil at El-Tahrir Province Sector during two successive winter-growing seasons of 2005/2006 and 2006/2007 to study the response of wheat growth, yield and some yield components to inoculation with N₂-fixing bacteria under different levels of mineral N-fertilization using sprinkler irrigation system. Wheat seeds (*Triticum aestivum* L., cv. Sids 1) were inoculated shortly before planting by *Azorhizobium caulinodans*, *Gluconacetobacter diazotrophicus*, *Bacillus polymyxa* as a single treatment or seeds inoculated with mixture of such strains. The tested bacterial strains were interacted with different levels of mineral N fertilizer (zero, 25, 50, 75 and 100 kg N/fed). Nitrogen fertilizer was added in the form of ammonium sulphate (20.5% N). Superphosphate (15.5% P₂O₅) and potassium sulphate (48% K₂O) were added at rates of 200 kg/fed and 100 kg/fed, respectively as a recommended dose. The experimental design was a split plot design with three replications and the plot size was 6 m².

Regarding the response to inoculation with N₂-fixing bacteria, results of both tested seasons showed that there are significant increases in all wheat vegetative growth, yield and some yield components due to inoculation with any tested diazotrophs. However, using mixture strains followed by *Bacillus polymyxa* treatments surpassed other inoculated treatments or uninoculated ones. As general, a comparison between inoculants on all wheat growth aspects as well as wheat yield and its yield components showed the order: Mixture > *Bacillus polymyxa* > *Gluconacetobacter diazotrophicus* > *Azorhizobium caulinodans* > uninoculated treatment.

The response of wheat growth, yields and yield components to increasing application rate of mineral N fertilizer was significant. Using higher rates of mineral N-fertilizer (100 kg N/fed) showed higher values of all tested parameters under investigation in both seasons.

Taking the interaction between inoculation and different N fertilizer levels into consideration, the best results of wheat growth, yield and its components were achieved when wheat plants inoculated with mixture strains followed by *Bacillus polymyxa* in combination with 50 kg N/fed, which showed a positive response and gave values nearly similar to or higher than using the full dose of mineral N-fertilizer (100 kg N/fed). This trend was true in both tested seasons.

From these results it could be concluded that the inoculation with N₂-fixing bacteria, particularly in a mixture form, may be acting as a good practice for enhancing wheat growth aspects and improving the crop yield and yield components. Also, it could compensate 50% of the recommended inorganic-N used in sandy loam soil with no decrease in wheat quantity or quality.

INTRODUCTION

Wheat is very important grain crops in Egypt and represents the major source of food. Every year Egypt has to import more than 5 million tons of wheat to cover the needs of increasing country population. Extensive cultivation of wheat in the newly reclaimed soils in Egypt seemed to be imperative to circumvent the problem of insufficiency in wheat grain supply. However, intensive agricultural methods have introduced undesirable and sometimes catastrophic consequences by polluting air, soil and aquatic systems as well as food stuffs. The efforts to decrease chemical fertilization by using biofertilizers may improve economy and reduce environmental pollution.

Undoubtedly, considerable attention is focused on fertilization management in cereals production to produce higher yield with minimal input cost and minor environmental pollution. Biological nitrogen fertilizers may act as a safe and cheap practice that contributes productivity directly by a supplement of fixed nitrogen and growth hormones to wheat plants, and indirectly by the enhancement of soil fertility. Therefore, it has become essential to use biofertilizers as partial substitutes for chemical nitrogen fertilizers. Many workers studied the possibility of using N₂-fixing bacteria to supply plants with a apart of their requirements and consequently reduce the amount of chemical N-fertilizer (Srinivasan *et al.*, 1997, Atalla *et al.*, 2005 and Abo El-Soud *et al.*, 2007). They conclude that associative N₂-fixing bacteria are effective in reducing N fertilizer for wheat.

Studies on biological nitrogen fixation (BNF) have emphasized the role of free-living N₂-fixing bacteria with cereals. Several investigators showed that inoculation with associative N₂-fixers have a great importance to improve growth and increase yield of cereal crops not only due to high N₂-fixation activity but related as well to the ability of these bacteria to produce antibiotics, phytohormones, siderophores and the ability to solubilize phosphate (El-Hawary *et al.*, 1998, Swedrzyńska, 2000, Galal *et al.*, 2001, Saubidet *et al.*, 2002, Kloepper, 2003 and Mekhemar *et al.*, 2006).

Azorhizobium caulinodans capable of free-living nitrogen fixation, is the microsymbiont in the stem and root nodules of the tropical legumes *Sesbania rostrata*. Microscopic examination of short lateral roots of inoculated plants showed invasion of *Azorhizobium* between the cells of the cortex, with the xylem and root meristem. Acetylene reduction assay combined with analysis of tissue nitrogen levels indicated that colonization led to measurable nitrogenase activity (Kenneth *et al.*, 1997 and Sabry *et al.*, 1997). The beneficial effects of *Azorhizobium* are related not only to its N₂-fixing proficiency but also to the ability of producing two major classes of plant growth regulators (auxin and gibberellins), antibacterial and antifungal. These results were supported with wheat plants inoculated with *Azorhizobium caulinodans* (Sabry *et al.*, 1997, Antoun *et al.*, 1998 and Sabry *et al.*, 2000). Recent research has been focused on the response of non-legume crops to inoculation with *Azorhizobium*. Sabry *et al.* (1997), Webster *et al.* (1997), Badawi (2003) and Mekhemar *et al.* (2006) reported that inoculation of wheat with *Azorhizobium caulinodans* resulted in significant increases in wheat

growth parameters, plant N-content, yield and some yield components compared to the uninoculated plants.

Gluconacetobacter diazotrophicus has a long-standing history of bacterial-plant interrelationship as a symbiotic endophyte capable of fixing atmospheric nitrogen. The bacterium apparently responsible for the plant-associated BNF has unique physiological properties for a diazotroph such as tolerance to low pH, and high sugar and salt concentrations, lack of nitrate reductase and nitrogenase activity, which tolerates short term exposure to ammonium (Boddey *et al.*, 1991). Extensive knowledge gained through past studies claimed that *G. diazotrophicus* is an important nitrogen fixer, imparting nitrogen nutrition to sugarcane and other crop plants (Boddey *et al.*, 2003 and Kennedy *et al.*, 2004). Lastly, the plant growth-promoting traits identified in this group of bacteria, including N₂-fixation, phytohormones such as indole acetic acid and gibberellic acid, *in vitro* solubilization of plant macro and micronutrients like P and Zn and bio-control of the phytopathogens (Bastian *et al.*, 1998, Dobbelaere *et al.*, 2003 and Saravanan *et al.*, 2007). This drew attention to reassessing the roles played by this bacterium in PGP aspects.

Bacillus polymyxa is commonly found in soil where it colonizes important crops species including wheat (*Triticum aestivum* L.). It plays a dual role by fixation of atmospheric N and producing antimicrobial agents against deleterious rhizobacteria (Kloepper, 2003). Many investigators have shown that *B. polymyxa* has properties rather than nitrogen fixation. These properties could be summarized as follows: solubilization of phosphate and enhancement of nutrient uptake (Gaikwad and Wani, 2001); production of plant growth regulators (Lebuhn *et al.*, 1997); production of antibiotics (Gajda and Kurzawinska, 2004). Many investigators confirmed the superiority of inoculation with *B. polymyxa* for enhancing wheat growth, yield and its yield components (Egamberdiyeva and Hoflich, 2004 and Khalil, 2006).

The present work is postulated to study the response of wheat growth, yield and some yield components to inoculation with N₂-fixing bacteria under different levels of mineral N-fertilization in sandy loam soil.

MATERIALS AND METHODS

Two field experiments were carried out on a sandy loam soil at El-Tahrir Province Sector during two successive winter-growing seasons of 2005/2006 and 2006/2007 to study the response of wheat growth, yield and some yield components to inoculation with N₂-fixing bacteria under different levels of mineral N-fertilization using sprinkler irrigation system.

Representative soil samples were collected from the experimental fields, sieved and air dried. The main physical and chemical properties of the soil were determined according to Black *et al.* (1965), and presented in Table (1).

Azorhizobium caulinodans, *Gluconacetobacter diazotrophicus*, *Bacillus polymyxa* strains were supplied by Microbiology Department, Soils, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt.

Table(1):Some physical and chemical characteristics of the experimental soil in both seasons

character	Season 2005/2006	Season 2006/2007
Particle size distribution (%):		
Sand	66.8	67.0
Silt	27.0	26.7
Clay	6.2	6.3
Texture grade	Sandy loam	Sandy loam
S.P (%)	40.2	39.8
pH	7.35	7.39
E.C. (dS/m at 25°C)	0.38	0.37
Soluble cations and anions (meq/L):	1.80	1.76
Ca ⁺⁺	0.94	0.92
Mg ⁺⁺	0.81	0.76
Na ⁺	--	--
K ⁺	0.84	0.81
CO ₃ ⁼	0.82	0.77
HCO ₃ ⁼	2.19	2.14
Cl ⁻		
SO ₄ ⁼	0.38	0.39
Organic-C (%)	0.033	0.034
Total N (%)	11.52	11.47
C/N ratio	33.6	34.8
Total soluble-N (ppm)	8.2	8.1
Available-P (ppm)		
DTPA extractable (ppm):		
Fe	4.0	3.8
Mn	3.0	2.9
Zn	1.8	1.9
Cu	0.5	0.7

Azorhizobium caulinodans (strain IRBG314) was grown in TGYE medium (Ladha *et al.*, 1989). *Gluconacetobacter diazotrophicus* was grown on standard medium (MYP) according to Sievers and Swings (2005). *Bacillus polymyxa* was grown on nutrient broth medium as described by Clark (1965). Cultures were incubated at 28°C for three days on rotary shaker until early log phase had been developed to 10⁹ viable cells ml⁻¹, then the cultures were transferred to sterile carrier material. Vermiculite supplemented with 10% Irish peat was packed in polyethylene bags (300g/bag). Bags were then sealed and sterilized by gamma irradiation (5x10⁶ rads). Bacterial culture was injected into sterilized carrier to satisfy 60% of the maximal water holding capacity.

The experiments were arranged as a split plot design with three replications. Since nitrogen treatments represent the main plots, while inoculation treatments represent the subplots, each plot was 6 m². All plots received the recommended dose of superphosphate (15.5% P₂O₅) at a rate of 300 g plot⁻¹ (200 kg fed⁻¹) before sowing and ploughed. Potassium sulphate (48% K₂O) was added at a rate of 150 g plot⁻¹ (100 kg fed⁻¹) after 15 and 30 days from sowing in two equal split doses. Ammonium sulphate (20.5% N) was used as a source of mineral N fertilizer at rates of (zero, 182.9, 365.9,

548.8 and 731.8g ammonium sulphate plot⁻¹) equal to zero, 25, 50, 75 and 100 kg N fed⁻¹. Each N-level was splitted to four equal doses after 10, 20, 30, 40 days from sowing.

Wheat seeds (*Triticum aestivum* cv. Sids-1) supplied by the Wheat Research Dept., Field Crops Research Institute, ARC, Giza, was sown in rows at a rate of 90 g plot⁻¹ (60 kg seeds fed⁻¹). Wheat seeds were inoculated shortly before planting by *Azorhizobium caulinodans*, *Gluconacetobacter diazotrophicus*, *Bacillus polymyxa* as a single treatment or seeds inoculated with mixture of such strains at a rate of 0.9 g inoculant per 90 g seeds plot⁻¹ (600 g inoculant per 60 kg seeds fed⁻¹). Arabic gum solution (16%) was used as a sticking agent. The following treatments were studied:

- Uninoculated.
- Inoculation with *Azorhizobium caulinodans*.
- Inoculation with *Gluconacetobacter diazotrophicus*.
- Inoculation with *Bacillus polymyxa*.
- Inoculation with mixture strains of *Azorhizobium caulinodans* + *Gluconacetobacter diazotrophicus* + *Bacillus polymyxa*.

These treatments were applied either alone or combined with different nitrogen levels, i.e., 25, 50, 75 and 100 kg N fed⁻¹.

After 80 days of planting, ten plants were uprooted from each plot to estimate the plant height, dry weight of roots and shoots and their nitrogen contents.

At harvest, a wood frame (0.5 x 0.5m) was used, immediately before harvest, to take the samples from each plot to guarantee recording data of plant height, number of spikes/m² (as average of two samples for each plot area). After harvest, the following traits were also determined: number of kernels/spike, 1000-kernel weight (g), grain yield (ardab/fed) and straw yield (ton/fed). Also, crude protein percentage in both grains and straw were determined.

The oven dried plant materials were wet digested by using a mixture of sulfuric and perchloric acids at a ratio of 1:1 according to Jackson (1973). Total-N concentration was determined using Microkjeldahl method as described by Page *et al.* (1982). The crude protein percentage in wheat grains and straw was determined by multiplying the nitrogen percentage by 5.7 factor according to A.O.A.C. (1960).

The obtained data were subjected to Analysis of Variance (ANOVA) and L.S.D test was used to compare the treatment means according to the procedures outlined by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

I. Plant growth:

Data in Table (2) show the effect of different N₂-fixing bacteria, different N-levels and their combination on some growth aspects (plant height, dry weight of roots and shoots) of 80-day old wheat plants grown in sandy loam soil. Data mostly showed that all growth parameters significantly responded to different studied treatments.

Table (2): Effect of inoculation, different N-levels and their interaction on plant height, dry weight of roots and shoots of field grown wheat after 80 days from planting in both tested seasons

Treatments	Plant height (cm)		Dry weight of root (g/plant)		Dry weight of shoot (g/plant)		
	Season 2005/2006	Season 2006/2007	Season 2005/2006	Season 2006/2007	Season 2005/2006	Season 2006/2007	
Effect of inoculation (Inoc.)							
Uninoculated	69.20	71.00	0.90	0.91	2.13	2.22	
<i>Azorhizobium caulinodans</i>	73.00	74.00	1.09	1.21	2.61	2.76	
<i>Gluconacetobacter diazotrophicus</i>	77.20	78.20	1.16	1.27	2.77	3.09	
<i>Bacillus polymyxa</i>	77.60	80.60	1.31	1.40	3.07	3.51	
Mixture	80.40	83.40	1.40	1.48	3.25	3.59	
L.S.D. _{0.05}	2.273	1.041	0.052	0.057	0.151	0.322	
Effect of different nitrogen levels (N)							
Zero	60.00	68.20	0.94	1.01	1.94	2.04	
25 kg N/fed	70.00	75.80	1.14	1.23	2.66	3.02	
50 kg N/fed	77.80	81.40	1.26	1.35	2.97	3.32	
75 kg N/fed	81.60	80.80	1.24	1.33	3.10	3.39	
100 kg N/fed	83.40	81.80	1.28	1.36	3.16	3.41	
L.S.D. _{0.05}	2.273	1.041	0.052	0.057	0.151	0.322	
Interaction effect (Inoc. x N)							
Uninoculated	Zero	58.00	61.00	0.74	0.79	1.77	1.82
	25 kg N/fed	65.00	69.00	0.81	0.84	1.82	1.89
	50 kg N/fed	71.00	74.00	0.98	0.82	2.13	2.32
	75 kg N/fed	74.00	76.00	0.96	0.99	2.45	2.53
	100 kg N/fed	78.00	75.00	1.03	1.11	2.47	2.55
<i>Azorhizobium caulinodans</i>	Zero	63.00	68.00	0.85	0.91	1.87	1.93
	25 kg N/fed	67.00	71.00	0.94	0.99	2.54	2.88
	50 kg N/fed	75.00	79.00	1.24	1.42	2.85	2.91
	75 kg N/fed	79.00	77.00	1.18	1.35	2.88	3.00
	100 kg N/fed	81.00	79.00	1.23	1.37	2.91	3.10
<i>Gluconacetobacter diazotrophicus</i>	Zero	66.00	70.00	0.88	0.94	1.92	1.98
	25 kg N/fed	70.00	72.00	1.13	1.32	2.59	2.89
	50 kg N/fed	81.00	83.00	1.27	1.44	3.10	3.41
	75 kg N/fed	85.00	84.00	1.25	1.31	2.99	3.54
	100 kg N/fed	84.00	82.00	1.29	1.33	3.23	3.62
<i>Bacillus polymyxa</i>	Zero	65.00	70.00	1.03	1.19	1.99	2.06
	25 kg N/fed	74.00	81.00	1.37	1.44	3.14	3.74
	50 kg N/fed	78.00	84.00	1.41	1.47	3.29	3.99
	75 kg N/fed	84.00	82.00	1.39	1.49	3.51	3.97
	100 kg N/fed	87.00	86.00	1.37	1.43	3.42	3.84
Mixture	Zero	68.00	72.00	1.21	1.24	2.15	2.40
	25 kg N/fed	77.00	86.00	1.45	1.53	3.19	3.71
	50 kg N/fed	84.00	87.00	1.41	1.58	3.46	3.95
	75 kg N/fed	86.00	85.00	1.44	1.51	3.69	3.91
	100 kg N/fed	87.00	87.00	1.48	1.56	3.75	3.96
L.S.D. _{0.05}	N.S	2.327	0.116	0.127	0.336	N.S	

a) Effect of inoculation:

Data clearly showed that wheat plant heights increased significantly as affected by inoculation with any tested N₂-fixing bacteria. Uninoculated treatment gave shorter wheat reaching the minimal heights of 69.20 and 71.00cm in the first and second seasons, respectively. However, using mixture strains of *A. caulinodans* + *G. diazotrophicus* + *B. polymyxa* followed by single inoculation with *B. polymyxa*, *G. diazotrophicus* or *A. caulinodans* recorded significant increases over the control treatment to be 16.18, 12.14, 11.56 and 5.49% in the first season and 17.46, 13.52, 10.14 and 4.23% in the

second one. This trend indicated that the combination of three diazotrophs gave a positive effect on wheat plant height that may due to the growth promoting substances. These results are in accordance with those obtained by Srinivasan *et al.* (1997), Bai *et al.* (2002), Abo-Kora (2004) and Abo El-Soud *et al.* (2007) who showed that inoculation with mixed culture of nitrogen fixing bacteria gave significant increases in all wheat plant growth. They attributed this enhancement to the possible production of plant growth promoting substances by the N₂-fixing bacteria.

Root and shoot dry weights gave a similar effect of inoculation treatments as those recorded by wheat plant height (Table 2). Values of root dry weight in both tested seasons were (1.40 and 1.48 g/plant), (1.31 and 1.40 g/plant), (1.16 and 1.27 g/plant) and (1.09 and 1.21 g/plant) by using mixture strains, *B. polymyxa*, *G. diazotrophicus* and *A. caulinodans*, respectively. While, uninoculated treatment recorded the lowest values of root dry weights in both seasons to be 0.90 and 0.91 g/plant, respectively. Also, the corresponding values of shoot dry weight at both growing seasons were (3.25 and 3.59 g/plant), (3.07 and 3.51 g/plant), (2.77 and 3.09) and (2.61 and 2.76 g/plant) in the same order mentioned above. The lowest values of shoot dry weight (2.13 and 2.22 g/plant) were obtained by uninoculated treatment. This confirmed again the superiority of using triple inoculation treatment followed by single inoculation with relatively higher values by using *B. polymyxa*. Generally, a comparison between inoculants on wheat growth aspects could arranged in descending order as follows: Mixture (triple inoculation contained three N₂-fixers) > *B. polymyxa* > *G. diazotrophicus* > *A. caulinodans*. These results indicate that inoculation of wheat with mixture of diazotrophs resulted in consistent plant growth promotion. This promotion in wheat growth could be attributed to N₂-fixation and/or certain growth promoting substances such as indole acetic acid and gibberellic acids, which positively affect plant growth. These results stand in accordance with those of Dileep Kumar *et al.* (2001), Saubidet *et al.* (2002), Kennedy *et al.* (2004) and Abo El-Soud *et al.* (2007). They came to same conclusion with pea and wheat crops.

b) Effect of different N-levels:

Data in Table (2) indicate that there were increases in all growth parameters as a result of increasing application rate of mineral N-fertilizer from zero to 100 kg N/fed. Values of plant height fluctuated between 60 to 83.40cm and 68.20 to 81.80cm in the first and second seasons, respectively. The corresponding values of root dry weight ranged from 0.94 to 1.28 g/plant and from 1.01 to 1.36 g/plant in both seasons, respectively. Dry weight of shoot in the first season ranged from 1.94 to 3.16 g/plant and in the second one ranged from 2.04 to 3.41g/plant. The increase in wheat plant growth with increasing the application rate of inorganic N fertilizer may due to the vital role of N-fertilizer in increasing vegetative organs and enhancing plant growth. Such explanation was suggested by Sabry *et al.* (2000), Abo-Kora (2004) Atalla *et al.* (2005), Khalil (2006) and Mahmoud *et al.* (2006) who mentioned that application of N-fertilizer can affect growth and development of plant not

only directly as a constituent of protein, but also indirectly by changing the phytohormone balance, which reflecting by changing in plant morphology.

c) Interaction effect between inoculation and N-fertilization:

Data in Table (2) displayed that all growth parameters responded significantly to the interaction effect between N₂-fixing bacteria and different N-levels, except the plant height in the first season and shoot dry weight in the second one, which were insignificantly responded. It is clear that using any tested bacterial strain in combination with any level of inorganic-N fertilizer gave higher values than using the same N-level without inoculation. In general, using half dose of N-fertilizer in combination with any bacterial strain gave almost similar values to those obtained by using the recommended dose of N-fertilizer (100 kg N/fed) with no significant differences by increasing N-level up to 50 kg N/fed. Meanwhile, wheat plants inoculated with mixture strains followed by *B. polymyxa*, *G. diazotrophicus* or *A. caulinodans* as a single inoculation when combined with half dose of N-fertilizer gave values similar to or higher than using the recommended dose of N-fertilizer. The corresponding increases in plant height at both seasons were (18.31 and 17.57%), (9.86 and 13.51%), (14.08 and 12.16%) and (5.63 and 6.76%), respectively, over the uninoculated treatment received half dose of N-fertilizer (50 kg N/fed). The corresponding increases, over the uninoculated treatment received 50 kg N/fed, of root dry weight in both growing seasons were (43.88 and 92.68%), (43.88 and 79.27%), (29.59 and 75.61%) and (26.53 and 73.17%) as a result of using half dose of N fertilizer and inoculation with the mixture, *B. polymyxa*, *G. diazotrophicus* and *A. caulinodans*, respectively. The increases in shoot dry weight in both seasons were (62.44 and 70.26%), (54.46 and 71.98%), (45.54 and 46.98%) and (33.80 and 25.43%), respectively, in the same order. The variation between two seasons may be related to the differences of weather and other environmental conditions. Such promotive effect of the integrated nitrogen fixing bacteria with half dose of inorganic-N fertilizer (50-60 kg N/fed) was demonstrated by many investigators in newly reclaimed soils (El-Hawary *et al.*, 1998 , Mahmoud *et al.*, 2006 and Abo El-Soud *et al.*, 2007).

II- Nitrogen content in plant tissues:

Data in Table (3) displayed that total N-content in both roots and shoots of 80-day old wheat plants had significantly affected by inoculation, different N-levels and their combination in both growing seasons.

a) Effect of inoculation:

Obtained results declared that the wheat inoculation accumulated higher N than the uninoculated plants. However, the splendid significant effect was observed with the use of the mixture culture followed by single inoculation with *B. polymyxa* for root and shoot nitrogen contents. This trend was true in both growing seasons. The corresponding higher values of root N-content in both growing seasons (15.71 and 16.72 mg N/plant) and (13.60 and 14.93 mg N/plant) were obtained due to the use of the mixture strains and *B. polymyxa*, respectively. While, uninoculated treatment recorded the lowest values (8.21 and 8.69 mg N/plant) in both tested seasons, respectively.

The higher values of shoot N-content in both seasons were (67.36 and 77.08 mg N/plant) and (59.77 and 71.19 mg N/plant) by using the N₂-fixing bacteria mixture and *B. polymyxa*, respectively, as compared to uninoculated treatment, which gave the lowest values of 27.83 and 30.50 mg N/plant. These results suggest the possibility that microbial inoculation, which released plant growth regulators in the rhizosphere may affect on root development and consequently their function in the uptake of both water and nutrients. Such results are in conformity with those of Bai *et al.* (2002), Kennedy *et al.* (2004), Mekhemar *et al.* (2006) and Abo El-Soud *et al.* (2007). They confirmed the promotion of wheat and corn N-contents by N₂-fixing bacteria inoculation.

Table (3): Effect of inoculation, different N-levels and their interaction on root and shoot N-contents of field grown wheat after 80 days from planting in both tested seasons

Treatments		N-content of root (mg/plant)		N-content of shoot (mg/plant)	
		Season 2005/2006	Season 2006/2007	Season 2005/2006	Season 2006/2007
Effect of inoculation (Inoc.)					
Uninoculated		8.21	8.69	27.83	30.50
<i>Azorhizobium caulinodans</i>		11.11	12.62	47.10	49.72
<i>Gluconacetobacter diazotrophicus</i>		12.06	13.89	51.19	60.53
<i>Bacillus polymyxa</i>		13.60	14.93	59.77	71.19
Mixture		15.71	16.72	67.36	77.08
L.S.D. _{0.05}		0.545	0.665	3.204	5.484
Effect of different nitrogen levels (N)					
Zero		7.99	9.02	27.28	29.25
25 kg N/fed		10.29	11.71	43.19	52.19
50 kg N/fed		13.63	14.81	59.29	68.21
75 kg N/fed		14.10	15.25	60.68	68.99
100 kg N/fed		14.66	16.07	62.52	70.38
L.S.D. _{0.05}		0.545	0.665	3.204	5.484
Interaction effect (Inoc. x N)					
Uninoculated	Zero	5.49	6.37	18.93	19.71
	25 kg N/fed	6.69	7.39	20.75	23.13
	50 kg N/fed	8.92	7.73	26.91	30.73
	75 kg N/fed	9.31	10.04	34.06	37.57
	100 kg N/fed	10.64	11.91	38.52	41.36
<i>Azorhizobium caulinodans</i>	Zero	6.97	7.80	26.28	28.44
	25 kg N/fed	8.37	9.63	39.92	48.17
	50 kg N/fed	13.52	14.75	55.76	57.23
	75 kg N/fed	12.75	15.27	56.16	56.91
	100 kg N/fed	13.94	15.64	57.41	57.86
<i>Gluconacetobacter diazotrophicus</i>	Zero	7.31	8.37	28.24	29.31
	25 kg N/fed	9.87	12.72	42.71	50.20
	50 kg N/fed	13.98	16.21	61.70	73.34
	75 kg N/fed	14.30	15.66	59.51	72.06
	100 kg N/fed	14.83	16.49	63.76	77.71
<i>Bacillus polymyxa</i>	Zero	9.03	10.87	29.52	31.03
	25 kg N/fed	12.93	14.16	54.94	68.72
	50 kg N/fed	15.09	16.17	71.93	86.87
	75 kg N/fed	15.66	16.41	72.42	85.84
	100 kg N/fed	15.26	17.05	70.04	83.48
Mixture	Zero	11.17	11.66	33.40	37.76
	25 kg N/fed	13.58	14.65	59.18	70.71
	50 kg N/fed	16.64	19.17	80.13	92.87
	75 kg N/fed	18.48	18.85	81.25	92.55
	100 kg N/fed	18.65	19.25	82.85	91.48
L.S.D. _{0.05}		1.217	1.488	7.165	12.260

b) Effect of different N-levels:

Data in Table (3) demonstrated that increasing of N-level applied to wheat plants (from zero to 100 kg N/fed) led to gradual increases in N-content accumulated in their tissues. This trend was true in both growing seasons. The corresponding values of root N-content ranged from 7.99 to 14.66 mg N/plant and from 9.02 to 16.07 mg N/plant in both seasons, respectively. Values of shoot N-content fluctuated between 27.28 to 62.52 mg N/plant and 29.25 to 70.38 mg N/plant, respectively. However, the addition of full dose of N fertilizer stimulated wheat growth and subsequently had a positive effect on root growth and the absorption sites, which enhance absorption of nutrients. This results was in consistent with those obtained by Galal *et al.* (2001), Badawi (2003), Abo-Kora (2004), Atalla *et al.* (2005) and Mahmoud *et al.* (2006), who mentioned that the increasing in N-uptake of wheat plants were in parallel to increasing the application rate of N-fertilizer from zero to 120 kg N/fed.

c) Interaction effect between inoculation and N-fertilization:

Table (3) showed that the response of both root and shoot N-contents to the interaction effect followed a similar pattern to that of wheat plant growth. The values of accumulated N in root tissues ranged from 5.49 to 18.65 mg N/plant and from 6.37 to 19.25 mg N/plant in the first and second season, respectively. The corresponding values of shoot N-contents fluctuated between 18.93 and 82.85 mg N/plant in the first season and between 19.71 and 92.87 mg N/plant in the second one. In general, wheat plants inoculated with any tested bacterial strains and fertilized with any level of N-fertilizer gave values higher than using the same N-fertilizer level without inoculation. Also, all treatments inoculated with any bacterial strains with 50 kg N/fed gave higher root and shoot N-content values as compared to using 50 kg N/fed alone, while increasing N-level above 50 kg N/fed with inoculation showed insignificant differences. However, using half dose of N-fertilizer along with the mixture bacteria strains followed by *B. polymyxa* gave higher increases in both parameters under investigation. The corresponding increases in root N-contents were 86.55 and 147.99% and 69.17 and 109.18% in both growing seasons, respectively, over those received half dose of mineral N alone. The increases in shoot N-content were 197.77 and 202.21% and 167.30 and 182.69% in the same order. The increases in accumulated N in wheat tissues due to the interaction effect could be explained through the role of available N supplemented by the inoculating organisms and the production of growth promoting substances. These results support those obtained by Dobbelaere *et al.* (2001), Abo-Kora (2004) and Mahmoud *et al.* (2006). They showed a positive effect of the combined application of diazotrophs and nitrogen fertilizer for wheat and agronomically important cereal crops.

III. Some yield parameters:

Data in Table (4) show the effect of inoculation with N₂-fixing bacteria, different levels of mineral N-fertilizer and their interaction on some yield parameters at harvest time of the field grown wheat under sandy loam soil conditions. Obtained results revealed that all yield parameters had significantly affected by inoculation and fertilization treatments in both tested

seasons. While, the response to the interaction were significant in some parameters and insignificant in other ones during the two growing seasons (2005/2006 and 2006/2007).

a) Effect of inoculation:

Data in Table (4) confirmed that all yield parameters at harvest time increased significantly by bacterial inoculation. It is clear that mixture strains followed by *B. polymyxa* inoculants were superior in this regard because they recorded the best results. In general, a comparison between inoculants showed the order: Mixture > *B. polymyxa* > *G. diazotrophicus* > *A. caulinodans* > uninoculated treatment. The corresponding increases in the plant height over the uninoculated control in the first and second season were (13.62 and 16.11%) and (11.91 and 13.59%) by using mixture and *B. polymyxa*, respectively. Increases in No. of spikes/m² were (32.21 and 36.62%) and (24.56 and 32.40%) in the same order, respectively. Increases of No. of kernels/spike in both seasons were (16.04 and 20.48%) and (11.98 and 13.11%), respectively. While, the corresponding increases in 1000-kernel weight were (25.04 and 21.37%) and (17.96 and 17.88%) in both seasons, respectively. These results confirmed again the important role of inoculation with N₂-fixing bacteria in enhancing wheat yield parameters under field conditions. This enhancement could be due to the biological role of such diazotrophs in promoting plant growth, N₂-fixation and phosphorus solubilization. Claims to the promotion effect of inoculation with diazotrophs on wheat yield parameters were reported by many workers (Swedrzynska, 2000; Galal *et al.*, 2001; Saubidet *et al.*, 2002; Atalla *et al.*, 2005 and Abo El-Soud *et al.*, 2007).

b) Effect of different N-levels:

Data in Table (4) showed that values of plant height (cm) fluctuated between (92.20 to 104.80) and (94.60 and 107.80); No. of spikes/m² (267.53 to 351.73) and (282.60 and 371.33); No. of kernels/spike (38.20 to 45.80) and (38.80 to 47.80) and 1000-kernel weight (g) (47.06 to 51.55) and (49.16 to 55.39) in the first and second season, respectively. It is of worth to note that the rate of promotion increased significantly as the rate of mineral N-fertilizer increased. This trend was true in both tested seasons. The pronounced increases in wheat yield parameters, due to N-application could be related to the role of nitrogen nutrient in the anabolic plant processes, which has very important function as main constituent of protein, amino acids and many essential compounds in plant system. The results are in consonance with the findings of Sabry *et al.* (1999), Atalla *et al.* (2005) and Khalil (2006) who reported that wheat plants exhibited marked increases in spikes number/m², grains number/spike, spike grains weight and 1000-grain weight with increasing N fertilization levels up to 75 kg N/fed.

Table (4): Effect of inoculation, different N-levels and their interaction on some yield parameters of field grown wheat in both tested seasons

Treatments	Plant height (cm)		No. of spikes/m ²		No. of kernels/spike		1000-kernel weight (g)		
	Season 2005/2006	Season 2006/2007	Season 2005/2006	Season 2006/2007	Season 2005/2006	Season 2006/2007	Season 2005/2006	Season 2006/2007	
	Effect of inoculation (Inoc.)								
Uninoculated	94.00	94.73	276.07	277.80	39.47	40.67	44.37	47.03	
<i>Azorhizobium caulinodans</i>	98.60	99.20	327.07	355.13	40.80	42.40	49.03	51.44	
<i>Gluconacetobacter diazotrophicus</i>	101.53	103.20	325.20	363.67	43.40	45.00	50.50	52.78	
<i>Bacillus polymyxa</i>	105.20	107.60	343.87	367.80	44.20	46.00	52.34	55.44	
Mixture	106.80	110.00	365.00	379.53	45.80	49.00	55.48	57.08	
L.S.D. 0.05	2.312	3.483	4.729	11.270	2.187	1.640	1.372	2.324	
Effect of different nitrogen levels (N)									
Zero	92.20	94.60	267.53	282.60	38.20	38.80	47.06	49.16	
25 kg N/fed	100.40	103.00	332.73	355.20	42.27	44.27	50.10	52.60	
50 kg N/fed	104.80	103.53	339.47	371.33	43.10	46.80	51.53	53.34	
75 kg N/fed	103.93	105.80	351.73	365.80	45.80	47.80	51.48	53.28	
100 kg N/fed	104.80	107.80	345.73	369.00	44.00	45.40	51.55	55.39	
L.S.D. 0.05	2.312	3.483	4.729	11.270	2.187	1.640	1.372	2.324	
Interaction effect (Inoc. x N)									
Uninoculated	Zero	84.00	87.00	233.33	259.00	35.00	37.00	42.40	44.60
	25 kg N/fed	92.00	93.00	257.67	267.33	41.33	39.33	44.60	45.70
	50 kg N/fed	98.00	94.67	275.00	281.67	41.00	41.00	46.10	44.00
	75 kg N/fed	97.00	99.00	301.33	287.00	41.00	44.00	43.60	46.10
	100kg N/fed	99.00	100.00	313.00	294.00	39.00	42.00	45.13	54.47
	<i>Azorhizobium caulinodans</i>	Zero	89.00	91.00	272.00	276.67	38.00	38.00	44.40
25 kg N/fed		97.00	95.00	334.00	345.00	39.00	41.00	47.60	51.90
50 kg N/fed		103.00	100.00	338.67	398.00	41.00	44.00	51.57	52.40
75 kg N/fed		102.00	104.00	353.33	375.00	43.00	47.00	52.00	53.10
100kg N/fed		102.00	106.00	337.33	381.00	43.00	42.00	49.60	53.60
<i>Gluconacetobacter diazotrophicus</i>		Zero	95.00	97.00	267.67	296.33	39.00	39.00	47.60
	25 kg N/fed	100.00	105.00	329.33	387.00	42.00	45.00	50.80	52.30
	50 kg N/fed	105.00	101.00	331.00	384.00	43.00	45.00	51.70	54.50
	75 kg N/fed	102.67	104.00	356.00	374.00	47.00	49.00	49.50	53.30
	100kg N/fed	105.00	109.00	342.00	377.00	46.00	47.00	52.90	53.80
	<i>Bacillus polymyxa</i>	Zero	97.00	99.00	276.00	282.00	39.00	39.00	49.20
25 kg N/fed		105.00	109.00	358.33	383.00	43.00	44.00	49.60	54.90
50 kg N/fed		109.00	113.00	360.67	389.00	45.00	51.00	53.20	57.80
75 kg N/fed		107.00	110.00	366.00	393.00	49.00	49.00	55.10	55.90
100kg N/fed		108.00	107.00	358.33	392.00	45.00	47.00	54.60	57.50
Mixture		Zero	96.00	99.00	288.67	299.00	40.00	41.00	51.70
	25 kg N/fed	108.00	113.00	384.33	393.67	46.00	52.00	57.90	58.20
	50 kg N/fed	109.00	109.00	392.00	404.00	47.00	53.00	55.10	57.70
	75 kg N/fed	111.00	112.00	382.00	400.00	49.00	50.00	57.20	58.00
	100kg N/fed	110.00	117.00	378.00	401.00	47.00	49.00	55.50	57.60
	L.S.D. 0.05	N.S	N.S	10.570	25.190	N.S	3.667	3.068	N.S

c) Interaction effect between inoculation and N-fertilization:

Table (4) shows the interaction effect of inoculation and different N-levels on some yield parameters of field grown wheat. In general, inoculation with any tested bacterial strains with any level of N-fertilizer gave higher values than uninoculated treatment received the same level of N-fertilizer. Data of plant height in both seasons ranged from (84.00 to 111.00 cm) and (87.00 to 117.00 cm), respectively. Although the response of plant height in both seasons was insignificant, the inoculation with any of bacterial strains in combination with half dose of N-fertilizer gave higher values of wheat plant height, particularly by using the bacteria mixture strains followed by using *B. polymyxa*. Also, data of No. of spikes/m² behaved the same trend obtained in wheat plant height at both seasons under investigation. The highest values of number of spikes/m² were attained by using the bacteria mixture strains plus 50 kg N/fed to be (392 and 404) followed by *B. polymyxa* plus 75 kg N/fed (366 and 393) in the first and second seasons, respectively. Regarding the number of kernels/spike and 1000-kernel weight, data in Table (4) declared that No. of kernels/spike insignificantly responded to such interaction in the first season and significantly responded in the second one. The highest values in the first season were achieved by inoculation with mixture treatment followed by single inoculation with *B. polymyxa* in combination with 75 kg N/fed being (49 and 49 kernel/spike), respectively. While, the highest values of No. of kernels/spike in second season were obtained by using the bacteria mixture strains followed by *B. polymyxa* but in combination with 50 kg N/fed to be (53 and 51 kernel/spike), respectively. Also, 1000-kernel weight took similar trend obtained above. The response of 1000-kernel weight in the first season was significant and gave higher values by using the bacteria mixture strains followed by *B. polymyxa* combined with any level of N-fertilizer (from 25 to 100 kg N/fed). Although the response of 1000-kernel weight to such interaction was insignificant in the second season, data behaved similar trend obtained in the first season. The favorable effects of the combination between N fertilizer and diazotrophs bacteria may be explained on the basis of the beneficial effect of bacteria on the nutrient availability, vital enzymes, hormonal stimulating effect on plant growth or the increasing of photosynthetic activity (Milosev, 1997, El-Shouny *et al.*, 2003, Abo-Kora, 2004 and Mahmoud *et al.*, 2006).

IV. Wheat grain and straw yields and their crude protein percent:

Table (5) shows the effect of inoculation, different N-levels and their interaction on wheat grain and straw yields and their crude protein percent of field grown wheat under sandy loam soil conditions in both seasons of 2005/2006 and 2006/2007.

a) Effect of inoculation:

Obtained results showed that inoculation of wheat with single or the mixture of bacterial strains exerted a valuable improvement in wheat yield components, which resulted in significant increases in grain and straw yields and their crude protein percentage in inoculated treatments in comparison to uninoculated one. The superiority of tested bacterial strains in enhancing wheat yield components under consideration could be arranged as follows:

mixture > *B. polymyxa* > *G. diazotrophicus* > *A. caulinodans*. The corresponding increases in grain yield over the control in both seasons were (52.77 and 46.20%), (41.47 and 32.88%), (21.15 and 20.71%) and (16.54 and 15.92%). Also, straw yield took a similar trend that mentioned above and attained the magnitude of increases by using mixture strains (68.37 and 73.90%) and *B. polymyxa* (40.14 and 54.58%) over the control in both growing seasons. However, the increase in wheat yield over uninoculated control is not necessarily due to the nitrogen fixation by the added inoculum but might be due to the secretion of plant growth hormones and some other factors including a tropical growth condition, which might be supporting such increase due to inoculation. Also, the response of crude protein percent in both grains and straw was in parallel with those of wheat grain and straw yields (Table 5). The increases in grain crude protein percent in both seasons were (25.90 and 24.49) and (22.39 and 18.20) due to the inoculation with the bacteria mixture and *B. polymyxa*, respectively. The corresponding increases percent in straw crude protein in both tested seasons were (37.75 and 17.93) and (43.71 and 14.68), in the same order. These increments in grain and straw crude protein are attributed to increasing the available nitrogen in the root zone and at the same time the increase of N absorption rate by plant. These results are in agreement with those obtained by El-Hawary *et al.* (1998), Nguyen *et al.* (2002), Abo-Kora (2004), Abo El-Soud *et al.* (2007) and Mekhemar *et al.* (2007) who explained that improving effects, arising from microbial inoculation, are due to producing growth promoting substances such as auxins, gibberellins and cytokinins.

b) Effect of different N-levels:

Data in Table (5) demonstrated that all yield components increased significantly with increasing the application rate of mineral N-fertilizer. This trend was true in both growing seasons. Values of grain yield fluctuated between (7.03 and 14.94 ardab/fed) and (6.70 and 14.64 ardab/fed) in both seasons, respectively. Values of straw yield ranged from (2.41 to 4.41 ton/fed) and from (2.48 to 4.68 ton/fed), respectively. Crude protein percentages of grains ranged from (10.71 to 12.96) in the first season and from (10.64 to 13.24) in the second season. Crude protein percentages of straw fluctuated between (3.55 and 4.11) and (3.71 and 4.11) in the first and second season, respectively. It is merit to mention that using full dose of mineral N-fertilizer (100 kg N/fed) achieved the highest values of wheat grain yield. While, wheat plants fertilized with 75 kg N/fed recorded higher values of straw yield as well as crude protein percent of grains and straw. The increase in yield with increasing in N supply might due to the increase in the dry weight of vegetative organs, which could be considered as a criterion for the photosynthetic efficiency of the plant. In addition, increasing application rate of N-fertilizer improved the plant content of amino acids, enzymes, some growth regulators such as auxins and incorporation into protein. These results are in harmony with those obtained by Badawi (2003), El-Wakil and Abd-Alla (2004), Atalla *et al.* (2005), Khalil (2006) and Mahmoud *et al.* (2006) who reported that elevating the levels of applied N up to 50 kg N/fed raised the wheat grain and straw yields and its protein contents.

c) Interaction effect between inoculation and N-fertilization:

Data in Table (5) show the combined effect of inoculation and different N-levels on grain, straw yields and its crude protein percent of field grown wheat in both tested seasons (2005/2006 and 2006/2007). Obtained results confirmed that wheat inoculated with any tested bacterial strains in combination with any tested N-fertilizer level gave values higher than uninoculated treatment fertilized with the same N-fertilizer level. However, triple inoculation treatment followed by single inoculation with *B. polymyxa* gave higher values of such tested wheat yield components when combined with half dose of N-fertilizer. Such treatments gave values nearly similar to or higher than uninoculated treatment received the recommended dose of N-fertilizer. The corresponding increases in grain yield over the uninoculated treatment received 50 kg N/fed (77.24 and 49.70%) and (66.91 and 48.90%) were obtained by using the bacteria mixture strains and *B. polymyxa* in combination with 50 kg N/fed in both tested seasons, respectively. Although the response of straw yield to such interaction was insignificant, using mixture strain plus 50 kg N/fed showed an increase in straw yield (72.73 and 69.03%) over using the same N-fertilizer level without inoculation in the first and second seasons, respectively. While, wheat inoculated with *B. polymyxa* combined with 75 kg N/fed showed increases in straw yield to be (48.90 and 48.01%) over those of uninoculated treatment received 75 kg N/fed in the first and second seasons, respectively.

Also, crude protein percent in both grain and straw had insignificantly responded to such interaction either in season 2005/2006 or season 2006/2007. Crude protein percentage of grains fluctuated from 9.25 to 14.12 in the first season and from 9.66 to 14.61 in the second one. The corresponding crude protein percentage in straw ranged from 2.40 to 4.93 and from 3.34 to 4.54, respectively. Obtained results confirmed again the superiority of using the mixture strains followed by *B. polymyxa* in combination with 25 or 50 kg N/fed in achieving higher percentages of crude protein percent of both wheat grains and straw. The non-significant effect of the interaction treatments on the straw yield and crude protein percent may due to the clear independent effect of the two factors under investigation. Generally, these results imply that wheat plants supplied with 50 kg N/fed and inoculated with the mixture strains of (*A. caulinodans*, *G. diazotrophicus* and *B. polymyxa*) exerted prominent superiority for increasing seed yield, straw yield and its crude protein percent indicating to extending their promotive effect to comprise the productivity and quality of wheat yield. Many workers confirmed that inoculation of wheat with efficient N₂-fixing inoculants could satisfy 50% of their nitrogen requirements (Srinivasan *et al.*, 1997, Oliveira *et al.*, 2002, Abou El-Soud *et al.*, 2007 and Mekhemar *et al.*, 2007).

From these results it could be concluded that the inoculation with N₂-fixing bacteria, particularly in a mixture form, may be acting as a good practice for enhancing wheat growth aspects and improving the crop yield and yield components. Also, it could compensate 50% of the recommended inorganic-N used in sandy loam soil with no decrease in wheat quantity or quality as well as lead to the reduction of environmental pollution due the intensive use of mineral nitrogen fertilizers.

Table (5): Effect of inoculation, different N-levels and their interaction on grain and straw yields and its crude protein percent of field grown wheat in both tested seasons

Treatments	Grain yield (ardab/fed)		Straw yield (ton/fed)		Crude protein of grain (%)		Crude protein of straw (%)		
	Season 2005/2006	Season 2006/2007	Season 2005/2006	Season 2006/2007	Season 2005/2006	Season 2006/2007	Season 2005/2006	Season 2006/2007	
Effect of inoculation (Inoc.)									
Uninoculated	9.55	9.61	2.94	2.95	10.54	10.82	3.02	3.68	
<i>Azorhizobium caulinodans</i>	11.13	11.14	3.46	3.63	12.14	11.93	3.77	3.68	
<i>Gluconacetobacter diazotrophicus</i>	11.57	11.60	3.90	3.96	12.48	12.63	3.99	4.00	
<i>Bacillus polymyxa</i>	13.51	12.77	4.12	4.56	12.90	12.79	4.34	4.22	
Mixture	14.59	14.05	4.95	5.13	13.27	13.47	4.16	4.34	
L.S.D. 0.05	0.841	0.749	0.585	0.564	0.699	0.719	0.346	0.204	
Effect of different nitrogen levels (N)									
Zero	7.03	6.70	2.41	2.48	10.71	10.64	3.55	3.71	
25 kg N/fed	11.16	11.27	3.81	4.09	12.07	12.28	3.84	4.06	
50 kg N/fed	13.29	12.97	4.34	4.52	12.66	12.63	3.88	4.05	
75 kg N/fed	13.93	13.64	4.41	4.68	12.96	13.24	4.11	4.11	
100 kg N/fed	14.94	14.64	4.40	4.46	12.93	12.85	3.91	3.99	
L.S.D. 0.05	0.841	0.749	0.585	0.564	0.699	0.719	0.346	0.204	
Interaction effect (Inoc. x N)									
Uninoculated	Zero	5.82	5.73	1.68	1.70	9.25	9.66	2.40	3.34
	25 kg N/fed	8.62	8.37	2.80	2.73	9.60	9.81	2.84	3.65
	50 kg N/fed	9.58	10.02	3.19	3.52	10.28	10.64	3.12	3.79
	75 kg N/fed	10.85	11.11	3.44	3.46	11.46	11.80	3.38	3.88
	100 kg N/fed	12.87	12.98	3.59	3.32	12.10	12.19	3.36	3.76
<i>Azorhizobium caulinodans</i>	Zero	6.84	5.99	2.06	2.26	11.20	10.52	3.46	3.37
	25 kg N/fed	9.94	9.98	3.22	3.57	11.59	10.87	3.70	3.89
	50 kg N/fed	11.54	11.96	3.87	3.89	12.48	12.39	3.95	3.75
	75 kg N/fed	12.95	13.05	4.11	4.32	13.06	13.52	3.91	3.65
	100 kg N/fed	14.36	14.75	4.04	4.10	12.41	12.37	3.85	3.72
<i>Gluconacetobacter diazotrophicus</i>	Zero	6.97	6.19	2.29	2.34	10.87	10.94	3.74	3.81
	25 kg N/fed	10.25	10.84	3.97	4.05	12.30	12.91	3.94	3.97
	50 kg N/fed	12.38	12.93	4.42	4.59	12.57	13.12	4.05	4.12
	75 kg N/fed	13.58	13.44	4.35	4.61	13.56	13.58	4.26	4.00
	100 kg N/fed	14.67	14.62	4.47	4.21	13.12	12.61	4.00	4.12
<i>Bacillus polymyxa</i>	Zero	7.64	7.08	2.45	2.81	11.00	10.90	3.98	3.92
	25 kg N/fed	12.26	12.38	4.14	4.93	12.95	13.21	4.39	4.38
	50 kg N/fed	15.99	14.92	4.71	4.66	14.12	13.00	4.15	4.21
	75 kg N/fed	15.78	14.61	4.75	5.21	12.98	13.73	4.93	4.54
	100 kg N/fed	15.88	14.87	4.55	5.19	13.45	13.11	4.23	4.05
Mixture	Zero	7.86	8.52	3.57	3.29	11.21	11.19	4.18	4.09
	25 kg N/fed	14.72	14.76	4.92	5.17	13.91	14.61	4.33	4.41
	50 kg N/fed	16.98	15.00	5.51	5.95	13.90	13.98	4.15	4.40
	75 kg N/fed	16.49	15.99	5.42	5.78	13.75	13.59	4.07	4.49
	100 kg N/fed	16.92	15.97	5.33	5.48	13.58	13.96	4.09	4.31
L.S.D. 0.05	1.880	1.674	N.S	N.S	N.S	N.S	N.S	N.S	

REFERENCES

- Abo El-Soud, A.A.; B.A.A. Kandil and B.A. Hasouna (2007). Response of wheat growth and yield to N₂-fixer bacteria combined with plant growth promoting rhizobacteria. *Egypt. J. of Appl. Sci.*, 22: 670-681.
- Abo-Kora, Hanaa A. (2004). Studies on nitrogen fixers producing exopolysaccharides in the rhizosphere of some economic plants. Ph.D. Thesis, Fac. Agric., Minufiya Univ., Egypt.
- Antoun, H.; J. Chantal., N. Goussard., R. Chabot and R. Ialande (1998). Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Raphanus sativus* L.). *Plant and Soil*. 204: 57-67.
- A.O.A.C. (1960). Official Methods of Analysis. 9th ed. Association of Official Analytical Chemists, Washington D.C., USA.
- Atalla, R.A.A.; M.Kh. Moshref and A.M.M. Biomy (2005). Evaluation the efficiency of seeding rate and inoculation with some diazotrophs on growth, yield and protein content of wheat crop (*Triticum aestivum* L.) grown in sandy soils under graded levels of N-fertilizer. *J. Agric. Sci., Mansoura Univ.*, 30: 5527-5538.
- Badawi, F.Sh.F. (2003). Studies on bio-organic fertilization of wheat under newly reclaimed soils. Ph.D. Thesis, Fac. Agric., Cairo Univ., Egypt.
- Bai, Y.; A. Souleimanov and D. L. Smith (2002). An inducible activator produced by *Serratia proteamaculans* strain and its soybean growth-promoting activity under greenhouse conditions. *J. Exp. Botany*. 53: 1495-1502.
- Bastian, F.; A. Cohen, P. Luna, V. Baraldi and R. Bottini (1998). Production of indole-3 acetic acid and gibberellins A₁ and A₃ by *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae* in chemically defined culture media. *Plant Growth Regul.*, 24: 7-11.
- Black, C.A.; D.D. Evans, L.E. Ensminger, J.L. White, F.E. Clark and R.C. Dinauer (1965). "Methods of Soil Analysis". II Chemical and Microbiological Properties. Amer. Soc. Agron. Inc. Bull., Madison, Wisconsin., USA.
- Boddey, R.M.; S. Urquiaga, B.J.R. Alves and V.M. Reis (2003). Endophytic nitrogen fixation in sugarcane: present knowledge and future applications. *Plant and Soil*. 252: 139-149.
- Boddey, R.M.; S. Urquiaga, V.M. Reis and J. Dobereiner (1991). Biological nitrogen fixation associated with sugarcane. *Plant and Soil*. 37: 111-117.
- Clark, F.E. (1965). Agar-plate methods for total microbial count. In: C.A. Clack *et al.* (eds), *Methods of Soil Analysis, Part 2 Agronomy*, 9: 1460-1466, Amer. Soc. of Agron., Inc., Madison, Wisconsin., USA.
- Dileep Kumar, B.S.; I. Berggren and A.M. Martensson (2001). Potential for improving pea production by co-inoculation with fluorescent *Pseudomonas* and *Rhizobium*. *Plant and Soil*. 229: 25-34.

- Dobbelaere, S.; A. Croonenborghs, A. Thys, D. Ptacek, P. Dutto, J. Vanderleyden, S. Brener, S. Burdman, G.C. Gonzalez, M.J. Caballero, A.J. Franciso, Y. Kapulnik, D. Kadouri, S. Saring and Y. Okon (2001). Response of agronomically important crops to inoculation with *Azospirillum*. *Australian J. Plant Physiol.*, 28: 871-879.
- Dobbelaere, S.; J. Vanderleyden and Y. Okon (2003). Plant growth promoting effects of diazotrophs in the rhizosphere. *Crit. Rev. in Plant Sci.*, 22: 107-149.
- Egamberdiyeva, D. and G. Hoflich (2004). Effect of plant growth promoting bacteria on growth and nutrient uptake of cotton and pea in a semi-arid region. *J. Arid. Environ.*, 56: 293-301.
- El-Hawary, F.; I. Ibrahim and F. Hammouda (1998). Effect of integrated bacterial fertilization on yield components of wheat in sandy soil. The regional Symposium on Agrotechnology based on Biological Nitrogen Fixation for Desert Agriculture. P. 78, El-Arish, North Sinai, Egypt.
- El-Shouny, K.A.; Sh.M. Selim, R.A. Mitkees, Sabah H. Abou El-Ela and Hala A.M. El-Sayed (2003). Selection in wheat under biofertilization with nitrogen fixing bacteria. *J. Environ. Sci.*, 6: 515-542.
- El-Wakil, Nadia A.R. and Maha M. Abd-Alla (2004). Influence of seeding rate and nitrogen fertilization level on yield and its attributes of some wheat cultivars. *Egypt. J. Appl. Sci.*, 19: 129-150.
- Gaikwad, R.M. and P.V. Wani (2001). Response of *Brinjal* (cv. *Krishna*) to phosphate solubilizing biofertilizers. *J. Maharashtra Agric. Univ.*, 26:29-32.
- Gajda, I. and P. Kurzawinska (2004). Biological protection of potato against *Helminthosporium solani* and *Rhizoctonia solani*. *Phytopathol. Polonica*. 34: 51-58.
- Galal, Y.G.M. ; I.A. El-Ghandour and E.A. El-Akel (2001). Stimulation of wheat growth and N₂-fixation through *Azospirillum* and *Rhizobium* inoculation.: A field trial with N¹⁵ technique. In: W.J. Horst *et al.* (Ed.) *Plant Nutrition: Food security and sustainability of Agro. Ecosystems*, p.666-667, Kluwer Academic Publishers, Dordrecht, the Netherland.
- Jackson, M.L. (1973). "Soil Chemical Analysis". Prentice- Hall of India Private Limited, New Delhi, India.
- Kennedy, I.R.; A.T.M.A. Choudhury and Mihaly L. Kecskes (2004). Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited. *Soil Biol. & Biochem.*, 36: 1229-1244.
- Kenneth, J.O.; R.D. Michael and E.C. Cocking (1997). Xylem colonization of the legume *Sesbania rostrata* by *Azorhizobium caulinodans*. *Proc. R. Soc. Land. B.*, 264: 1821-1826.
- Khalil, Zeinab M. (2006). Physiological studies on using nitrogen-fixing *Bacillus polymyxa* as a biofertilizer for wheat plant in sandy soil. Ph.D. Thesis, Fac. Sci., Cairo Univ., Egypt.
- Kloepper, J.W. (2003). A Review of Mechanisms for Plant Growth Promotion by PGPR. 6th Internat. PGPR Workshop, 5-10 October, Calcutta, India.

- Ladha, J.K.; M. Garcia ; S. Miyan ; A. Padre and I. Watanabe (1989). Survival of *Azorhizobium caulinodans* in the soils and rhizosphere of wetland rice under *Sesbania rostrata*-rice rotation. *Appl. Environ. Microbiol.*, 55:454-460.
- Lebuhn, M.; T. Heulin and A. Hartmann (1997). Production of auxin and other indolic and phenolic compounds by *Paenibacillus polymyxa* strains isolated from different proximity to plant roots. *FEMS Microbiol. Ecol.*, 22: 325-334.
- Mahmoud, M.M.; Nadia M.A. Ghalab, H.H. Abotaleb and S.F. Mansour (2006). Response of wheat plants to inoculation with diazotrophic bacteria under different levels and forms of mineral N-fertilizers in south Sinai soils. *Egypt. J. Appl. Sci.*, 21: 670-685.
- Mekhemar, G.A.A.; F.Sh.F. Badawi, T.E.E. Radwan and B.A. Hasouna (2007). Assessment of multi-strain PGPRs biofertilization as compared to sole-strain or mineral N-fertilization on wheat plants grown in clayey soil in Egypt. *Egypt. J. Biotechnol.*, 25: 27-44.
- Mekhemar, G.A.A.; Nadia M.A. Ghalab, F.Sh.F. Badawi and A.A. Abo El-Soud (2006). Response of wheat to inoculation with *Azorhizobium* and *Azospirillum* as affected by fungicides. *Egypt. J. of Appl. Sci.*, 21: 317-339.
- Milosev, D. (1997). Grain yield per spike as influence by nitrogen and temperature at grain formation in wheat. *Letopis Naucnih Radora (Yugoslaviva)*. 21: 30-41.
- Nguyen, T.H. ; I.R. Kennedy and R.J. Royghley (2002). The response of field-grown rice to inoculation with multi-strain biofertilizer in Hanoi district, Vietnam. In: Kennedy, I.R., Choudhury, A.T.M.A. (Eds.), *Biofertilizers in Action*. Rural Industries Research and Development Corporation, Canberra, Australia.
- Oliveira, A.L.M.; S. Urquiaga, J. Dobereiner and J.I. Baldani (2002). The effect of inoculating endophytic N₂-fixing bacteria on micropropagated sugarcane plants. *Plant and Soil*. 242: 205-215.
- Page, A.L.; R.H. Miller and D.R. Keeney (1982). "Methods of Soil Analysis". II- Chemical and Microbiological Properties. *Soil Sci. Amer.*, Madison Wisconsin, USA.
- Sabry, S.R.S.; E.M. Taha and A.A. Khattab (1999). Response of long spike wheat (*Triticum aestivum* L.) genotypes to nitrogen fertilizer levels in soil of Middle Egypt. *Bull. Fac. Agric. Cairo Univ.*, 50: 169-188.
- Sabry, S.R.S.; S.A. Saleh and A.A. Ragab (2000). Response of wheat (*Triticum aestivum* L.) to dual inoculation with *Azorhizobium caulinodans* and VA Mycorrhizae under different nitrogen fertilizer levels. *Zagazig J. Agric. Res.*, 47: 145-158.
- Sabry, S.R.S.; S.A. Saleh, C.A. Batchelor, J. Jones, J. Webster, S.L. Kothari, M.R. Davey and E.C. Cocking (1997). Endophytic establishment of *Azorhizobium caulinodans* in wheat. *Proc. of the Royal Society, London: Biological Sciences*. 264: 341-346.

- Saravanan, V.S.; P. Kalaiarasan, M. Madhaiyan and M. Thangaraju (2007). Solubilization of insoluble zinc compounds by *Gluconacetobacter diazotrophicus* and the detrimental action of zinc ion (Zn^{+2}) and zinc chelates on root knot nematode *Meloidogyne incognita*. Lett App. Microbiol., 44: 235-241.
- Saubidet, Maria I.; Nora Fatta and Atilio J. Barneix (2002). The effect of inoculation with *Azospirillum brasilense* on growth and nitrogen utilization by wheat plants. Plant and Soil. 245: 215-222.
- Sievers, M. and J. Swings (2005). Cultivation media for *Acetobacter*. In: Bergey's Manual of Systematic Bacteriology. Don J. Brenner, Noel R. Krieg and James T. Staley (eds.), 2nd Ed., Vol. 2, Part C, pp. 51-54, USA.
- Snedecor, G.W. and W.G. Cochran (1980). "Statistical Methods" 7th Ed., Iowa State Univ. Press, Amr., USA, pp. 255-269.
- Srinivasan, M.; D.J. Peterson and F.B. Holl (1997). Nodulation of *Phaseolus vulgaris* by *Rhizobium etli* is enhanced by the presence of *Bacillus*. Can. J. Microbiol., 43: 1-8.
- Swedrzyńska, D. (2000). Effect of inoculation with *Azospirillum brasilense* on development and yielding of winter wheat and oat under different cultivation conditions. Pollsk-Journal of Environmental Studies. 9: 423-428.
- Webster, G.; C. Gough, J. Vasse, C.A. Batchelor, K.L. Collaghan, S.L. Kothari, M.R. Davey, J. Demarie and E.C. Cocking (1997). Interaction of rhizobia with rice and wheat. Plant and Soil. 144: 115-122.

استجابة القمح لمستويات مختلفة من النيتروجين المعدني والتلقيح

بالبكتيريا المثبتة للنيتروجين

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أجريت تجربتين حقليتين فى أرض رملية سلتية بقطاع جنوب التحرير خلال الموسمين الشتويين المتتاليين 2006/2005 و 2007/2006 لدراسة استجابة النمو الخضري والمحصول وبعض مكونات المحصول لنباتات القمح للتلقيح بالبكتيريا المثبتة لنيتروجين الهواء الجوى متداخلة مع مستويات مختلفة من التسميد النيتروجينى المعدنى تحت نظام الرى بالرش. تم تلقيح حبوب القمح صنف سدس 1 قبل الزراعة بفترة قصيرة ببكتيريا الأزوريزوبيم ، الأستوباكتر ، الباسيلس بوليمكسا كل منهم على حدة كمعاملات فردية وكذلك المعاملة المشتركة (اللحاق الثلاثى المشترك). تم اختبار هذه اللقاحات متداخلة مع مستويات مختلفة من التسميد النيتروجينى المعدنى (صفر ، 25، 50، 75 و 100 كجم/ن/فدان). تم استخدام سماد كبريتات الأمونيوم (20.5 ن) لإضافة معاملات التسميد النيتروجينى المعدنى. كما تم إضافة المعدل الموصى به للفدان من سماد السوبر فوسفات (15.5% فو2أ5) وكبريتات البوتاسيوم (48% بوأ2) بمعدل 200 كجم و 100 كجم سماد، على التوالى. وكان تصميم التجارب هى قطع منشقة مرة واحدة ومساحة القطعة التجريبية كانت 6م². بالنسبة لاستجابة نباتات القمح للتلقيح بالبكتيريا المثبتة لنيتروجين الهواء الجوى ، أشارت نتائج الموسمين أن هناك زيادة معنوية فى جميع صفات النمو الخضري والمحصول وبعض مكونات المحصول نتيجة التلقيح بأي نوع من مثبتات الأزوت الجوى. أظهرت المعاملة المشتركة (معاملة

اللقاح الثلاثي يتبعها معاملة التلقيح الفردي بالباسيلس بوليمكسا تفوقاً ملحوظاً على باقى المعاملات فى جميع الصفات تحت الدراسة. وبمقارنة تأثير معاملات التلقيح المختلفة على جميع الصفات الخضريّة والمحصول وبعض مكونات المحصول لنباتات القمح فقد أخذت الترتيب التالى: معاملة اللقاح الثلاثي < الباسيلس بوليمكسا < الأسيوباكتر < الأزوريزوبيم < المعاملة الغير ملقحة. استجابت معنوياً قياسات النمو الخضري والمحصول وبعض مكونات المحصول لنباتات القمح للزيادة من معدل التسميد النيتروجيني المعدنى. وعموماً فإن استخدام المعدل العالى من السماد النيتروجيني المعدنى (100 كجم/ن/فدان) أعطى أعلى قيم لجميع الصفات تحت الدراسة فى كلا الموسمين.

أشارت النتائج الخاصة بالتأثير المشترك لكل من التلقيح ومستويات النتروجين المعدنى المختلفة الى الحصول على أفضل نتائج للنمو والمحصول وبعض مكونات المحصول لنباتات القمح باستخدام معاملة التلقيح المشترك (معاملة اللقاح الثلاثي) يليها التلقيح الفردي بالباسيلس بوليمكسا فى وجود 50 كجم/ن/فدان (نصف الجرعة الكاملة). وكان لهذه المعاملات تأثيراً إيجابياً وأعطت نتائج تشابه تقريباً أو تتفوق على النتائج المتحصل عليها من استخدام الجرعة الكاملة من التسميد النيتروجيني المعدنى (100 كجم/ن/فدان) وكان هذا التأثير واضح خلال موسمى الزراعة تحت الدراسة.

ومن هذه النتائج يمكن استنتاج أن التلقيح بالبكتيريا المثبتة لنيتروجين الهواء الجوى (خصوصاً اللقاح الثلاثي) هو إجراء جيد يعمل على تحسين صفات النمو والمحصول وبعض مكونات المحصول لنباتات القمح. كذلك يمكن أن يعوض ما يوازي 50% من الكمية الموصى بها من السماد النيتروجيني المعدنى المستخدمة فى الأراضى الرملية دون التأثير على إنتاجية وجودة محصول القمح.