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Effect of Inoculation with Diazotrophs on Wheat Productivity under Different N-Fertilizer Levels in Sandy Soils

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ABSTRACT

Two field experiments were carried out on a sandy soil at Ismailia Agricultural Research Station located at 30º 35´ 41.901˝ N for Latitude and 32º 16´ 45.834˝ E for Longitude, Egypt, during two winter seasons of 2013/14 and 2014/15, to study the effect of inoculation with diazotrophs (*Herbaspirillum* sp., *Azorhizobium caulinodans* and *Azospirillum brasilense*) applied as a single treatment and\or as a mixture treatments under different levels of nitrogen, i.e., zero, 25, 50, 75 and 100 kg N/fed on the growth, yield and some yield components of wheat crop. Obtained results showed that there were significant increases in all wheat vegetative growth characters, yield and some yield components due to inoculation with any tested diaztotrophs. However, using mixture strains followed by *Azospirillum brasilense* surpassed other inoculated treatments or uninoculated one. Generally, all the inoculated treatments were the superior to in enhancing all wheat growth parameters, wheat yield and its components in the order of mixture> *Azospirillum brasilense*> *Herbaspirillum* sp.> *Azorhizobium caulinodans*> uninoculated.All wheat growth characters yield and its components increased significantly along with increasing N-fertilizer level. Due to the interaction between inoculation and N-fertilizer levels, the splendid results of wheat growth parameters, yield and its components were for wheat plants inoculated with the bacterial mixture inoculum followed by *Azospirillum brasilense* in combination with 50 kg N/fed, which showed significantly a positive response and gave values nearly similar to or higher than those given by the use of full N-fertilizer level (100 kg N/fed). It is of worth to note that inoculation with diazotrophs having excellent potential to be used with non-legumes not only because of their N_2-fixing proficiency but also to their ability to acts as PGPR. The triple diazotrophs treatment may be acting as a good practice for sustaining wheat grain yield and reduce the reliance on chemical fertilizers in agriculture, to minimize the environmental pollution and to have safe healthy food.

Keywords: Wheat (*Triticum aestivum*), Diazotrophic bacteria, *Herbaspirillum* sp, *Azorhizobium caulinodans*, *Azospirillum brasilense*, Inorganic-N fertilizer and Sandy soil.

INTRODUCTION

Wheat is one of the most important crops within the cereals grown for green forage for feeding animals on straw as well as its grains also as food for more than 33% of the world's population. The world's overpopulation has resulted in a wide gab between production and consumption of most of the cereals and field crops. Intensive crop cultivation led to the use of huge amounts of chemical fertilizers, which are not only so expensive but also do as agroecosystem's pollutants. Efforts are being done to minimize the amounts of chemical fertilization by using biofertilizers, which might improve the cleanness of agricultural products and decrease the environmental pollution.

Use of nitrogen fertilizer is of great importance in agriculture, as nitrogen is the major factor limiting growth under most conditions. Since agriculture is expected to move toward environmentally sustainable methods, much attention has been paid to natural methods of biological nitrogen fixation (Mohanta *et al.,* 2010). Much information has accumulated on the association of diazotrophs and the root of non-legumes with the expectation that these bacteria would fix dinitrogen gas and provide combined nitrogen to the plant for enhanced cereal crop production. Plant growth promoting rhizobacteria (PGPR) that microorganisms colonize the rhizosphere and have definite beneficial role in the fertility of soil, rhizosphere and root seedlings (Verma *et al.,* 2010 and Santi *et al.,* 2013). The diazotrophic PGPR in detail, highlighting their mechanisms of action including BNF, plant growth promotion by production of auxins, cytokinins, gibberellins and ethylene, P-solubilization, increased nutrient uptake, enhanced stress resistance, vitamin production and biocontrol (Compant *et al.,* 2010 and Mohanta *et al.,* 2010). The practical reason for studying the diazotrophic bacteria is that they provide a portion of the nitrogen needed by the plants, diminishing the need for the use of chemical fertilizers in agricultural fields as well as may influence plant growth, alter root bacterial enzyme activities and alter plant root growth due to bacteria produced growth relating substances (Mohanta *et al.,* 2010).

There are a large number of different diazotrophic as well as non-diazotrophic species may contribute to the beneficial effects on the growth and yield of cereals, including *Herbasirillum*, presumably by stimulating greater plant root growth (Kennedy *et al.,* 2004). *Herbaspirillum* is an endophyte which colonizes sugarcane, rice, maize, sorghum and other cereals (James *et al.,* 2000 and Magnani *et al.,* 2010). Endophytic diazotrophs such as *Herbaspirillum* sp. was found in the intercellular space of stems in sugarcane and rice (Mohanta *et al.,* 2010 & Reinhold-Hurek and Hurek, 2011). Sabry and Saleh (2001) and Carvalho *et al.* (2014) confirmed the superiority of inoculation with *Herbaspirillum* plus inorganic-N fertilizer for enhancing wheat growth, yield and its yield components.

Azorhizobium caulinodans which induces root and stem nodules on the tropical legumes *Sesbania rostrata* is considered a highly promising microbe for fixing atmospheric nitrogen in cereals due to its ability to fix N_2 in the free living state as well as in symbiosis. It has the ability to enter cereals root by crack entry infection intercellularly between adjacent cells and not by the formation of infection threads at the tip of root hairs (Sabry *et al.,* 1997 and Sabry *et al.,* 2000). The response of non-legume crops to inoculation with *Azorhizobium* was studied by many investigators (Sabry *et al.,* 2000; Mekhemar, 2008 and Islam *et al.,* 2009). They found that wheat growth parameters, yield and some yield components significantly increased due to inoculation of wheat seeds with *Azorhizobium caulinodans*. These beneficial effects are related not only to its N_2-fixing proficiency but also to the ability of producing two major classes of plant growth regulators (auxin and gibberellins), antibacterial and antifungal.

Bacteria of the genus *Azospirillum* are associative nitrogen (N_2)-fixing rhizobacteria that are found in close association with plant roots. Microscopical evidence as to the endophytic nature of *Azospirillum* has been showed by Baldani *et al.* (1993). No gross changes in root morphology, but cross section of roots related altered and more irregular arrangements of cells in the outer four to five layers of the cortex. This suggests that *Azospirillum* softens the middle lamellae through the action of pectionlytic enzymes, thus enhancing nutrient elements absorption throughout surface of cortex cells in a kind of sponge effect (Okon and Labandero-Gonzalez, 1994). It is able to exert beneficial effects on plant growth and yield of many agronomic crops under a variety of environmental and soil conditions. The bacteria affect plant growth by producing some phytohormones or plant growth regulators (PGRs), fix nitrogen, increase phosphorus availability, enhance mineral uptake and/or suppress plant pathogens (Saubidet *et al.,* 2002; Islam *et al.,* 2009 and Carvalho *et al.,* 2014).

The present work is to study the effect of different of diazotrophs inoculation and different nitrogen fertilizer levels on wheat growth, yield and some yield components to in sandy soil.

MATERIALS AND METHODS

Bacterial strains and growth condition:

Herbaspirillum sp., *Azorhizobium caulinodans* (strain ORS 571) and *Azospirillum brasilense* (strain SP 245) were supplied by Microbiology Department, Soils, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt. *Herbaspirillum* was grown on nutrient broth medium (Difico Manual, 1984), *Azorhizobium* was grown in TGYE medium (Ladha *et al.,* 1989) and *Azospirillum* was grown in YEP medium (Vanstockem *et al.,* 1987). Cultures were incubated at 28^oC for three days on rotary shaker until early log phase was developed to 10^9 viable cells ml^{-1}, and then the cultures were transferred to sterile carrier material.

Preparation of inoculants:

Vermiculite supplemented with 10% Irish peat was packed in polyethylene bags (300 g bag^{-1}). Bags were then sealed and sterilized by gamma irradiation ($5x10^6$ rads). Bacterial culture was injected into sterilized carrier to satisfy 60% of the maximal water holding capacity.

Field experiments:

Two field experiments were conducted in the sandy soil farm at EL-Ismaillia Experimental Research Station (ARC), EL-Ismaillia Governorate (Latitude 30° 35′ 41. 901″ N and Longitude 32° 16′ 45. 843″ E), Egypt, during two winter seasons of 2013/14 and 2014/15 to study the effect of inoculation with diazotrophs on the growth, yield and some yield components of wheat crop (*Triticum aestivum* cv. Giza 168) under different nitrogen fertilizer levels using sprinkler irrigation system. The main initial physical and chemical properties of the experimental soil (Piper, 1950 and Page *et al.,* 1982) are shown in Table (1).

The experiments were arranged as a split plot design with three replications, each plot was 8.4 m^2. Nitrogen fertilizer levels represent the main plots and the biofertilizer represents the sub plots. Mature compost at rate of (10 ton fed^{-1}) was applied to all studied treatments during soil preparation. The main chemical and biological properties (Page *et al,*. (1982) and its seed germination test was evaluated according to Pare *et al.* (1997) using cress seeds (*Lepidium staivum* L., local variety) (Table 2).

All plots received nitrogen at rates of zero, 25, 50, 75 and 100 kg N fed^{-1} in the form of Ammonium sulphate (20.5% N), while, both phosphorus and potassium were applied at recommended doses of 200 and 100 kg fed^{-1} in the form of superphosphate (15% P_2O_5) and Potassium sulphate (48% K_2O). Nitrogen was applied in four equal doses at10, 20, 30 and 40 days from sowing, phosphorus was added during soil preparation and potassium was added after 15 and 30 days from sowing in equal two doses.

Wheat seeds (Field Crops Res. Inst., ARC, Giza, was sown in rows at a rate of 60 kg seeds fed^{-1}. They were inoculated shortly using coating technique before sowing by *Herbaspirillum* sp., *Azorhizobium caulinodans* and *Azospirillum brasilense* as a single treatment or seeds inoculated with mixture of such strains at a rate of 600 g inoculum per 60 kg seeds fed^{-1}).

The experiment includes the following treatments:

- Uninoculated.
- Inoculation with *Herbaspirillum* sp.
- Inoculation with *Azorhizobium caulinodans*.
- Inoculation with *Azospirillum brasilense*.
- Inoculation with mixture of (*Herbaspirillum +Azorhizobium caulinodans+ Azospirillum brasilense*).

These inoculation treatments were interacted with the different applied nitrogen levels.

Obtained results were subjected to analysis of variance (ANOVA) and L.S.D test was used to compare the treatment means according to the procedure of Snedecor and Cochran (1980).

At 80 days from sowing, ten wheat plants were uprooted from each plot and assayed for plant height, dry weight of roots and shoots as well as their nitrogen content.

Table 1. Initial physical and chemical properties of the experimental soil

Property	Season 2013/14	Season 2014/15
Particle size distribution (%):		
Sand	89.8	90.2
Silt	3.9	3.7
Clay	6.3	6.1
Texture grade	Sandy	Sandy
Saturation percent (S.P %)	20.20	20.33
pH (soil paste)	7.39	7.37
E.C (dS m^{-1}, at 25°C)	0.31	0.33
Soluble cations (meq/L) :		
Ca^{++}	0.51	0.58
Mg^{++}	0.36	0.39
Na^{+}	1.51	1.57
K^{+}	0.68	0.67
Soluble anions (meq/L) :		
$CO^{=}_{3}$	0.00	0.00
HCO^{-}_{3}	0.86	0.89
Cl^{-}	0.63	0.69
$SO^{=}_{4}$	1.57	1.63
Organic matter (%)	0.24	0.28
Organic-C (%)	0.14	0.16
Total-N (%)	0.021	0.024
C/N ratio	7.00	6.67
Total soluble- N (mg kg^{-1})	15.90	16.46
Available- P (mg kg^{-1})	6.49	6.58
Available-K (mg kg^{-1})	39.33	40.25
DTPA-extractable (mg kg^{-1}):		
Fe	1.29	1.26
Mn	0.32	0.33
Zn	0.47	0.49
Cu	0.27	0.25

DTPA: Di-ethylene tri-amine penta acetic acid

Table 2.The main characteristics of the used compost

Property	Value
pH	6.75
EC (dS/m)	5.90
Organic-C (%)	26.96
Organic matter (%)	46.37
Total-N (%)	1.47
C/N ratio	18.34
Total-P (%)	1.11
Total-K (%)	0.96
Total soluble-N (mg kg^{-1})	763.1
Available-P (mg kg^{-1})	262
Available-K (mg kg^{-1})	869.3
DTPA*-extractable Fe (mg kg^{-1})	598.2
DTPA-extractable Mn (mg kg^{-1})	66.2
DTPA-extractable Zn (mg kg^{-1})	45.6
DTPA-extractable Cu (mg kg^{-1})	6.9
Total count of bacteria (cfu/g)	13×10^{7}
Total count of fungi (cfu/g)	12×10^{6}
Total count of actinomycetes (cfu/g)	2.4×10^{6}
Dehydrogenase activity (mg TPF**/g)	131.4
Nitrogenase activity (n mol C_2H_4/g/h)	86.5
Germination test of cress seeds*** (%)	81.65

*** Di-ethylene tri-amine penta acetic acid**
****Tri-Phenyl-Formazan ***Cress seeds incubated for 48 hr.**

At harvest, plant samples with wooden frame (0.5 x 0.5m) were immediately collected before harvest to record data of plant height, number of spikes m^{-2} (as an average of two samples for each plot area). After harvest, the following traits were also determined: number of kernels $spike^{-1}$, 1000-kernel weight (g) as an average of four 100-kernel random samples, grain yield ardab/fed (one ardab = 150 kg) and straw yield (ton/fed) and one feddan = 4200 m^2.

Nitrogen contents of wheat shoots, roots, grains and straw were determined according to Jackson (1973) while Crude protein percentage in both grains and straw were also determined by multiplying the nitrogen percentage by 5.7 factor according to A.O.A.C. (1960).

RESULTS AND DISCUSSION

I- Effect of inoculation with diazotrophs on growth and N-accumulation in plant tissues under different N-levels in sandy soil:

1) Plant growth:

Wheat plants characters, i.e., plant height (cm), dry weight of roots and dry weight of shoots after 80 days of sowing as affected by diazotrophic bacterial inoculation (*Herbaspirillum* sp., *Azorhizobium caulinodans* and *Azospirillum brasilense*), different N-fertilizer levels (zero, 25, 50, 75 and 100 kg N/fed) and their combinations are shown in Table (3). Results revealed that these characters increased significantly in response to these treatments.

a) Effect of inoculation:

Obtained results revealed that uninoculated treatment gave shorter wheat plants (66.47 and 68.20 cm) than those inoculated with any tested bacterial strain. Wheat plants singly inoculated with *Herbaspirillum, Azorhizobium* or *Azospirillum* gave relative plant height of 75.27 and 76.87 cm, 70.20 and 73.00 cm and 75.13 and 75.40 cm in both seasons, respectively. However, wheat plants inoculated with mixture of the tested bacterial strains exerted a salient superiority in values of plant height (78.13 and 80.60cm) in both seasons, respectively. The promotive effect of such inoculation treatments, particularly the triple diazotrophs treatment, may be presumed by high potency of used bacteria to produce physiologically active quantities of auxins, which may exert pronounced effects on plant growth and establishment (Mekhemar, 2008 and Verma *et al.*, 2010).

Root and shoot dry weights gave a similar effect of inoculation treatments as those recorded by wheat plant height. Data in Table (3) demonstrated that inoculation with any tested bacterial strain increased significantly the root and shoot dry weights compared to those of uninoculated plants with relatively higher values due to the use of *Azospirillum brasilense*. However, the inoculation of wheat plants with bacterial mixture of *Herbaspirillum* sp., *Azorhizobium caulinodans* and *Azospirillum brasilense* flourished greatly the growth of wheat plants and gave increases in root dry weight (67.09 and 86.67%) and shoot dry weight (44.78 and 38.61%) over those recorded by the uninoculated plants in both seasons, respectively. In fact, the promotion effects of inoculation with diazotrophs could be attributed to N_2-fixation and/or certain growth promoting substances such as indole acetic acid and gibberellic acids, which play a beneficial role through mechanisms acting together on root development, including development and function of roots, number and length of lateral roots (Mekhemar, 2008, Compant *et al*,. 2010 and Mohanta *et al.*, 2010).

Table 3. Effect of bacterial inoculation, different N-levels and their combination on some wheat growth parameters after 80 days from sowing in sandy soil

Treatments		Season 2013/14			Season 2014/15		
		Plant height (cm)	Root dry weight (g/plant)	Shoot dry weight (g/plant)	Plant height (cm)	Root dry weight (g/plant)	Shoot dry weight (g/plant)
Main effect of biofertilizer inoculation (Inoc.)							
Uninoculated		66.47	0.79	2.01	68.20	0.75	2.02
Herbaspirillum sp.		75.27	0.93	2.47	76.87	1.04	2.50
Azorhizobium caulinodans		70.20	0.89	2.53	73.00	0.89	2.61
Azospirillum brasilense		75.13	1.02	2.78	75.40	1.32	2.79
Mixture		78.13	1.32	2.91	80.60	1.40	2.80
L.S.D. $_{0.05}$		1.480	0.033	0.023	0.437	0.066	0.040
Main effect of different nitrogen levels (N)							
Zero		60.67	0.70	1.80	65.47	0.87	1.83
25 kg N/fed		68.47	0.83	2.30	70.00	0.95	2.19
50 kg N/fed		76.07	1.11	2.80	79.40	1.24	2.82
75 kg N/fed		80.07	1.15	2.88	79.20	1.15	2.93
100 kg N/fed		79.93	1.17	2.91	80.00	1.21	2.94
L.S.D. $_{0.05}$		1.480	0.033	0.023	0.437	0.066	0.040
Interaction effect (Inoc. x N)							
Uninoculated	Zero	53.33	0.63	1.62	56.00	0.66	1.67
	25 kg N/fed	61.00	0.69	1.72	64.00	0.71	1.75
	50 kg N/fed	70.33	0.87	1.97	73.00	0.77	1.94
	75 kg N/fed	74.00	0.88	2.33	73.00	0.79	2.34
	100 kg N/fed	73.67	0.91	2.42	75.00	0.84	2.41
Herbaspirillum sp.	Zero	63.33	0.65	1.73	69.33	0.85	1.79
	25 kg N/fed	70.00	0.81	2.33	73.00	0.93	2.32
	50 kg N/fed	76.67	1.05	2.74	81.00	1.33	2.76
	75 kg N/fed	82.67	1.07	2.75	79.00	1.05	2.83
	100 kg N/fed	83.67	1.08	2.78	82.00	1.06	2.77
Azorhizobium caulinodans	Zero	61.00	0.66	1.79	64.00	0.78	1.83
	25 kg N/fed	68.00	0.77	2.37	69.00	0.84	2.36
	50 kg N/fed	72.00	0.99	2.83	77.00	0.92	2.95
	75 kg N/fed	76.00	1.02	2.85	78.00	0.91	2.95
	100 kg N/fed	74.00	1.03	2.82	77.00	0.99	2.93
Azospirillum brasilense	Zero	61.00	0.76	1.93	67.00	0.95	1.91
	25 kg N/fed	70.33	0.87	2.44	70.00	1.05	2.43
	50 kg N/fed	77.33	1.14	3.16	81.00	1.48	3.15
	75 kg N/fed	83.00	1.16	3.17	79.00	1.49	3.21
	100 kg N/fed	84.00	1.16	3.20	80.00	1.65	3.24
Mixture	Zero	64.67	0.82	1.95	71.00	1.09	1.95
	25 kg N/fed	73.00	1.00	2.65	74.00	1.21	2.09
	50 kg N/fed	84.00	1.48	3.28	85.00	1.69	3.30
	75 kg N/fed	84.67	1.63	3.32	87.00	1.53	3.31
	100 kg N/fed	84.33	1.66	3.33	86.00	1.48	3.33
L.S.D. $_{0.05}$		3.310	0.073	0.052	0.978	0.147	0.089

Mixture: (*Herbaspirillum* sp. + *Azorhizobium caulinodans* + *Azospirillum brasilense*)

b) Effect of different N-levels:

Results in Table (3) reveled that the application of mineral-N fertilizer induced significant increase in all studied wheat growth parameters and the promotion effect increased as the rate of mineral N-fertilizer increased up to 100 kg N/fed. Unfertilized wheat plants gave shorter wheat plant height (60.67 and 65.47 cm) and lower root and shoot dry weights (0.70 and 0.87 g/plant) and (1.80 and 1.83 g/plant), respectively in both seasons. However, increasing mineral N-fertilizer up to 100 kg N/fed achieved higher values of all growth parameters in both tested seasons. The average wheat height, root dry weight and shoot dry weight were (79.93 cm), (1.17 g/plant) and (2.91 g/plant) in the first season and (80.00 cm), (1.21 g/plant) and (2.94 g/plant) in the second one, respectively. The increase of vegetative growth with increasing N-fertilizer level might be due to the prominent role of nitrogen in increasing the meristematic activities and its important function as main constituent of protein, amino acids and many essential compounds in plant system. Many workers confirmed the promotion effect of N-fertilizer on wheat growth parameters (Attala *et al.*, 2005; Mahmoud *et al.,* 2006 and Mekhemar, 2008). They recorded significant increases in wheat growth parameters by increasing application rate of mineral-N fertilizer up to 100-120 kg N/fed in sandy soil.

c) Effect of interaction involved in this study:

Data in Table (3) showed that all growth parameters responded significantly to such interaction.

The use of any mineral N fertilizer levels in combination with any of the tested bacterial strains gave plant height, dry weight of root and shoot values higher than those recorded by the same N-level without bacterial inoculation. The shorter wheat plant height as well as the lower root and shoot dry weights were achieved by the plants uninoculated or inoculated with any tested bacterial strain without mineral N-fertilization. Generally, using any tested bacterial strain in combination with 50 kg N/fed gave almost similar values to those obtained by using the recommended dose of N-fertilizer (100 kg N/fed). In the first season, values of plant height (76,67, 72.00 and 77.33 cm), root dry weights (1.05, 0.99 and 1.14 g/plant) and shoot dry weights (2.74, 2.83, 3.16 g/plant) were obtained from wheat plants inoculated with *Herbaspirillum*, *Azorhizobium* and *Azospirillum* and received 50 kg N/fed, respectively. The corresponding values in the second season were (81.00, 77.00 and 81.00 cm), (1.33, 0.92 and 1.48 g/plant) and (2.76, 2.95 and 3.15 g/plant), respectively, in same order. More enhancing in wheat growth parameters, due to the inoculation with strains to the half dose of N-fertilizer (50 kg N/fed). Values of wheat plant height were (84.00 and 85cm), root dry weights (1.48 and 1.69g/plant) and shoot dry weights (3.28 and 3.30 g/ plant), respectively with no significant differences by increasing N-level up to 100 kg N/fed. It is clear that wheat plant vigour exhibited significant augment due to joint application of N_2-fixing bacteria and mineral N-fertilizer, with a salient surpassing of mixed inoculum. Such promotive effect of the integrated diazotrophic bacteria with half dose of inorganic-N fertilizer (50 kg N/fed) was found by many investigators (Mahmoud *et al.,* 2006; Abo El-Soud *et al.,* 2007 and Mekhemar, 2008).

2) Nitrogen accumulation in plant tissues:

Table (4) show the effect of bacterial inoculation, different N-levels and their combination on root and shoot N-content of 80-day old wheat plants in sandy soil. Obtained data showed that both root and shoot N- contents were significantly responded to all treatments under investigation in both seasons.

a) Effect of inoculation:

Data of Table (4) declared that uninoculated plants recorded low plant nitrogen contents as compared to using any tested bacterial strain. Values of root N-contents ranged from 6.44 to 12.60 mg N/plant in the first season and from 6.37 to 13.16 mg N/plant in the second one. The corresponding values of shoot N-contents fluctuated between 18.32 to 42.23 mg N/plant and from 19.98 to 43.94 mg N/plant in the first and second season, respectively. However, the splendid significant effect in promoting nitrogen accumulation in wheat plant tissues was observed with the use of the mixture culture followed by single inoculation with *Azospirillum*. The corresponding increases in root N-contents over uninoculated treatment were (95.65 and 37.58%) in the first season and (106.59 and 99.06%) in the second season by using mixture and *Azospirillum* treatments, respectively. The increases in shoot N-contents were (130.51 and 117.14%) in the first season and (118.97 and119.92%) in the second one in the same order. In fact, this improvement in wheat N-content could be attributed to the ability of these rhizobacteria to fix nitrogen in association or endophytic manners and/or certain growth promoting substances, which positively affect root development and consequently their function in the uptake of both water and nutrients. Such results are in conformity with those of Abo El-Soud *et al.* (2007), Mekhemar (2008) and Islam *et al.* (2009). They showed better development in N-accumulation in wheat and maize tissues inoculated with diazotrophs.

b) Effect of different N-levels:

Table (4) show the main effect of different N-levels on N-accumulation in wheat tissues, data clearly indicated that the total-N content in both roots and shoots are in parallel with dry matter results. Values of root N-contents ranged from 4.66 to 11.94 mg N/plant and from 6.66 to 12.54 mg N/plant in the first and second season, respectively. The corresponding values for shoots were 14.95 to 43.40 mg N/plant and from 18.94 to 46.04 mg N/plant, respectively. In general, increasing N-level from zero to 100 kg N/fed led to gradual increase in N-content accumulated in their tissues. Such increases in the N-content mainly attributed to the increases in both root and shoot dry weights as well as the increases in the percentage of N. These results are in accordance with those of Mahmoud *et al.* (2006) and Mekhemar *et al.* (2007) who found that increasing the application rate of N-fertilizer from zero to 120 kg N/fed increased NPK uptake of wheat plants. They added that increasing N levels had positive effect on root growth and the absorption sites which enhance absorption of nutrients.

c) Effect of interaction involved in this study:

Regarding the interaction effect between bacterial inoculation and N-levels on total N-content of shoots and roots (Table 4), results show that the values of root N-content ranged from 3.99 to 19.19 mg N/plant and from 4.60 to 17.93 mg N/plant in the first and second seasons, respectively. The corresponding values of shoot N-content fluctuated between 10.83 to 55.66 mg N/plant and from 12.70 to 56.01 mg N/plant in the first and second season, respectively. It is clear that the total N-content in both root and shoot tissues were positively affected by inoculation, irrespective of the N-fertilizer level. This was evident from the significant to highly significant increases in the root and shoot N-contents over the uninoculated treatments, even at higher N-fertilizer level (100 kg N/fed). In other words, uninoculated treatment received different levels of N-fertilizer (from zero to 100 kg N/fed) gave values lower than using the same level of N and inoculated with any tested bacterial strain. However, wheat plants inoculated with mixed inoculum followed by single inoculation with *Azospirillum* in combination with any level of mineral N-fertilizer were significantly surpassed the uninoculated or other single inoculation treatments fertilized with the same N-level. As general, the highest values of the root N-contents (14.44 and 16.40 mg

N/plant) and shoot N-contents (52.26 and 55.77 mg N/plant) were attained in case of addition of the mixed supernatant to half dose of N-fertilizer (50 kg N/fed) with no significant differences with increasing N-levels up to 100 kg N/fed. The promotive effect of such combination between N fertilizer and diazotrophs inoculation on enhancing the accumulated N in wheat tissues may be related to improve root development via stimulating the proliferation of lateral root hairs, which resulted in better nutrient uptake capability and increased N-supply. These results are in complete harmony with those obtained by Mahmoud *et al.* (2006) and Mekhemar (2008) who recorded a synergistic effect of combined application of N_2-fixing bacteria and suitable mineral N-level (half dose) in enhancing N-accumulation of wheat tissues.

Table (4): Effect of bacterial inoculation, different N-levels and their combination on root and shoot N-contents of 80 day old wheat plants in sandy soil

Treatments		Season 2013/14		Season 2014/15	
		N-content of root (mg/plant)	**N-content of shoot (mg/plant)**	**N-content of root (mg/plant)**	**N-content of shoot (mg/plant)**
		Main effect of biofertilizer inoculation (Inoc.)			
Uninoculated		6.44	18.32	6.37	19.98
Herbaspirillum sp.		8.25	33.08	9.69	37.35
Azorhizobium caulinodans		6.80	29.40	7.39	33.19
Azospirillum brasilense		8.86	39.78	12.68	43.94
Mixture		12.60	42.23	13.16	43.75
L.S.D. $_{0.05}$		0.368	1.564	0.715	0.833
		Main effect of different nitrogen levels (N)			
Zero		4.66	14.95	6.66	18.94
25 kg N/fed		6.13	23.19	7.85	27.63
50 kg N/fed		9.91	39.27	11.54	41.41
75 kg N/fed		10.29	41.99	10.71	44.19
100 kg N/fed		11.94	43.40	12.54	46.04
L.S.D. $_{0.05}$		0.368	1.564	0.715	0.833
		Interaction effect (Inoc. x N)			
Uninoculated	Zero	3.99	10.83	4.60	12.70
	25 kg N/fed	4.92	12.70	5.46	14.44
	50 kg N/fed	7.02	16.90	6.45	17.92
	75 kg N/fed	7.59	23.07	7.17	25.44
	100 kg N/fed	8.65	28.07	8.17	29.40
Herbaspirillum sp.	Zero	4.53	14.47	6.63	19.24
	25 kg N/fed	6.02	22.65	7.91	30.97
	50 kg N/fed	10.26	42.33	12.48	41.72
	75 kg N/fed	9.24	42.17	9.49	46.37
	100 kg N/fed	11.19	43.79	11.98	48.44
Azorhizobium caulinodans	Zero	4.09	13.25	5.66	19.45
	25 kg N/fed	5.06	20.19	6.71	27.66
	50 kg N/fed	6.94	35.95	7.71	39.19
	75 kg N/fed	8.94	36.29	7.93	38.88
	100 kg N/fed	8.95	41.32	8.96	40.78
Azospirillum brasilense	Zero	5.05	17.60	7.63	21.17
	25 kg N/fed	6.36	27.24	9.07	34.80
	50 kg N/fed	10.91	48.93	14.66	52.46
	75 kg N/fed	10.23	52.79	14.12	55.70
	100 kg N/fed	11.71	52.37	17.93	55.57
Mixture	Zero	5.63	18.59	8.80	22.14
	25 kg N/fed	8.26	33.17	10.12	30.28
	50 kg N/fed	14.44	52.26	16.40	55.77
	75 kg N/fed	15.46	55.66	14.84	54.56
	100 kg N/fed	19.19	51.47	15.64	56.01
L.S.D. $_{0.05}$		0.823	3.498	1.599	1.862

Effect of inoculation with diazotrophs on some yield parameters of wheat under graded levels of mineral N-fertilization in sandy soil:

Data of Table (5) show the effect of bacterial inoculation, different N-levels and their combination on some wheat yield parameters in sandy soil. Obtained results showed that all yield parameters significantly responded to inoculation and fertilization treatments in both seasons.

a) Effect of inoculation:

Data in Table (5) indicate that the inoculation with different bacterial strains either individually or in a mixture stimulate significantly all the tested parameters over the control treatments in both tested seasons. However, the splendid significant effect was observed with the use of mixture strains followed by *Azospirillum brasilense* inoculants. In general, a comparison between inoculants showed the order: Mixture> *Azospirillum brasilense* > *Herbaspirillum* sp.> *Azorhizobium caulinodans*> uninoculated. The corresponding increases in the plant height over the uninoculated treatment in the first and second season were (14.08 and 12.99%) and (10.74 and 12.15%) by using mixture and *Azospirillum brasilense*, respectively. Increases in No. of spikes/m^2 were (33.60 and 42.43%) and (26.77 and 27.62%) in the same order, respectively. Increases of

No. of kernels/spike in both seasons were (57.50 and 52.60%) and (50.76 and 42.90%), respectively. While, the corresponding increases in 1000-kernel weight were (22.23 and 25.97%) and (13.90 and 17.77%) in both seasons, respectively. These results confirmed the important role of inoculation with diazotrophic bacteria in enhancing wheat yield parameters. This enhancement could be due to the biological role of such diazotrophs in promoting plant growth, N_2-fixation performance as well as P mobilization, phytohormone production and saving the bio-protection against phytopathogens. Such promotion is confirmed by many investigators (Abo El-Soud *et al.*, 2007; Mekhemar, 2008 and Verma *et al.*, 2010).

Table (5): Effect of bacterial inoculation, different N-levels and their combination on some yield parameters of wheat plants in sandy soil

Treatments		Season 2013/14 Plant height (cm)	No. of spikes/m^2	No. of kernels/ spike	1000-kernel weight (g)	Season 2014/15 Plant height (cm)	No. of spikes/m^2	No. of kernels/ spike	1000-kernel weight (g)
Main effect of biofertilizer inoculation (Inoc.)									
Uninoculated		83.80	227.40	30.73	43.37	86.20	224.20	30.93	43.17
Herbaspirillum sp.		87.33	277.60	44.53	48.32	90.20	272.87	43.87	49.19
Azorhizobium caulinodans		88.07	250.47	43.07	47.45	88.60	248.87	41.93	47.53
Azospirillum brasilense		92.80	288.27	46.33	49.40	96.67	286.13	44.20	50.84
Mixture		95.60	303.80	48.40	53.01	97.40	319.33	47.20	54.38
L.S.D. $_{0.05}$		1.288	4.112	1.259	0.576	0.664	9.165	1.398	0.529
Main effect of different nitrogen levels (N)									
Zero		81.60	216.20	29.87	45.33	86.20	230.53	29.47	45.43
25 kg N/fed		87.20	257.33	40.53	47.56	90.80	254.00	40.60	47.94
50 kg N/fed		91.73	284.07	46.60	49.59	92.27	286.53	44.80	50.68
75 kg N/fed		93.93	294.73	47.67	49.64	93.80	290.13	45.80	50.24
100 kg N/fed		93.13	295.20	48.40	49.44	96.00	290.20	47.47	50.82
L.S.D. $_{0.05}$		1.288	4.112	1.259	0.576	0.664	9.165	1.398	0.529
Interaction effect (Inoc. x N)									
Uninoculated	Zero	75.00	190.00	24.33	40.82	79.00	202.00	26.33	41.21
	25 kg N/fed	81.00	207.67	28.00	41.93	85.00	213.33	27.33	42.31
	50 kg N/fed	86.00	225.00	32.00	44.76	87.00	224.67	31.00	44.51
	75 kg N/fed	89.00	251.33	33.33	44.58	87.00	237.00	33.00	43.40
	100 kg N/fed	88.00	263.00	36.00	44.77	93.00	244.00	37.00	44.41
Herbaspirillum sp.	Zero	83.00	222.00	29.00	46.21	87.00	220.00	29.00	46.61
	25 kg N/fed	85.00	276.67	42.67	47.45	90.00	275.00	44.00	48.81
	50 kg N/fed	91.00	288.67	49.00	49.36	91.00	281.00	48.00	50.37
	75 kg N/fed	92.00	306.67	51.00	49.58	90.00	294.00	47.00	49.26
	100 kg N/fed	85.67	294.00	51.00	48.98	93.00	294.33	51.33	50.88
Azorhizobium caulinodans	Zero	80.00	214.33	28.00	44.12	86.00	212.67	29.67	42.43
	25 kg N/fed	86.00	235.67	43.33	46.73	87.00	229.67	43.00	46.82
	50 kg N/fed	86.67	263.00	48.00	48.48	87.00	264.33	45.00	49.71
	75 kg N/fed	92.67	265.33	49.00	48.73	91.00	268.00	45.00	49.77
	100 kg N/fed	95.00	274.00	47.00	49.21	92.00	269.67	47.00	48.92
Azospirillum brasilense	Zero	82.00	226.00	32.00	47.21	88.00	224.00	29.00	47.43
	25 kg N/fed	91.00	278.33	44.67	49.37	97.00	265.33	44.00	48.16
	50 kg N/fed	97.00	310.67	51.00	50.13	97.33	318.67	49.00	52.17
	75 kg N/fed	96.00	312.33	50.00	50.10	100.00	311.00	50.00	53.92
	100 kg N/fed	98.00	314.00	54.00	50.21	101.00	311.67	49.00	52.53
Mixture	Zero	88.00	228.67	36.00	48.30	91.00	294.00	33.33	49.48
	25 kg N/fed	93.00	288.33	44.00	52.30	95.00	286.67	44.67	53.59
	50 kg N/fed	98.00	333.00	53.00	55.23	99.00	344.00	51.00	56.65
	75 kg N/fed	100.00	338.00	55.00	55.21	101.00	340.67	54.00	54.83
	100 kg N/fed	99.00	331.00	54.00	54.01	101.00	331.33	53.00	57.37
L.S.D. $_{0.05}$		2.881	9.194	2.815	1.287	1.485	20.490	3.125	1.185

b) Effect of different N-levels:

Data in Table (5) showed that values of plant height (cm) ranged between (81.60 & 93.93) and (86.20 & 96.00); No. of spikes/m^2 (216.20 &295.20) and (230.53 & 290.20); No. of kernels/spike (29.87& 48.40) and (29.47 & 47.47) and 1000-kernel weight (g) (45.33 & 49.64) and (45.43 & 50.82) 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. 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 Attala *et al.* (2005) and Mekhemar (2008) who reported that wheat plants exhibited marked increases in spikes number/m^2, grains number/spike, spike grains weight and 1000-grain weight with increasing N fertilization levels up to 120 kg N/fed.

c) Effect of interaction involved in this study:

Table (5) show that all yield parameters significantly responded to the combined effect between bacterial inoculation and mineral N-levels in both seasons. It is clear that the response of wheat yield parameters to such interaction behaved similar trend obtained in wheat plant growth parameters mentioned before. Values of wheat plant height (cm) ranged from (75.00 to 100.00), number of spikes/m^2 (190.00 to

338.00), number of kernels/spike (24.33 to 55.00) and 1000-kernel weight (g) (40.82 to 55.23) in the first season, respectively. In the second season, the corresponding values of plant height ranged from (79.00 to 101.00), number of spikes/m^2 (202.00 to 344.00), number of kernels/spike (26.33 to 54.00) and 1000-kernel weight (g) (41.21 to 57.37). As mentioned before, using mixture treatment followed by single inoculation with *Azospirillum* gave higher values of wheat yield components, particularly when combined with 50 kg N/fed. Inoculation of wheat plants with mixture strains and *Azospirillum* in combination with 50 kg N/fed gave an increases in wheat plant height (13.95 and 12.79%), number of spikes/m^2 (48.00 and 38.07%), number of kernels/spike (65.63 and 59.38%) and 1000-kernel weight (23.39 and 11.99%) in the first season, respectively, over the uninoculated plants received 50 kg N/fed. In the second season, the increases in plant height were (13.79 and 11.87%), number of spikes/m^2 (53.11 and 41.84%), number of kernels/spike (64.52 and 58.06%) and 1000-kernel weight (27.27 and 17.21%), respectively in the same order. 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 (Mahmoud *et al.,* 2006 and Mekhemar, 2008).

Effect of inoculation with diazotrophs on wheat yield and its crude protein percentage under graded levels of mineral N-fertilization in sandy soil:

Table (6) shows the effect of bacterial inoculation, different N levels and their interaction on wheat yield and its crude protein percentage in sandy soil. Obtained data revealed that wheat yield and its crude protein significantly responded to such treatments under investigation, except the crude protein of straw insignificantly responded to the combined effect between inoculation and different N-levels.

a) Effect of inoculation:

Data in Table (6) indicate that inoculation of wheat with single or the mixture of bacterial strains exerted a valuable improvement in wheat yield component in comparison to uninoculated one. The promotive effect on such yield characters was clear in presence of seed bacterization with mixture of diazotrophs followed by *Azospirillum* treatments. In the first season, mixture strains treatment gave an increases in grain yield over than using single inoculation with *Herbaspirillum*, *Azorhizobium* or *Azospirillum* (14.48, 16.19 and 7.43%), respectively. The increases in straw yield were (35.17, 38.69 and 14.34%), crude protein of grain (5.06, 10.57 and 3.48%), crude protein of straw (5.60, 6.45 and 3.66%), respectively in the same order. In the second season, grain yield (13.33, 16.31 and 7.37%), straw yield (30.33, 40.71 and 13.57%), crude protein of grain (1.77, 6.12 and 1.93%) and crude protein of straw (6.45, 8.20 and 5.04%), respectively. The promotive action of inoculation in wheat yield 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. Also, 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 **Carvalho *el al.* (2014)** who explained that improving effects, arising from microbial inoculation, are due to producing growth promoting substances such as auxins, gibbrillins and cytokinins.

b) Effect of different N-levels:

Data in Table (6) demonstrate that all yield components increased significantly with increasing the application rate of mineral N-fertilizer from zero to 100 kg N/fed. Values of grain yield fluctuated between (3.83 to 11.33 ardab/fed) and from (4.04 to 11.33 ardab/fed) in both seasons, respectively. Values of straw yield ranged from (1.06 to 3.38 ton/fed) and from (0.98 to 3.37 ton/fed), respectively. Crude protein percentages of grains ranged from (10.54 to 13.54%) in the first season and from (10.47 to 13.46%) in the second one. Crude protein percentages of straw fluctuated between (3.36 to 3.82%) and from (3.56 to 3.84%), in the first and second season, respectively. The increase in yield with increasing 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 Attala *et al.* (2005), Mahmoud *et al.* (2006) and Mekhemar (2008) who reported that elevating the levels of applied N up to 120 kg N/fed raised the wheat grain and straw yields and its protein contents under sandy soil conditions.

c) Effect of interaction involved in this study:

Due to seed and straw yields, data reported in Table (6) 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. Inoculation with *Herbaspirillum* or *Azorhizobium* combined with 75 kg N/fed and inoculation with *Azospirillum* or mixture of strains combined with 50 kg N/fed achieved appreciable values for grain and straw yield nearly to or higher than value of full dose of N-fertilizer. In the first season, using mixture strains in combination with 50 kg N/fed gave increases in grain yield (84.86, 18.18, 20.54, 8.72%) and straw yield (84.95, 44.87, 54.88 and 12.06%) over than uninoculated, *Herbaspirillum, Azorhizobium* and *Azospirillum* in combination with 50 kg N/fed, respectively. In the second season, the increases in grain yield were (74.21, 20.28, 22.09 and 11.66%) and in straw yield were (87.62, 43.02, 49.80 and 7.06%), respectively. Generally, wheat plants fertilized with 50 kg N/fed and inoculated with tested bacterial strains took the following order: mixture> *Azospirillum*> *Herbaspirillum*> *Azorhizobium*> uninoculated. On the other hand, wheat contained variable quantities of proteins in their seeds ranged from (9.36 to 14.23%) and (9.32 to 14.07%) in the first and second season, respectively. Values of straw crude protein fluctuated between (2.39 to 4.01%) and (3.35 to 4.01), respectively. Crude protein of grains significantly responded to such interaction and took similar trend obtained above. The insignificant effect of the interaction treatments on the straw crude protein may due to the clear independent effect of the two factors under investigation and took a similar pattern of the superiority of using mixture treatment followed by *Azospirillum* in combination with 50 kg N/fed or above. Generally, these results imply that wheat plants supplied with 50 kg N/fed and inoculated with mixed inoculum 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 N_2-fixing inoculants could satisfy 50% of their nitrogen requirements (Abou El-Soud *et al.,* 2007; Mekhemar *et al.,* 2007 and Mekhemar, 2008).

It is worthy to note that inoculation with diazotrophs have excellent potential to be used with non-legumes not only because of their N_2-fixing proficiency but also to their ability to acts as PGPR. Diazotrophic bacteria, particularly in a mixture form, may be acting as a good practice for sustaining wheat grain yield and reduce the reliance on chemical fertilizers in agriculture, to minimize the environmental pollution and to have safe healthy food.

Table (6): Effect of bacterial inoculation, different N-levels and their combination on wheat yield and its crude protein percentage in sandy soil

Treatments		Season 2013/14 Grain yield (ardab/fed)	Straw yield (ton/fed)	Crude protein of grain (%)	Crude protein of straw (%)	Season 2014/15 Grain yield (ardab/fed)	Straw yield (ton/fed)	Crude protein of grain (%)	Crude protein of straw (%)
		Main effect of biofertilizer inoculation (Inoc.)							
Uninoculated		6.69	1.93	10.58	3.01	6.83	1.79	10.75	3.19
Herbaspirillum sp.		8.84	2.36	12.45	3.75	9.00	2.44	12.43	3.72
Azorhizobium caulinodans		8.71	2.30	11.83	3.72	8.77	2.26	11.92	3.66
Azospirillum brasilense		9.42	2.79	12.64	3.82	9.50	2.80	12.41	3.77
Mixture		10.12	3.19	13.08	3.96	10.20	3.18	12.65	3.96
L.S.D. $_{0.05}$		0.158	0.077	0.366	0.174	0.252	0.084	0.291	0.147
		Main effect of different nitrogen levels (N)							
Zero		3.83	1.06	10.54	3.36	4.04	0.98	10.47	3.56
25 kg N/fed		7.81	2.09	11.52	3.61	7.97	2.11	11.24	3.75
50 kg N/fed		10.00	2.87	12.14	3.72	10.02	2.91	12.28	3.84
75 kg N/fed		10.81	3.17	12.85	3.82	10.93	3.11	12.71	3.82
100 kg N/fed		11.33	3.38	13.54	3.76	11.33	3.37	13.46	3.83
L.S.D. $_{0.05}$		0.158	0.077	0.366	0.174	0.252	0.084	0.291	0.147
		Interaction effect (Inoc. x N)							
Uninoculated	Zero	2.10	0.61	9.36	2.39	2.42	0.50	9.32	3.35
	25 kg N/fed	5.00	1.53	9.74	2.83	4.85	1.42	9.81	3.64
	50 kg N/fed	6.54	2.06	10.28	3.11	6.98	2.02	10.63	3.77
	75 kg N/fed	8.84	2.50	11.47	3.36	9.03	2.30	11.80	3.87
	100 kg N/fed	10.95	2.96	12.05	3.38	10.87	2.70	21.17	3.81
Herbaspirillum sp.	Zero	3.43	0.86	11.00	3.44	3.59	0.83	10.94	3.48
	25 kg N/fed	8.09	1.87	11.91	3.69	8.42	2.12	11.61	3.69
	50 kg N/fed	10.23	2.63	12.29	3.88	10.11	2.65	12.91	3.77
	75 kg N/fed	11.14	3.08	13.00	3.91	11.44	3.16	13.12	3.81
	100 kg N/fed	11.31	3.34	14.07	3.85	11.45	3.45	13.58	3.86
Azorhizobium caulinodans	Zero	3.55	0.79	10.87	3.35	3.42	0.74	10.52	3.38
	25 kg N/fed	7.80	1.91	11.13	3.68	8.00	1.85	10.88	3.79
	50 kg N/fed	10.03	2.46	11.21	3.77	9.96	2.53	12.33	3.75
	75 kg N/fed	11.01	2.98	12.70	3.87	11.20	2.89	12.36	3.65
	100 kg N/fed	11.16	3.37	13.22	3.92	11.26	3.28	13.52	3.72
Azospirillum brasilense	Zero	4.85	1.30	10.64	3.65	5.24	1.19	10.65	3.70
	25 kg N/fed	8.73	2.41	11.97	3.87	8.85	2.47	12.27	3.71
	50 kg N/fed	11.12	3.40	13.00	3.92	10.89	3.54	12.45	3.88
	75 kg N/fed	11.21	3.39	13.48	3.93	11.27	3.32	12.73	3.78
	100 kg N/fed	11.22	3.49	14.12	3.74	11.24	3.51	13.97	3.76
Mixture	Zero	5.20	1.72	10.83	3.98	5.53	1.64	10.94	3.87
	25 kg N/fed	9.43	2.77	12.85	3.98	9.72	2.72	11.61	3.92
	50 kg N/fed	12.09	3.81	13.91	3.92	12.16	3.79	13.10	4.01
	75 kg N/fed	11.86	3.91	13.58	4.01	11.72	3.86	13.52	4.00
	100 kg N/fed	12.02	3.75	14.23	3.91	11.85	3.88	14.07	3.99
L.S.D. $_{0.05}$		0.352	0.172	0.818	N.S	0.564	0.187	0.651	N.S

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تأثير التلقيح بمثبتات الأزوت الجوى على إنتاجية القمح تحت مستويات مختلفة من السماد النيتروجينى فى الأراضى الرملية

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أجريت تجربتين حقليتين فى أرض رملية بمحطة البحوث الزراعية بالإسماعيلية خلال الموسمين الشتويين 2014/2013 و2014 /2015 لدراسة استجابة النمو الخضرى لنباتات القمح وكذا المحصول وبعض مكوناته للتلقيح البكتيرى بمثبتات الأزوت الجوى متداخلة مع مستويات مختلفة من السماد النيتروجينى المعدنى. حيث تم تلقيح حبوب القمح صنف جيزة 168 قبل الزراعة بفترة قصيرة ببكتيريا الهيرباسبيريللم ، الأزوريزوبيم والأزوسبيريللم أى منها منفردا كمعاملات فردية أو التلقيح بخليط من هذه السلالات (اللقاح الثلاثى المشترك). وقد تم اختبار هذه اللقاحات متداخلة مع مستويات مختلفة من السماد المعدنى (سلفات الأمونيوم 5 ,20 %) هى صفر ، 25 ، 50 ، 75 و 100 كجم ن/فدان).أظهرت النتائج أن هناك زيادة معنوية فى جميع صفات النمو الخضرية والمحصول وبعض مكونات المحصول للقمح نتيجة التلقيح بأى نوع من مثبتات الأزوت الجوى المختبرة. ومع ذلك، فإن استخدام معاملة التلقيح المشتركة يتبعها معاملة التلقيح الفردى بالأزوسبيريللم اظهرت تفوقاً ملحوظاً على باقى معاملات التلقيح الأخرى او المعاملة الغير ملقحة في جميع الصفات تحت الدراسة. وبمقارنة تأثير معاملات التلقيح المختلفة على جميع الصفات الخضرية والمحصول وبعض مكونات المحصول لنباتات القمح فقد أخذت الترتيب التالى: معاملة اللقاح الثلاثى> الأزوسبيريللم> الهيرباسبيريللم> الأزوريزوبيم> المعاملة الغير ملقحة استجابت معنوياً جميع قياسات النمو الخضرى والمحصول وبعض مكونات المحصول لنباتات القمح للزيادة من معدل التسميد النتيروجينى المعدنى. عموماً فأن استخدام المعدل العالى من السماد النيتروجينى المعدنى (100 كجم ن/فدان) أعطى أعلى قيم لجميع الصفات تحت الدراسة فى كلا الموسمين.أشارت النتائج الخاصة بالتأثير المشترك لكل من التلقيح ومستويات النيتروجين المعدنى المختلفة الى أفضل نتائج للنمو والمحصول وبعض مكونات المحصول لنباتات القمح باستخدام معاملة التلقيح المشترك (معاملة اللقاح الثلاثى) يليها التلقيح الفردى بالأزوسبيريللم 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 النتروجينى المعدنى (100 كجم ن/فدان).و عموما فإن هذه النتائج تؤكد أهمية التلقيح بمثبتات الأزوت الجوى ليس فقط لدورها في تثبيت النتروجين الجوى ولكن لقدرتها على انتاج العديد من المواد المشجعة للنمو. ولذلك فإن استخدام البكتيريا المثبتة لنيتروجين الهواء الجوى (خصوصاً اللقاح الثلاثى) هو إجراء جيد يعمل على تحسين صفات النمو والمحصول وبعض مكونات المحصول لنباتات القمح و تقليل الاعتماد على الأسمدة الكيماوية في الزراعة ، للحد من التلوث البيئي والحصول على غذاء صحي آمن.