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EFFECT OF *Azotobacter chroococcum* AND *Azospirillum brasilense* INOCULATION AND ANHYDROUS AMMONIA ON ROOT COLONIZATION, PLANT GROWTH AND YIELD OF WHEAT PLANT UNDER SALINE ALKALINE CONDITIONS

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ABSTRACT

A field experiment was conducted to study the effect of different rates of nitrogen (anhydrous ammonia at 0, 20, 40, 60 and 80 Kg N fed^{-1}) and single or dual inoculation with *Azospirillum brasilense* and *Azotobacter chroococcum* on plant growth and N-uptake of wheat at Abo Eash (Fayoum Governorate) during the growing season of 2000-2001. The experimental design was split-split plot in a randomized complete blocks with three replicates. The obtained data showed significant increases in dry weight and yield either by increasing the rate of nitrogen application or with dual inoculation. All the inoculants gave significantly higher grain and straw yield and nitrogen uptake by wheat plant than other uninoculated treatment. It was observed that dual inoculation with *Azotobacter chroococcum* and *Azospirillum brasilense* performed significantly better followed by single inoculation with *Azotobacter* or *Azospirillum*. The maximum benefit was obtained with combined inoculation at all levels of N-fertilizer and increased all values of wheat yield and its components. The results illustrated that, incremental rates of applied nitrogenous fertilizer to wheat plants gradually increased the means of N-uptake by both grains and straw. It could be concluded that a substantial amount of chemical N fertilizer could be saved by using biofertilizer which in turn minimizes the production costs and pollution factors, which can occur by the excess use of chemical fertilizers.

Keywords: Anhydrous ammonia, *Azospirillum brasilense* and *Azotobacter chroococcum*, plant-bacteria interaction, wheat growth.

INTRODUCTION

Wheat is one of the major crops in Egypt. Any effort to increase wheat yield to face the continual increasing of consumption is highly appreciated. It is well known that nitrogen is considered as one of the limiting factors to achieve the high yield of wheat crop. With the steadily increasing prices of nitrogen fertilizers and the pollution problems, the atmospheric dinitrogen –fixing systems are becoming more and more important. The efforts to decrease utilization of chemical fertilizers by using biofertilizers might reduce financial costs. Fixation as an alternative or supplementary source of nitrogen for wheat has been the major approach in soil fertility management of nitrogen for wheat (Kotb, 1998). Hence to obtain optimum yields of cereal –crops, the maintenance of soil fertility at a high level is utmost important. Since chemical fertilizers are very expensive for medium and marginal farmers, the use of nitrogen fixing bacteria assumes a greater important (Darmwal and Gaur, 1988).

Application of *Azotobacter* and *Azospirillum* to wheat, maize, sorghum, rice, millets, vegetables, potatoes, cotton and sugarcane grown under both irrigated and rainfed conditions, with and without application of NPK, increased yields of these crops (Kapulink *et al.*, 1981; Rai & Gaur, 1982; Saha *et al.*, 1985; Eweda & Vlassak, 1988; Okon & Labandera-Gonzalez, 1994 and Fallik & Okon, 1996). The beneficial effect of *Azotobacter* and *Azospirillum* are related not only to their N_2-fixing proficiency but also with their ability to produce antifungal compounds, growth regulators and siderophores (Pandey and Kumar, 1989). Single or dual inoculation of wheat seedlings with *Azotobacter chroococcum* and *Azospirillum brasilense* in sterilized soil have been extremely variable-from significantly negative (Barber *et al.*, 1976 and Albrecht *et al.*, 1977) to significantly positive stimulation of their population in the rhizosphere, and also stimulated plant growth and significantly increased the concentration of indole acetic acid, P, Mg, N and total soluble sugars in wheat shoots (Bazzicalupo *et al.*, 1985; Charyulu *et al.*, 1985; Hegazi and Saleh, 1985 and ElShanshoury, 1995).

On the other hand, the use of chemical N-fertilizer especially NH_3 gave higher grain yield, straw and uptake efficiencies than other N chemical fertilizer (Raun, 1986; Raun *et al.*, 1989 and Darwish, 1989 & 1993).

Therefore the present investigation was undertaken to study the effect of inoculation with *Azospirillum brasilense* and *Azotobacter chroococcum* on the growth, N-uptake as well as bacterial colonization and the yield of wheat at different nitrogen levels (anhydrous ammonia) under saline alkaline field conditions.

MATERIALS AND METHODS

Experimental conditions:

A field experiment was carried out at Abo Eash (Fayoum Governorate) during the season of 2000-2001. The experiment was aimed to study the effect of the inoculation with two strains of assymbiotic N_2-fixing bacteria, *Azospirillum brasilense* or/and *Azotobacter chroococcum* on the growth, N-uptake, bacterial colonization and wheat yield at different rates of anhydrous ammonia under field conditions. The experimental plots (3.5 x 3.0 m) were planted with wheat grains (c.v. Sakha 69). Potassium and phosphorus were added at sowing date with the rates of 48 and 15 kg fed^{-1}, as K_2O and P_2O_5, respectively. The nitrogen fertilizer used were anhydrous ammonia (82% N). The anhydrous ammonia was injected directly at the rates of (0, 20, 40, 60 and 80 Kg fed^{-1}) into the moderately moist soil at 15 cm depth with 30 cm spacing between points of injection before planting.

Preparation of inocula:

Azospirillum brasilense (isolated from rhizosphere of wheat grown in saline alkaline soil, Ali, 1997) was grown on liquid N-deficient medium (Dobereiner *et al.*, 1976) with shaking at 28-30°C for 48 h. *Azotobacter* *chroococcum* previously isolated from wheat soil in 1999. Then two strains were purified, characterized and checked to nitrogenase activity before used. After that, these strains were grown in modified Asby's medium (Abdel-Malek and Ishac, 1968) with shaking at 30°C for 24 h.

Seed preparation:

Wheat seeds were inoculated by soaking in liquid culture with either sole or a combined inoculum of *Azospirillum brasilense* (10^8 cells ml^{-1} approximately) and *Azotobacter chroococcum* (10^8 cells ml^{-1} approximately). Arabic gum was added to the liquid culture as an adhesive agent before treating seeds. Inoculated seeds were spread over a plastic sheet for short time to dry before planting. Uninoculated seeds were enriched with autoclaved culture. All treatments were carried out in three replicates.

Bacterial counts:

Soil samples at 30, 60, 90 and 120 days from sowing were taken from the rhizosphere of plants, and (10 g) root free soil were shaken for 1 hr in 90 ml sterilized tap water and ten fold dilution were made.

1. Enumeration of *Azospirillum* in soil samples was done using semi solid malate medium (Dobereiner *et al.*, 1976) and MPN technique.
2. Numbers of *Azotobacter* in soil samples were determined on modified Ashby's liquid medium (Abdel-Malek & Ishac, 1968) and using MPN technique.
3. Total N_2-fixers were determined by using Watanabe & Bawraquia, 1979 and pouring plate method technique.
4. Total colony counts were made on Bunt & Rovira (1955) modified soil extract agar medium and incubation at 30°C for 7 days.

The counts of *Azospirillum*, *Azotobacter*, total N_2-fixers as well as total bacteria were related to dry weight of soil samples at 105°C.

Plant growth characters:

Plant samples at 60, 90 and 120 days from sowing were taken from inner area of each plot (to minimize border effect). Dry matter yield (g/plant), N-uptake at harvest time, yield component of the inner 1 m^2 of each plot, *i.e.*, grain yield (Ard. Fed^{-1}), straw yield (ton. fed^{-1}), N-uptake of dry matter yield and straw were determined (Jackson, 1973). All data were calculated on dry weight basis at 70°C.

The soil was analyzed for mechanical and some chemical properties according to the standard method (Black, 1965) and the data was given in Table (1).

Table (1): The mechanical and some chemical properties of soil used.

Properties	Soil
1. Particle size distribution of soil	
Sand %	40
Silt %	20
Clay %	40
O.M %	1.04
$CaCO_3$%	8.40
Texture	Clayey
2- Chemical analysis of soil	
a-Soluble cations (meq L^{-1})	
Na^+	3.3
K^+	0.17
Ca^{++}	1.03
Mg^{++}	1.24
b-Soluble anions (meq L-1)	
CO^{3-}	---
HCO^{3-}	1.27
Cl^-	1.10
SO_4^{--}	3.37
PH	8.2
E.C (m mohs/cm at 25°C)	0.56
CEC meq/100 g soil.	32.0

Total N ppm 620.

RESULTS AND DISCUSSION

This experiment was conducted in saline alkaline soil of El-Fayoum Governorate to study the associative effect of *Azospirillum brasilense* and *Azotobacter chroococcum* under different levels of inorganic nitrogen on yield and N-uptake by wheat plants.

1. Effect of inoculation on some groups of microorganisms in wheat grown soil:

To ensure the establishment of *Azospirillum* and *Azotobacter* inoculum in wheat grown soil as affected with different N-levels, the total number of *Azospirillum* and *Azotobacter* in soil were estimated by MPN-method and total N_2-fixers and total microbial count in pouring plate method after 30, 60, 90 and 120 days of sawing.

a- Effect of bacterial inoculation on *Azospirillum* counts:

Inoculation with *A. brasilense* increased the counts of *Azospirillum* in soil at high level of inorganic nitrogen 80 kg N fed^{-1} which reached 3.26×10^6 cells g^{-1} dry soil of wheat after 60 days of sowing, thereafter gradually decreased to reach up to 10^4 after 120 days. In the case of *Azotobacter* inoculation, the numbers are less than those of the above which reached 2.3×10^5 at 40 kg N fed^{-1} and decreased slowly. In combination of *Azospirillum* and *Azotobacter* as well as in uninoculated one, the numbers of *Azospirillum* at the end of cultivation periods (120 days) tended to increase more than the single inoculation (Table, 2).

Table (2): Periodical changes in counts of azospirilla in root free soil* of wheat (cells / g dry soil x 10^4).

Treatments	Time in days			
	30	60	90	120
1. *Azospirillum* inoculation				
a- 80**	45	326	6.3	2.4
b- 60	39	9	11.3	2.8
c- 40	23	17	14.0	2.9
d- 20	27	17	8.4	3.6
e- 00	20	9.0	8.5	1.9
2. *Azotobacter* inoculation				
a- 80	9.8	22.0	15.0	0.22
b- 60	19.2	8.0	0.6	1.3
c- 40	3.0	12.0	23.0	2.7
d- 20	12.0	8.6	10.3	4.3
e- 00	10.0	13.0	14.0	7.0
3. Dual inoculation				
a- 80	9.0	8.0	13.0	13.0
b- 60	36.0	23.0	21.0	21.0
c- 40	24.0	23.0	14.0	13.3
d- 20	20.0	39.0	12.3	11.6
e- 00	18.0	12.0	14.0	9.1
4. Uninoculated treatment				
a- 80	3.0	8.0	2.4	13.0
b- 60	27.0	16.0	9.0	8.1
c- 40	23.0	16.0	14.0	14.0
d- 20	5.0	5.6	23.0	23.0
e- 00	2.8	0.6	22.0	23.0

* Initial in soil 0.79 x 10^4

** (a, b, c, d and d) doses of anhydrous ammonia kg N fed^{-1}

b) Effect of bacterial inoculation on *Azotobacter* counts:

Table (3) show that in *Azospirillum* inoculation, the numbers of *Azotobacter* increased gradually up to 90 days at 60 kg N fed^{-1} then decreased sharply up to 120 days, whereas at 20 kg N fed^{-1} the numbers were higher which reached to 16.5 x 10^4 after 30 days then decreased slowly to reach 0.4 x 10^4 at 120 days. In the case of *Azotobacter* inoculation the numbers were more than that of *Azospirillum* inoculation at 40 kg N fed^{-1} 45.2 x 10^4 after 30 days decreased sharply to reach 0.03 x 10^4 at the end of 120 days. Therefore, it was clear that mixed inoculation caused light increase in numbers than those of *Azospirillum* inoculation especially at low nitrogen levels. Also, it was noticed that the uninoculated treatment recorded high number than the mixed inoculation till 60 days then the numbers were decreased at 20 and 40 kg N fed^{-1}.

Table (3): Periodical changes in counts of *Azotobacter* in root free soil* of wheat (cells / g dry soil x 10^4).

Treatments	Time in days			
	30	60	90	120
1. *Azospirillum* inoculation				
a- 80**	4.6	12.0	24.7	0.15
b- 60	3.0	17.0	25.5	0.29
c- 40	4.3	15.0	20.8	2.22
d- 20	16.5	12.0	15.4	0.4
e- 00	5.0	5.0	13.6	0.022
2. *Azotobacter* inoculation				
a- 80	13.0	4.0	26.0	0.19
b- 60	6.5	12.0	32.0	0.054
c- 40	45.2	17.3	29.5	0.03
d- 20	5.5	2.4	19.0	0.10
e- 00	10.0	9.0	24.0	1.33
3. Dual inoculation				
a- 80	12.5	5.4	14.2	0.35
b- 60	5.4	5.6	9.5	0.23
c- 40	5.3	0.7	14.2	0.055
d- 20	3.8	0.95	16.5	0.056
e- 00	2.3	0.59	26.0	0.15
4. Uninoculated treatment				
a- 80	1.4	6.2	4.4	0.12
b- 60	2.3	2.0	0.97	0.12
c- 40	27.0	1.8	0.99	0.03
d- 20	3.1	12.6	1.9	3.9
e- 00	3.5	3.1	3.1	0.03

* Initial in soil 0.05 x 10^4
** (a, b, c, d and d) doses of anhydrous ammonia kg N fed^{-1}

c. Effect of bacterial inoculation on total N_2-fixers count:

As indicated in Table (4) there are pronounced increased in total count of nitrogen fixers with inoculation than those without inoculation. In the case of *Azotobacter* inoculation the numbers reached up to 10^7 after 30 days under different levels of inorganic nitrogen and decreased slowly to the end of cultivation periods (120 days). Also in the case of *Azospirillum* inoculation the numbers of nitrogen fixers was found in the same trend as in *Azotobacter* inoculation but the numbers are lower than those of *Azotobacter* (up to 10^6 cells g^{-1} dry soil). It was clearly noticed that mixed inoculation gave lower number than single inoculation after 30 days, further, it take the same trend with single inoculation which numbers reach up to 10^6 after 60 days then gradually decreased at the end of 120 days. Also, it was noticed that the mixed inoculation gave higher numbers than those found with single inoculation at the end of cultivation with moderate levels of inorganic nitrogen (40 and 20 kg N fed^{-1}).

Table (4): Periodical changes in counts of total N_2-fixers in root free soil* of wheat (CFU / g dry soil x 10^5).

Treatments	Time in days			
	30	60	90	120
1. *Azospirillum* inoculation				
a- 80**	97.1	11.6	10.2	1.56
b- 60	96.2	6.8	17.7	0.8.4
c- 40	17.6	14.6	40.3	0.82
d- 20	78.2	24.7	10.6	0.95
e- 00	18.1	9.2	11.2	1.55
2. *Azotobacter* inoculation				
a- 80	233.0	9.2	15.1	2.79
b- 60	40.5	11.0	14.6	2.91
c- 40	50.0	16.8	13.7	2.5
d- 20	113.0	21.6	15.3	1.0
e- 00	93.0	14.7	17.7	5.66
3. Dual inoculation				
a- 80	15.6	25.4	1.64	2.71
b- 60	13.63	22.8	2.32	3.15
c- 40	11.87	11.0	1.96	3.00
d- 20	10.35	16.8	1.76	4.78
e- 00	0.58	10.0	2.5	0.207
4. Uninoculated treatment				
a- 80	25.9	10.3	6.13	0.089
b- 60	1.17	0.82	3.9	0.240
c- 40	0.32	1.14	2.38	0.284
d- 20	0.159	10.5	5.098	0.3196
e- 00	0.136	3.07	3.0	0.225

* Initial in soil 0.32 x 10^5

** (a, b, c, d and d) doses of anhydrous ammonia kg N fed^{-1}

d) Effect of bacterial inoculation on total microbial counts:

It could be seen from Table (5) that inoculation with either *Azospirillum brasilense* or *Azotobacter chroococcum* increased the total microbial counts than those in mixed inoculation, which gave higher numbers reached 138 x 10^6 and 394 x 10^6 cells g^{-1} dry soil, respectively, compared with mixed inoculation and uninoculated treatment which gave (18 x 10^6 cells g^{-1} dry soil at 80 kg N fed^{-1} after 60 days and 20.5 x 10^6 cells g^{-1} dry soil after the same time of cultivation as well as at the same level of inorganic nitrogen, respectively.

Table (5): Periodical changes in total colony counts in root free soil* of wheat (CFU / g dry soil x 10^6).

Treatments	Time in days			
	30	**60**	**90**	**100**
1. *Azospirillum* inoculation				
a- 80**	117.0	20.7	5.8	8.0
b- 60	99.7	25.0	10.8	2.5
c- 40	138.0	105.0	15.0	3.0
d- 20	97.0	23.0	9.6	3.1
e- 00	118.0	21.0	11.0	0.9
2. *Azotobacter* inoculation				
a- 80	394.0	6.00	8.5	1.52
b- 60	253.0	11.0	10.0	2.8
c- 40	82.5	10.5	1.92	1.34
d- 20	207.0	16.0	4.23	2.1
e- 00	186.0	19.3	7.1	2.14
3. Mixed inoculation				
a- 80	1.36	18.0	2.55	1.46
b- 60	2.5	11.0	1.36	1.56
c- 40	1.82	14.2	1.50	1.33
d- 20	1.6	11.0	1.30	1.06
e- 00	1.53	6.00	1.56	0.124
4. Uninoculated treatment				
a- 80	59.5	20.5	1.50	0.32
b- 60	2.35	2.30	2.24	0.109
c- 40	0.98	3.00	3.40	0.165
d- 20	1.05	2.66	2.90	0.18
e- 00	0.97	2.28	1.19	0.12

* Initial in soil 0.18 x 10^6

** (a, b, c, d and d) doses of anhydrous ammonia kg N fed^{-1}

2. Effect of anhydrous ammonia and single or dual inoculation with *Azospirillum brasilense* and *Azotobacter chroococcum* on the growth, yield and N-uptake of wheat plants:

Data presented in Table (6) show that the dry weight at different stages increased by increasing the rate of nitrogen application. Higher values were observed when inorganic nitrogen (NH_3) was used with combined inoculation followed by *Azospirillum* inoculation and *Azotobacter* inoculation treatments. Similar results were obtained by El-Borollosy & Refaat (1982) which observed that inoculation with a mixture of *A. chroococcum* and *Azospirillum* sp that is in fresh and/or dry form gave highest weights of maize plants, followed by inoculation with *Azotobacter* or *Azospirillum* in decreased order.

Table (8): Effect of anhydrous ammonia and inoculation with *Azospirillum*, *Azotobacter*, and a mixture of nitrogen uptake and protein content at harvesting.

Treatments	Grain		Straw		Total N-uptake kg/fed	Protein content %	
	N%	N-uptake	N%	N-uptake		Grain	Straw
1- 80 kg N fed^{-1}							
a- *Azospirillum* inoculation	1.94	66.38	0.46	22.33	88.71	12.13	2.88
b- *Azotobacter* inoculation	1.90	65.50	0.42	21.95	87.45	11.88	2.63
c- Mixed inoculation	2.10	72.99	0.50	27.31	100.30	13.13	3.13
d- Uninoculated treatment	1.85	61.61	0.24	22.22	83.83	11.56	1.50

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Table (6): Effect of anhydrous ammonia and inoculation with *Azospirillum*, *Azotobacter*, and a mixture of them on wheat plant dry weight (g/plant).

Treatments	Time in days		
	60	90	120
1- 80 kg N fed^{-1}			
a- *Azospirillum* inoculation	2.71	6.51	8.44
b- *Azotobacter* inoculation	2.60	6.32	8.20
c- Mixed inoculation	2.85	6.80	8.69
d- Uninoculated treatment	2.80	6.20	7.45
Mean	2.85	6.80	8.69
2- 60 kg N fed^{-1}			
a- *Azospirillum* inoculation	2.62	6.10	7.98
b- *Azotobacter* inoculation	2.55	5.91	7.94
c- Mixed inoculation	2.75	6.32	8.03
d- Uninoculated treatment	2.45	6.00	6.92
Mean	2.61	6.08	7.72
3- 40 kg N fed^{-1}			
a- *Azospirillum* inoculation	2.53	5.97	7.13
b- *Azotobacter* inoculation	2.44	5.84	6.96
c- Mixed inoculation	2.66	6.11	7.46
d- Uninoculated treatment	2.17	5.45	6.43
Mean	2.45	5.84	7.00
4- 20 kg N fed^{-1}			
a- *Azospirillum* inoculation	2.28	5.64	6.41
b- *Azotobacter* inoculation	2.14	5.40	6.09
c- Mixed inoculation	2.39	5.94	6.82
d- Uninoculated treatment	2.07	5.03	5.65
Mean	2.22	5.50	6.24
5- 0 kg N fed^{-1}			
a- *Azospirillum* inoculation	1.70	4.85	5.64
b- *Azotobacter* inoculation	1.56	4.75	5.42
c- Mixed inoculation	1.82	4.94	5.74
d- Uninoculated treatment	1.53	4.33	5.14
Mean	1.65	4.72	5.49

L.S.D. at 0.05			
N rate :	0.045	0.101	0.234
Inoculation :	0.040	0.090	0.209
N x inoculation :	0.090	0.202	0.080

The results presented in Tables (7, 8) show that in all treatments, seed inoculation increased N-uptake of wheat plants over the controls but the mixed Inoculation gave the highest effect especially with high level of inorganic nitrogen followed by *Azospirillum* inoculation then *Azotobacter*.

stimulate seed germination, plant length, root length and subsequently increased nutrients uptake by plants.

It is also clear shown that the N-fertilization of wheat plants increased the protein quantity in the grains. This might be explained by the influence of N availability at critical stages of spike initiation and the development on plant metabolism in way leading to increase synthesis of amino acids and their incorporation into grain protein. Darwiche (1994) indicated that any increase in N-fertilization was followed by an increase in protein percentage in wheat grains.

Grain and straw yields were significantly increased with inoculation as compared to uninoculated treatments irrespective of inorganic nitrogen fertilizer levels applied (Table 9). The mean values as a result of application of 0, 20, 40, 60 and 80 kg N fed^{-1} were 2.10, 5.20, 13.10, 16.30 and 22.2 Ardab fed^{-1} for grain and 2.07, 3.97, 4.55, 4.70 and 5.05 ton fed^{-1} for straw yield respectively against the highest values of inoculated treatments mixed inoculation followed by *Azotobacter* inoculation then *Azosprillum* inoculation in order. These results may be attributed to the high efficiency of bacteria presence in inoculated seeds to fix atmospheric nitrogen and to produce some biologically active substances, *e. g.*, IAA, ALA, gibberllins and cytokinine-like substances. These results are in line with those reported by Zambre *et al.* (1984), Abbas *et al.* (1994); Attallah and El-karamity (1997) and Kotb (1998). They showed higher grain and straw yields when they use inoculated seeds of wheat than uninoculated ones in both silty clay loam and sandy soils.

It is quite clear from the data that grain and straw yields recorded highest values at 80, 60 Kg N fed^{-1} but it was noticed that the yield at 80 kg N fed^{-1} without inoculation gave the same result with inoculated treatment at 60 kg N fed^{-1} and the yield at 60 kg N fed^{-1} with inoculated treatments at 40 kg N fed^{-1} Thus, the inoculation save 20 units of N-fertilizer (25-33.5%) and that saving was economically feasible. Therefore, it seams from this data that a substantial amount of chemical N-fertilizer could be saved by using biofertilizer which in turn minimizes the production costs and pollution factors which can occur by the excess use of chemicals fertilizers.

The results of grain weight/spike, number of grains/spike, spike length, number of spklite /spike and 1000-grain weight (Table10) show the highest values for *Azospirillum* inoculation at high levels of inorganic nitrogen (80 and 60 kg N fed^{-1}) followed by either *Azotobacter* or mixed inoculation. But at low levels of N (0, 20 and 40 kg N fed^{-1}) the mixed inoculation and single with *Azotobacter* gave the highest results followed by *Azospirillum* inoculation in decreasing order more than the uninoculated and control, Shams El-Din and El-Habbak (1992), Shams El-Din and Abdrabou (1995) and Kotb (1998) stated significant increases in number and weight of grains/spike by inoculated wheat grains N_2-fixing bacteria.

It is worthy to mention that seed inoculation increased all values of wheat yield and its compcnents at all levels of N-fertilizer NH_3 (anhydrous ammonia).

Mean	27.77	85.18	108.00

L.S.D. at 0.05

N rate :	**1.951**	**2.833**	**11.38**
Inoculation :	**1.745**	**2.534**	**10.18**
N x inoculation :	**3.901**	**5.666**	**22.76**

Table (9): Effect of anhydrous ammonia and inoculation with *Azospirillum*, *Azotobacter*, and a mixture of them on grain and straw yields of wheat at harvesting.

Treatments	Grain yield Ard. Fed^{-1}	Straw yield ton. Fed^{-1}	Harvesting index*
1- 80 kg N fed^{-1}			
a- *Azospirillum* inoculation	22.81	5.03	0.410
b- *Azotobacter* inoculation	22.94	5.17	0.399
c- Mixed inoculation	23.17	5.47	0.389
d- Uninoculated treatment	22.20	5.05	0.397
Mean	22.78	5.18	
2- 60 kg N fed^{-1}			
a- *Azospirillum* inoculation	22.23	4.89	0.406
b- *Azotobacter* inoculation	22.72	5.00	0.405
c- Mixed inoculation	22.90	5.15	0.400
d- Uninoculated treatment	16.30	4.70	0.342
Mean	21.04	4.94	
3- 40 kg N fed^{-1}			
a- *Azospirillum* inoculation	18.93	4.75	0.360
b- *Azotobacter* inoculation	19.07	4.80	0.373
c- Mixed inoculation	19.33	4.44	0.370
d- Uninoculated treatment	13.10	4.55	0.302
Mean	17.61	4.76	
4- 20 kg N fed^{-1}			
a- *Azospirillum* inoculation	12.00	4.03	0.309
b- *Azotobacter* inoculation	22.00	4.20	0.304
c- Mixed inoculation	13.07	4.37	0.310
d- Uninoculated treatment	5.20	3.97	0.164
Mean	10.62	4.14	
5- 0 kg N fed^{-1}			
a- *Azospirillum* inoculation	6.08	3.20	0.222
b- *Azotobacter* inoculation	6.17	3.27	0.221
c- Mixed inoculation	6.40	3.33	0.224
d- Uninoculated treatment	2.10	2.07	0.132
Mean	5.188	2.97	

L.S.D. at 0.05

N rate :	0.1734	0.1333
Inoculation :	0.1551	0.1193
N x inoculation :	0.3468-7	0.2665

Table (10): Effect of anhydrous ammonia and inoculation with *Azospirillum*, *Azotobacter*, and a mixture of them on wheat yield components.

Treatments	Grain weight / spike (g)	Number of grains / spike	Spike length (cm)	Number of spiklete / spike	1000-grain weight (g)
1- 80 kg N fed^{-1}					
a- *Azospirillum* inoculation	2.45	48.13	11.25	20.13	50.98
b- *Azotobacter* inoculation	2.14	44.07	11.16	18.60	48.71
c- Mixed inoculation	2.14	43.43	11.18	19.53	48.86
d- Uninoculated treatment	2.06	45.90	11.15	17.30	44.88
Mean					
2- 60 kg N fed^{-1}					
a- *Azospirillum* inoculation	2.103	42.43	11.86	18.23	49.91
b- *Azotobacter* inoculation	1.830	37.70	11.10	16.47	49.43
c- Mixed inoculation	1.830	38.27	10.85	16.33	47.85
d- Uninoculated treatment	1.600	31.80	10.85	16.00	47.63
Mean					
3- 40 kg N fed^{-1}					
a- *Azospirillum* inoculation	1.97	40.53	10.38	19.50	48.32
b- *Azotobacter* inoculation	2.18	40.53	10.48	21.03	53.69
c- Mixed inoculation	2.32	43.38	11.12	21.30	50.58
d- Uninoculated treatment	2.13	45.40	11.35	22.20	48.09
Mean					
4- 20 kg N fed^{-1}					
a- *Azospirillum* inoculation	1.83	39.43	10.08	19.33	46.35
b- *Azotobacter* inoculation	2.18	45.10	10.72	19.70	48.25
c- Mixed inoculation	2.22	47.00	10.75	19.70	47.24
d- Uninoculated treatment	2.06	54.30	10.80	20.25	45.16
Mean					
5- 0 kg N fed^{-1}					
a- *Azospirillum* inoculation	1.43	32.13	8.80	16.27	44.44
b- *Azotobacter* inoculation	1.87	40.03	10.13	18.63	46.77
c- Mixed inoculation	2.03	41.93	10.37	19.47	48.37
d- Uninoculated treatment	1.32	43.50	11.30	19.90	44.32
Mean					

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تأثير التلقيح بالآزوتوباكتر أو الآزوسبيريللم والأمونيا الغازية علـــى نمـــو نبـــات القمح تحت ظروف الأراضي الملحية القلوية

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قسم بحوث الميكروبيولوجيا الزراعية -معهد بحوث الأراضى والمياه والبيئة-مركـــز البحـــوث الزراعية .

قسم تغذية النبات – معهد بحوث الأراضى والمياه والبيئة – مركز البحوث الزراعية .

تم إجراء هذا البحث بهدف دراسة تأثير التلقيح بالآزوتوباكتر أو الآزوسبيريللم أو خليط منهما فـــى وجود مستويات مختلفة من الآزوت المعدنى (الأمونيا الغازية) على نمـــو نبـــات القمـــح ، والنيـــتروجين الممتص ، ومكونات المحصول ، والمحتوى البروتينى للحبوب والقش تحت ظروف الأراضى الملحية القلوية

وقد أجريت تجربة زراعة حقلية خلال الموسم الشتوى ٢٠٠٠ – ٢٠٠١ بقرية أبو عيش بمحافظة الفيوم ، وكانت هناك أربعة معاملات للتلقيح البكتيرى :

١- تلقيح البذور بالآزوسبيريللم ٢- تلقيح البذور بالآزوتوبـــاكتر ٣- التلقيـــح بخليـــط متجـــانس مـــن الميكروبين ٤- بدون تلقيح (للمقارنة) .

بينما أستخدمت مستويات التسميد الأزوتى (صفر – ٢٠ – ٤٠ – ٦٠ – ٨٠ كجم نيـــتروجين / فدان) تم حقنها بالتربة قبل الزراعة ، وكانت أهم النتائج المتحصل عليها مايلى :

١- زادت أعداد الآزوسبيريللم فى التربة نتيجة للتلقيح المنفرد بالآزوسبيريللم وأعطت أعلى قيـــم للأعـــداد حتى ٦٠ يوم من الزراعة مقارنة مع التلقيح بالآزوتوباكتر ثم إنعكست الصورة فى الـ ٦٠ يوم التاليـــة حتى ١٢٠ يوم لتعطى معاملة التلقيح الخليط أعلى قيم للآزوسبيريللم عن بقية المعاملات . بينمـــا زادت أعداد الآزوتوباكتر نتيجة التلقيح المنفرد بالآزوتوباكتر عنه فى حالة التلقيح بالآزوسبيريللم وذلك عنـــد ٤٠ كجم آزوت / فدان وأعطت معاملة التلقيح الخليط زيادة طفيفة فى الأعـــداد عـــن معاملـــة التلقيـــح بالآزوسبيريللم وبخاصة عند المستويات المنخفضة من الآزوت المعدنى وقد لوحظـــت زيـــادة أعـــداد الآزوتوباكتر فى المعاملات الغير ملقحة عن معاملة التلقيح الخليط حتى ٦٠ يوم ثـــم إنخفضـــت حتـــى ١٢٠ يوم وذلك عند معدلات ٢٠ / ٤٠ كجم آزوت / فدان .

٢- أدى التلقيح سواء بالآزوسبيريللم أو الآزوتوباكتر إلى زيادة الأعداد الكلية للميكروبات فى التربة عنـــه فى حالة التلقيح الخليط والمعاملات الغير ملقحة ، كذلـــك زادت أعـــداد الميكروبـــات الكليـــة المثبتـــة للنيتروجين الجوى فى جميع معاملات التلقيح عن الغير ملقحة .

٣- حدثت زيادة معنوية فى الوزن الجاف والمحصول بزيادة معدل الآزوت المعدنى وخاصة مـــع التلقيـــح الخليط .

٤- جميع معدلات التلقيح أدت إلى زيادة فى محصول الحبوب والقش وأيضاً النيتروجين الممتص وبالتـــالى المحتوى البروتينى عن المعاملات بدون تلقيح .

٥- أعطت معاملة ٦٠ / كجم آزوت / فدان مع التلقيح نفس القيم المتحصل عليها من إســـتخدام ٨٠ كجـــم آزوت / فدان بدون تلقيح فى محصولى الحبوب والقش وأيضاً معاملة ٤٠ كجم آزوت / فدان مع التلقيح بمثبات النيتروجين قيم مماثلة تقريباً لمعاملة ٦٠ كجم آزوت للفدان بدون تلقيح مما يشير إلى أهمية دور التلقيح فى توفير حوالى ٢٥ – ٣٣% من التسميد الأزوتى .

٦- تفوقت معاملة التلقيح الخليط عند جميع مستويات التسميد الأزوتى فـــى الحصـــول علـــى أعلـــى قيـــم للمحصول ومكوناته (وزن الحبوب / سنبلة ، عدد الحبوب / سنبلة ، طول السنبلة ، عـــدد الأبـــراج / سنبلة ، وزن الألف حبة) وكذلك محتوى الحبوب والقش من النيـــتروجين الممتـــص وتبعـــها التلقيـــح المنفرد بأى من الآزوتوباكتر أو الآزوسبيريللم) .

تشير النتائج بوجه عام إلى أهمية التلقيح بمثبتات النيتروجين الجوى التـــى تعمـــل علـــى تقنيـــن إستخدام الآزوت المعدنى مما يقلل من تكاليف الإنتاج وأيضاً الحد من التلوث البيئى الناتج من الإفـــراط فـــى إستخدام الأسمدة المعدنية ، بالإضافة لما تلعبه الأسمدة الحيوية من دور هام فى إنتاج بعض منشطات النمـــو فى منطقة الريزوسفير مما يؤثر تأثيراً إيجابياً على نمو المجموع الجذرى ومـــايتبع ذلـــك مـــن زيـــادة فـــى المحصول الخضرى وإمتصاص العناصر الغذائية من التربة .