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EFFECT OF NITROGEN FERTILIZATION AND *Azospirillum* APPLICATION ON CORN YIELD, N P K CONTENTS AND SOIL BIOLOGICAL TESTS.

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ABSTRACT

Two field experiments were conducted at El-Gemmaiza Agricultural Research Station farm, middle Delta, region Egypt 30º 43ˉ latitude and 31º 07ˉ longitude during the two successive summer seasons of 2012 and 2013 to study the effect of different rates of nitrogen, biofertilizer and their combinations on yield, yield attributes of maize (cv three way cross 324), N, P and K content and available nutrients in the soil. . Split Split plot design was used with three replicates. The main plots were assigned for three nitrogen fertilization levels (80, 100 and 120 kg N fed $^{-1}$), the sub plots were assigned with three inoculation treatments (without inoculation, inoculation with isolate 1 Azospirillum and inoculation with isolate 2 Azospirillum). The sub sub plots were assigned for three spraying Azospirillum treatments (without spraying, spraying with isolate 1 and spraying with isolate 2). The obtained results can be summarized as follow:

Nitrogen fertilization level (80 kg N fed $^{-1}$) produced the highest values of corn grain yield (32.26 ardb fed $^{-1}$), stalk yield (10.14 ton fed $^{-1}$), 100 grains weight (41.15g), N % in the grains (1.82%), protein % and content in the grains, the highest T.B.C (total bacterial count), Azospirillum count and catalase activity. Meanwhile nitrogen fertilization level of 120 kg N fed $^{-1}$ had the lowest grain yield, stalk yield, protein % and content.

Inoculation with isolate 2 Azospirillum had the highest grain yield (32.53 ardb fed $^{-1}$), the highest N (2%), P (0.52%) and K (1.14%) in grain, the highest T.B.C, Azospirillum count and catalase activity comparing with isolate 1 and without inoculation treatments.

Spraying with isolate 2 Azospirillum gave the highest corn grain yield, stalk yield, 100 grain weight, protein % and content, T. B. C, Azospirillum count and catalase activity.

The interaction of 80 kg N fed $^{-1}$ + inoculation with Azospirillum isolate 2 + spraying with isolate 2 Azospirillum had the highest values of the studied parameters.

The treatment 100 kg N fed $^{-1}$ gave the highest available P after harvesting (7.04 ppm), T. B. C, Azospirillum count and catalase activity, while the treatment of 120 Kg N fed $^{-1}$ had the highest available K in the soil after harvesting.

Keywords: N fertilization, biofertilizers, corn, nutrient contents, available NPK and soil biological tests.

INTRODUCTION

Raising the demand for food production and urgent need for improvement of the agricultural products during the last decade of the 21th century needs an unprecedented spate of technological changes such as plant breeding, fertilizers and irrigation regime. So, increasing crop productivity of the unit of cultivated area has become the main goal of all workers in this field, especially under Egyptian conditions where the cultivated area is limited and it decreases annually due to housing. The agriculture development strategy must be conducted by increasing productivity of the land under cultivation, reduced costs of production and high efficiency use of inputs without causing harm to the environment.

Maize (Zea mays L.) is the most important crop among cereals after wheat and rice in respect of area, production, food and feed security.

Nitrogen is a major element and it is needed in large amount to increase growth and yield of maize. Nitrogen fertilization had significant positive effect on number of green leaves / plant, plant height and dry matter yield (Esmail and El- Sheikh, 1994& Quadros, 2009). Increasing nitrogen fertilizer levels led to increase grain yield and N-uptake by corn plants (Salem, 2000 & Barros Neto, 2008). Phosphorous uptake was also increased as the rate of nitrogen was increased (Hegab, 1990). Kandil, et, al., (1984) showed that K content in both blades and stems of corn plants was significantly increased by increasing N-levels, while K percentage in grain was not affected.

Chemical fertilizers have several negative impacts on environment and sustainable agriculture. Therefore, bio-fertilizers are recommended in these conditions and growth promoting bacteria uses as a partial replacement of chemical fertilizers (Wu, *et al.,* 2005). Growth promoting bacteria are bacteria that improve yield (Gholami, et, al., 2009 and Naserirad, et, al., 2011), growth promoting bacteria including *Azotobacter, Azospirillum and Pseudomonas* (Zahir et, al., 2004; Turan et, al.,2006 and Banerjee et, al., 2006). Tilak (1992) reported positive effect of double inoculation of *Azotobacter and Azospirillum* on dry matter of maize and sorghum.

Plant growth promoting bacteria have nitrogenase enzymatic complex system and are able to break the triple bond connecting the two nitrogen atoms and reduce N_2 to NH_3 , as well as increases in corn dry matter production and grain productivity in response to inoculation can be attributed to the stimulation that diastrophic bacteria provide to root system development, increasing root hair density, length, volume and number of lateral roots of corn resulting in higher capacity to absorb and use water and nutrients (Huergo *et al.,* 2010 and Hungria *et al.,* 2011).

A positive effect of the interaction between *Azospirillum* spp. and maize mainly when unimproved genotypes were involved in the presence of low nitrogen availability (Hungria *et al.,* 2010).

The objective of the present study is to investigate the effects of nitrogen fertilization levels on maize productivity and *Azospirillum* activity as well as the response of maize growth and productivity to two effective isolates of *Azospirillum* inoculation and spraying.

MATERIALS AND METHODS

Two field experiments were carried out at the experimental farm of El-Gemmeiza Agricultural Research Station, middle Delta, region Egypt (30 º 43 ¯ latitude and 31º 07¯ longitude) during the two successive seasons 2012 and 2013 to study the effect of different rates of nitrogen, bio fertilizer and their combinations on yield and yield attributes of maize (cv three way cross 324) N, P and K contents and available nutrients in the soil. The soil of the experiments site is clayey loam in texture and its some physical and chemical properties were determined according to the standard methods reported by Black *et al.,* 1965 and Jackson, (1967) are presented in Table 1

Table (1): Physical and chemical properties of the experimental soil

Analysis	**2012**	**2013**
Practical size distribution %		
Sand	15.07	15.07
Silt	42.41	42.41
Clay	42.52	42.52
Texture class	Clay loam	Clay loam
Chemical analysis		
pH (in 1:2.5 soil: water suspension)	8.00	8.00
E.C $dS.m^{-1}$ (soil past extract)	1.8	1.93
Organic matter (%)	1.52	1.79
Available N (ppm)	30	33
Available p (ppm)	8.00	7.8
Available K (ppm)	410	390

Isolation of *Azospirillum*

Samples from different sources (soil, leaves of zea maize and leaves of orange trees) were used to isolate *Azospirillum* . Nitrogen free bromo thymol blue (Nfb) semi solid medium was used to isolate the target microorganism, where the *Azospirillum* growth was indicated by the presence of subsurface white pellicles were streaked on solid (Nfb) medium, and incubated at 32 º C for 24 h. Morphologically different colonies of *Azospirillum* (white and yellow) were picked and re-streaked on the same medium, then transferred to nutrient agar slant and preserved at 4 º C for further work screening of isolate for the production of IAA, the colorimetric method according to Pilet and Chollet (1971). The potent two isolates were further identified by biochemical method and used to complete the experiment.

Enumeration of *Azospirillum* in soil.

For enumeration of *Azospirillum* cells, Nfb solid medium was used. After 48 h of incubation the colonies in the Nfb plates were counted by using Quebec colony counter.

Population density is expressed in terms of colony forming unit (CFU) per gram of soil with dilution factor .

$$\text{Number of cells / ml} = \frac{\text{Number of colonies}}{\text{Amount of plated X dilution}}$$ (Kanimozhi and Panneerselvam 2011)

Identification of the bacterial strains:

Bacterial strains were streaked four times for purification on malate agar medium supplemented with 20 mg l^{-1} yeast extract Dobereiner and Pedrosa, (1987). Eight isolates were recognized as belonging to the genus Azospirillum based on the morphological, cultural and some biochemical characterization by Tarrand *et al.,* (1978) and the schemes as described in 9th

edition of Bergey's manual of systematic Bacteriology (Krieg and Dobereiner, 1984).

Indole acetic acid (IAA) assay

For rapid quantitive determination of IAA, the colorometric method according to Pilet and Chollet., (1971) was used briefly: 24 h old culture of Azospirillum grown in nutrient broth media were used to inoculate Nfb media supplemented with 0.3 g/L tryptophan and incubated at 30 C for 72 h . The cultures were centrifuged at 4000 rpm for 15 min and two ml of supernatant were mixed with one ml of pilet-clhollet reagent. The mixture was incubated in the dark for 30 min. The absorbance of pink color was measured at 530 nm.

Determination of catalase activity:-

Catalase activity was determined by the method of Cohen et al. (1970) where decomposed hydrogen peroxide is measured by reacting it with excess of potassium tetraoxomanganate(VII), (KMnO4) and residual KMnO4 is measured spectrophotometrically at 480 nm.

The experiments were laid out in split-split plot design with three replicates. The main plots were assigned for three nitrogen levels, 80 (N1), 100 (N2) and 120 (N3) kg .fed $^{-1}$. (fed.= 4200 m ^{2}). Nitrogen was applied as urea 46.5% N The sub plots were assigned for two inoculating isolates, seed inoculation with Azospirillum isolates (S1) and (S2) in addition to control (without inoculation). The sub sub plots were assigned for two sprays of isolate S1 and S2 and without spraying. Grains of maize were sown in hills 30 cm apart with 70 cm between ridges. The plot area was 21 m ^{2} i.e., (6 ridges x 5 m length). Sowing date was 21 st June in both seasons. The recommended seed rate was divided into three parts, the first was noninoculated; the second was inoculated with Azospirillum isolate 1 and the third was inoculated by Azospirillum isolate 2. The amount of each N treatment was divided into two equal doses and added before the first and second irrigation. The recommended dose of phosphorus (30 kg P_2O_5. Fed $^{-1}$) was applied as single super phosphate 15.5% P_2O_5 at soil preparation and the recommended rate of potassium (24 kg K_2O. Fed $^{-1}$) was applied as potassium sulphate 48% K_2O with the second irrigation. Harvesting took place after 120 days from sowing in both seasons. Grain yield, straw yield and 100-grain weight were located at the harvesting.

`Samples of grains were oven dried at 70 ºC then milled and kept for chemical analysis. Nitrogen was determined using the microkjeldahl method as described by Jackson, (1967). Potassium was determined using a flame photometer method. Phosphorus was determined calorimetrically according to Snell and Snell (1967). All collective data for the two seasons were statically combined analyzed according to the procedure described by Gomez and Gomez, (1984) and the main values of the two seasons were tabulated in the Tables.

RESULTS AND DISCUSSION

Eight *Azospirillum* isolates were recovered from leave surface and soil Rhizosphere. After several sub-culturing, 8 isolates were identified as bacteria belonging to the genus *Azospirillum* based on the following common cultural and cell morphological characteristics (Table 2).

Table 2: Morphological, biochemical and indole acetic acid assay for bacterial isolates.

	S1	S2	S3	S4	S5	E1	E2	E3
Cell shape	**Curved**	**Curved**	**Ovoid**	**Rode**	**Curved**	**Ovoid**	**Curved**	**Curved**
Motility	+	+	+	+	+	+	+	+
Gram stain	-	-	-	-	-	-	-	-
Polymorphism	+	+	+	+	+	+	+	+
Growth in 3% NaCl	+	+	+	+	+	+	+	+
Growth in NFb medium	+	+	+	+	+	+	+	+
Catalase activity	+	+	+	+	+	+	+	+
Gelatin hydrolysis	-	-	-	-	+	-	-	-
Starch hydrolysis	-	-	-	-	-	+	-	-
Sole carbon source								
Malate	+	+	+	+	+	+	+	+
Succinate	+	+	+	+	+	+	+	+
IAA (µg. ml $^{-1}$)	34.77	39.02	23.93	25.00	20.76	19.97	28.02	27.05

On the basis of IAA production; two isolates (S1, S2) were used to complete the study under the name strain 1 (S1) and strain 2 (S2).

1-Maize grain and stalk yields & 100 grain weight :-

Data presented in Table 3 clearly show that nitrogen fertilization levels highly significant effect on grain yield and 100 grain weight which were reduced by increasing N rate. On the other hand no significant effect of nitrogen levels was detected on maize stalk yield was observed. The highest values of maize grain (32.26 ardab . fed. $^{-1}$), stalk (10.14 ton . fed. $^{-1}$) and 100 grain weight (41.15 g) were obtained with 80 kg N. fed. $^{-1}$ (feddan = 4200 m^{2}). On the other hand the lowest values of the above mentioned parameters were recorded with 120 kg N fed. $^{-1}$. This may be due to the high content of available nitrogen in the soil before the sowing (Table 1) and the contribution of the inoculation with *Azospirillum* by fixing some needed nitrogen. These results agreed with those obtained by Soleymanifard *et al.,* (2013).

In respect to the inoculation with Azospirillum data show highly significant effect of inoculation on maize grain yield in favor of isolate 2 (Table 3). The highest value (32.53 ardab. fed. $^{-1}$) was obtained with the inoculation with strain 2 *Azospirillum*, and the lowest value (28.55 ardab. fed. $^{-1}$) was observed with the control treatment (without inoculation. On the other hand no significant differences were noticed in stalk yield and 100 grain weight due to the Azospirillum inoculation.

Data in Table 3 show that spraying maize plants with Azospirillum strains high resulted in significant increases in grain, stalk yields and 100 grain weight in favor of spraying isolate 2. The highest values of grain yield, stalk yield and 100 grain weight 31.3 ardab. fed. $^{-1}$, 10.73 ton .fed . $^{-1}$ and 40. 82 g, respectively which were obtained with strain 2 and the lowest values were observed with the treatment of no spraying. These results are in harmony with those obtained by Qudors, (2009) and Soleymanifard *et al.,* (2013).

Table 3: Effect of nitrogen fertilization levels, Azospirillum inoculation and spraying on maize grain, stalk yields and 100 grain weight (mean values of the two seasons)

Treatment		**Crop yield * ardb .fed^{-1}**	**Stalks fresh w. Ton.fed^{-1}**	**100-grain Weight (g)**
A) nitrogen	80 N.fed^{-1}	32.26 a	10.14	41.15 a
	100 N.fed^{-1}	32.03 a	9.60	38.54 b
	120 N.fed^{-1}	28.31 b	9.25	37.61 c
F test L.S.D 0.05		** 0.65	n.s --	** 0.897
B) inoculation	Without	28.55 c	10.08	38.80
	With S1	31.52 b	9.46	39.06
	With S2	32.53 a	9.46	39.44
F test L.S.D 0.05		** 0.47	n.s --	n.s --
C) spray	Without	30.40 b	7.62 b	36.80 c
	With S1	30.90 ab	10.64 a	39.68 b
	With S2	31.30 a	10.73 a	40.82 a
F test L.S.D. 0.05		** 0.51	** 0.57	** 0.52
F test A*B L.S.D. 0.05		** 0.65	* 2.43	** 0.37
F test B*C L.S.D. 0.05		n.s --	n.s --	n.s --
F test A*C L.S.D. 0.05		n.s --	n.s --	n.s --
F test A*B*C L.S.D. 0.05		n.s --	n.s --	n.s --

***Ardb= 140 kg**

The interaction effect between the nitrogen fertilization levels and the inoculation with *Azospirillum* showed significant effects on maize grain and straw yields and 100 grain weight (Table 4 and Figure 1). The highest values were obtained with N1 C3 (80 kg N .fed. $^{-1}$ + inoculation with isolate 2 of *Azospirillum*) since 5172.1 kg grain .fed. $^{-1}$, 10.77 ton stalk .fed. $^{-1}$ and 43.68 g of 100 grains were reduced. The lowest values were recorded with N3 C2 (120 kg N .fed. $^{-1}$ + inoculation with isolate 1 of Azospirillum). These results are in agreement with the obtained by Campos and Genata, (2000) and Maria *et al.,* (2012).

Table 4 effect of the interaction between N levels and Azospirillum inoculation on maize grain and stalk yields and100 grain weight

Treatment	grain yield kg. fed. $^{-1}$	Stalks fresh w. Ton.fed. $^{-1}$.	100-grain weight (g)
N1C1	3370.383	9.5	36.86
N1C2	5006.283	10.16	42.91
N1C3	5172.067	10.77	43.68
N2C1	3563.467	10.54	38.92
N2C2	4931.967	9.22	38.4
N2C3	4956.35	9.04	38.29
N3C1	5056.683	10.19	40.62
N3C2	3301.783	8.99	35.87
N3C3	3532.433	8.57	36.35

C1= without inoculation, C2=inoculation with starin 1 and C3=inoculation with strain 2, N1= 80kg N .fed^{-1}, N2= 100 kg N .fed^{-1} and N3= 120 kg N .fed^{-1}

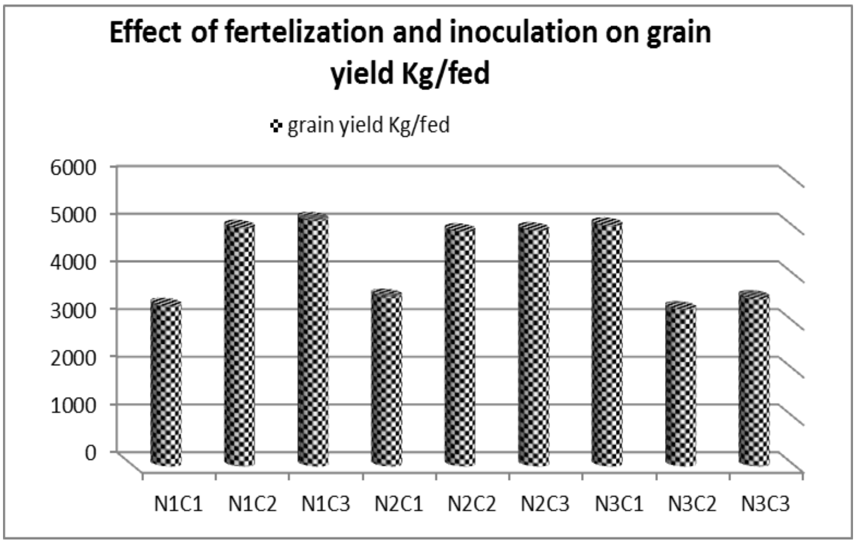

Fig.1. The interaction effect between nitrogen fertilization levels and Azospirillum inoculation on maize grain yield.

2-Nitrogen, P and K% in maize grain:

Data presented in Table 5 show that nitrogen fertilization levels significantly affected N%, P% and K% in maize grain. N% in corn grain was reduced by increasing N rate while P% and K% were increased by increasing N rate to 100 kg N. fed $^{-1}$, then declined with the highest N rate (120 kg N. fed^{-1}). These results agreed with those obtained by Dobbelaere *et al.,* (2003**).**

In respect to *Azospirillum* inoculation data in Table 5 show that, inoculation with Azospirillum significantly increased N%, P% and K% in maize grain in favour of isolated 2. The highest values of 2.0 % N, 1.14 % K and

0.52 %P were obtained with inoculation with strain 2 Azospirillum. On the other hand the lowest values were recorded with noninoculation treatment.

Table 5 effect of nitrogen fertilization levels and inoculation with Azospirillum strain and spraying on maize grain N, P and K percentage.

Treatment		T.N % in grain	K% in grain	P% in grain
A) nitrogen	80 N.fed^{-1}	1.89 a	1.13 a	0.46 b
	100 N.fed^{-1}	1.86 a	1.23 a	0.48 a
	120 N.fed^{-1}	1.79 b	0.97 b	0.43 b
F test L.S.D 0.05		0.066	** 0.098	* 0.033
B) inoculation	Without	1.57 b	1.06 b	0.39 c
	With S1	1.98 a	1.13 a	0.45 b
	With S2	2.00 a	1.14 a	0.52 a
F test L.S.D 0.05		** 0.058	* 0.059	** 0.036
C) spray	Without	1.76 c	1.03 b	0.42 b
	With S1	1.86 b	1.14 a	0.45 a
	With S2	1.92 a	1.16 a	0.50 a
F test L.S.D. 0.05		** 0.057	** 0.059	** 0.043
F test A*B L.S.D. 0.05		** 0.35	** 0.35	** 0.22
F test B*C L.S.D. 0.05		* 0.34	n.s --	n.s --
F test A*C L.S.D. 0.05		n.s --	n.s	n.s --
F test A*B*C L.S.D. 0.05		n.s --	* 0.35	n.s --

Spraying corn with *Azospirillum* spraying highly significant increased N, P and K % in maize grain. Isolate 2 was superior with the highest values of 1.92 % N, 1.16 % K and 0.5 %P, while Uninoculated treatment had the lowest values. This may be due to *Azospirillum* produce some hormones which enhanced plant shoot, root growth and nutrients uptake. This is in agreement with those obtained by Swedrzynska and Sawicka, (2000), Hungria *et al.,* (2010), Maria *et al.,* (2012) and Soleymainfard *et al.,* (2013).

Highly significant effect for the interaction between nitrogen fertilization levels and *Azospirillum* inoculation on N, P and K% in corn grain (Fig. 2) was detected. The highest N (2.15%) and K% (1.36) were obtained with 80 kg N.fed $^{-1}$ + inoculation with strain 2, while the highest value of P (0.61 %) was obtained with 100 kg N.fed $^{-1}$ + inoculation with strain 2 followed by N1C3 treatment. On the other hand the lowest values were recorded with 80 kg N.fed $^{-1}$ without inoculation. This due to Azospirillum strains had high activity under the low nitrogen level. These results agreed with those obtained by Campos and Genatta, (2000), Dobbelaere *et al.,* (2001) and Lopes, (2007), who concluded that the efficiency of biological nitrogen fixation in *Azospirillum spp*. is rapidly or even inhibited in the presence of higher concentrations of nitrogen compound in the soil. Hungria *et al.,* (2010) and Begheri, (2011) who concluded that Azospirillum can increase ion absorption e. g. K and NO_3

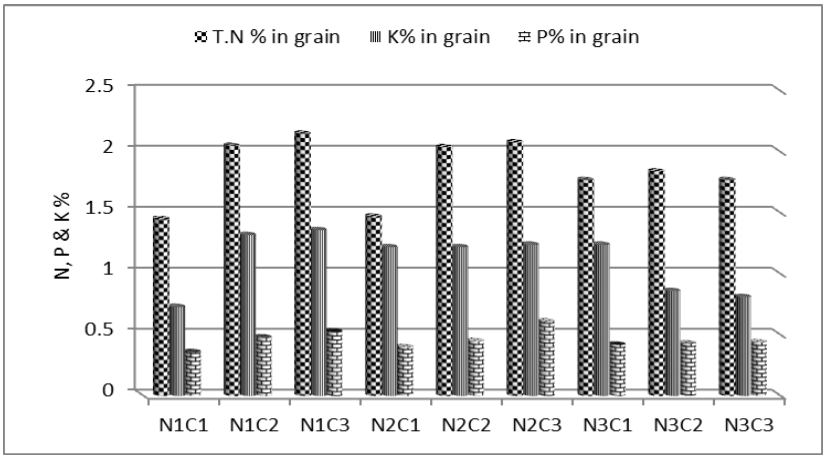

Fig.2: the interaction effects between nitrogen fertilization levels and Azospirillum inoculation on N, P and K% in maize grain.

C1= without inoculation, C2=inoculation with strain 1 and C3=inoculation with strain 2, N1= 80 kg N .fed^{-1}, N2= 100 kg N .fed^{-1} and N3= 120 kg N .fed^{-1}

Data presented in Figure 3 show that the interaction between inoculation and spraying Azospirillum significantly affected N % in corn grain.

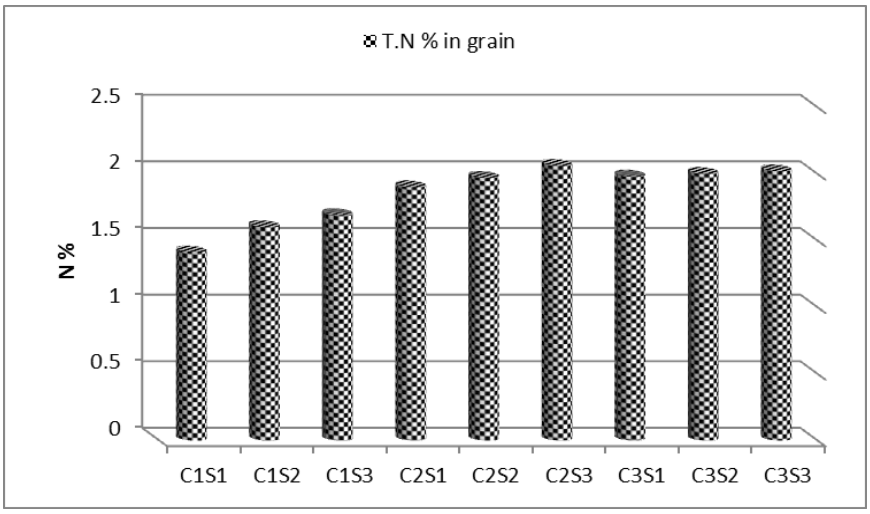

Fig. 3 The interaction effects between Azospirillum inoculation (C) and spraying (S) on N % maize grain (the mean values in the two seasons).

The highest values were obtained with C2 S3 (inoculation with strain 1 + spraying with strain 2) followed by C3 S3 (inoculation with strain 2 + spraying with strain 2) treatments. On the contrary the lowest value was recorded with C1S1 (without inoculation and without spraying). This may be due to *Azospirillum* one of PGPR group which increase nitrogen by fixation and increasing the N absorbance. This was in harmony with those obtained by Jain *et al.,* (2010) and Begheri, (2011).

3- Grain protein content:

Data presented in Table 6 show that nitrogen fertilization levels significantly affected protein % and protein content kg .fed^{-1} of maize grain. The highest values were obtained with the lower N rate 80 kg N .fed^{-1} showing a reduction by increasing N rate. This may be due to the fixed nitrogen and PGPR by Azospirillum in addition to native nitrogen in the soil which led to increase protein % and grain yield. Similar results were reported by Maria *et al.* (2012).

Table 6: Effect of nitrogen fertilization levels, Azospirillum inoculation and Azospirillum spraying on protein % and protein content of corn grain (mean values of the two seasons).

Treatment		% protein in grain	Protein content in grain kg.fed^{-1}	Grain yield kg.fed^{-1}
A) nitrogen	80 N.fed^{-1}	10.75 a	500.10 a	4516.24 a
	100 N.fed^{-1}	10.61 a	486.37 a	4483.93 a
	120 N.fed^{-1}	10.22 b	404.26 b	3963.63 b
F test L.S.D 0.05		* 0.37	** 24.00	** 91.299
B) inoculation	Without	8.92 b	363.42 c	3996.84 c
	With S1	11.26 a	501.30 b	4413.34 b
	With S2	11.40 a	526.00 a	4553.62 a
F test L.S.D 0.05		** 0.33	** 16.76	** 66.01
C) spray	Without	10.06 c	438.93 c	4256.51 b
	With S1	10.57 b	464.76 b	4325.46 ab
	With S2	10.96 a	487.04 a	4381.84 a
F test L.S.D. 0.05		** 0.32	** 13.3	** 71.02
F test A*B L.S.D. 0.05		** 0.81	** 9.62	** 10.39
F test B*C L.S.D. 0.05		* 0.796	* 4.7	n.s --
F test A*C L.S.D. 0.05		n.s --	n.s --	n.s --
F test A*B*C L.S.D. 0.05		n.s --	n.s --	n.s --

Azospirillum inoculation significantly increased protein % in maize grain and protein content (kg fed^{-1}) of maize grain (Table 6). The highest values of protein% (11.4 %) and protein content (526 kg protein fed^{-1}) were obtained with strain 2 *Azospirillum* inoculations. On the contrary control treatment recorded the lowest values (8.92 % and 363.42 Kg protein fed^{-1}). This may be due to that strain 2 of *Azospirillum* was more active which produce higher amount of nitrogen fixed and plant promoting growth agents. These results are harmony with those obtained by Naserirad *et al.* (2011) and Soleymanifard *et al.* (2013).

It is quite obvious from the data presented in Table 6 that *Azospirillum* spraying induced high significant effect on protein % and protein content (kg fed^{-1}) of maize grain. The highest values were obtained spraying with strain 2 *Azospirillum* (10.96 % and 487.04 kg fed^{-1}) respectively, while the lowest values were recorded with the control treatment. Showing that *Azospirillum* enhanced plant growth and protein accumulation. These results are in agree with those obtained by Allen et al. (1980) and Ribaudo et al. (2001) who concluded that the maize inoculated by Azospirillum showed increased activity of glutamate dehydrogenase (GDH) and glutamine synthestase (GS) in plant cells.

The interaction effect between the nitrogen levels and *Azospirillum* inoculation on protein % in corn grain is shown in Fig (4). The interaction had highly significant effect on protein% and, the highest value (12.27 %) was obtained with (N1C3) (80 Kg N fed^{-1} with inoculation by strain 2 *Azospirillum*). On the contrary the lowest values were observed with N1C1 treatment (80 kg N fed^{-1} without *Azospirillum* inoculation). This may be due to that *Azospirillum* activity depends on available N in soil, and it was more active under low available N. This is in harmony with those obtained by Ribaudo *et al.* (2001) and Maria *et al.* (2012).

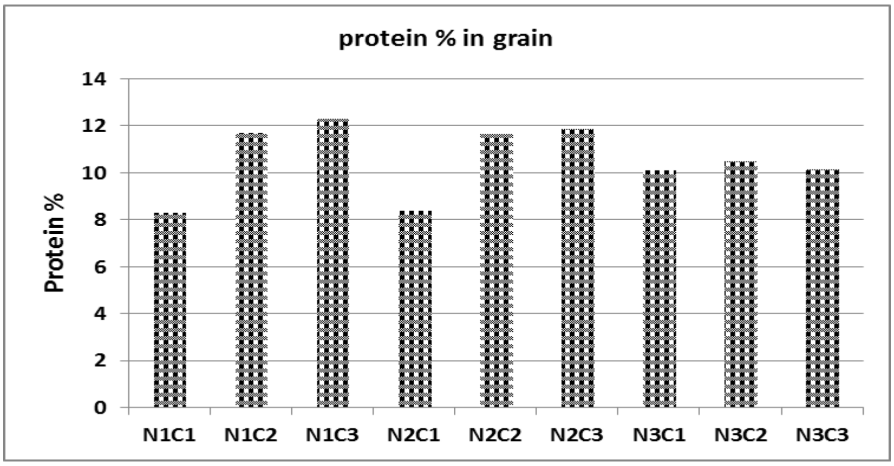

Fig. 4: The interaction effect between nitrogen levels and Azospirillum inoculation on protein % in corn grain

Data illustrated in Fig 5 show that the interaction between Azospirillum inoculation and Azospirillum spraying had significant effects on protein content (kg fed^{-1}) of maize grain. The highest value (539.05 kg fed^{-1}) was obtained with and C3S3. On the other hand the lowest value (319.53 kg fed^{-1}) was recorded with C1S1 treatment (neither *Azospirillum* inoculation nor spraying)

This may be due to that the presence of *Azospirillum* at root zone and on leaves enhanced plant hormones which affect growth, and synthesis and protein accumulation. These results are in agreement with those obtained by Ribaudo *et al.* (2001), Han *et al.* (2005) and Huergo *et al.* (2010).

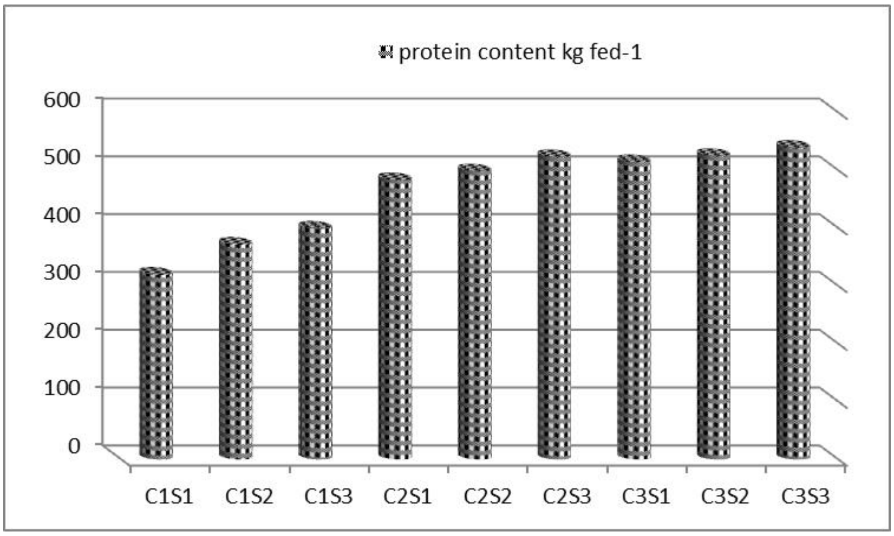

Fig 5: The interaction between Azospirillum inoculation and spraying on protein content in maize grain

4-Soil available N, P and K after harvest:

Data presented in Table 7 show that nitrogen fertilization levels induced highly significant effect on available P and K in the soil after maize harvesting. The highest available P value (7.04 mg kg^{-1}) soil was obtained with 100 kg N fed^{-1} level, while the lowest P value (5.61 mg kg^{-1}) soil was observed with 80 kg N level. In respect to available K, the highest value (486.83 mg kg^{-1}) soil was obtained with 120 kg N fed^{-1} , while the lowest value (307.37 mg kg^{-1}) soil was observed with 80 kg N fed^{-1}. This may be due to that the higher nitrogen levels led to slightly change in soil pH and/ or increased cation exchange which led to increased K released. Similar results were reported by Sami *et al.* (2008). On the other hand no significant differences were noticed in available N after maize harvesting due to nitrogen fertilization levels. Azospirillum inoculation had highly significant effect on available N, P and K in the soil after maize harvesting (Table7).

Table 7: Effect of nitrogen fertilization levels, Azospirillum inoculation and Azospirillum spraying on available N, P &K in the soil after maize harvesting

Treatment		K-available in soil (ppm)	P-available in soil (ppm)	N-available in soil (ppm)
A) nitrogen	80 N.fed^{-1}	307.37 a	5.61 b	43.83
	100 N.fed^{-1}	442.20 b	7.04 a	43.44
	120 N.fed^{-1}	486.83 c	6.98 a	41.46
F test L.S.D 0.05		** 28.83	** 0.69	n.s --
B) inoculation	Without	444.17 a	6.59 ab	41.16 b
	With S1	428.25 a	5.82 b	42.05 b
	With S2	363.97 b	7.21 a	45.52 a
F test L.S.D 0.05		** 25.94	** 0.78	** 2.13
C) spray	Without	399.83	6.18 b	42.01
	With S1	418.31	6.75 a	42.21
	With S2	418.25	6.70 a	44.51
F test L.S.D. 0.05		n.s --	** 0.35	n.s --
F test A*B L.S.D. 0.05		** 51.89	** 2.71	** 5.23
F test B*C L.S.D. 0.05		n.s --	n.s --	n.s --
F test A*C L.S.D. 0.05		** 61.65	n.s --	n.s --
F test A*B*C L.S.D. 0.05		* 63.77	n.s --	n.s --

The highest available (45.52 mg.kg^{-1}) soil and P (7.21 mg.kg^{-1}) were obtained with inoculation with isolate 2, while the highest available K value was obtained with uninoculated treatment. On the other hand the lowest available N value (41.16 mg.kg^{-1}) was recorded with uninoculated treatment, while the lowest available P value (5.82 mg.kg^{-1}) was observed with isolate 1 and the lowest available K value (363.97 mg.kg^{-1}) was observed with isolate 2. This may be due to isolate 2 of Azospirillum was effective on nitrogen fixation and producing substances affects phosphorus availability, but neither isolate 1 nor isolate 2 had effects on K availability. These results agreed with those obtained by Han *et al.,* (2005); Huergo *et al.,* (2010), Bashan and de-Bashan, (2010) and Araujo *et al.,* (2015).

Azospirillum spraying had no significant effects on available N and K in the soil after corn harvesting, significant effect on available P (Table 7) with no significant differences between the two isolates.

Data presented in Table 8 show that, the interaction between nitrogen fertilization levels and inoculation with Azospirillum significantly affected the available N, P and K in the soil after maize harvesting. The highest P value (8.55 mg.kg^{-1}) was obtained with N2C3 (100 kg N.fed^{-1} + inoculation with isolate 2), while the lowest P value was recorded with N3C2 (120 kg N.fed^{-1}

+ inoculation with isolate 1). In respect to available K the highest value was obtained with N3C1 (120 kg N.fed^{-1} without inoculation), but the lowest value was recorded with N1C1 (80 kg N.fed^{-1} without inoculation). In respect to available N the highest value (53.02 mg.kg^{-1}) was obtained with (80 kg N.fed^{-1}+ inoculation with isolate 2). The lowest value was recorded with N1C1 (80 kg N.fed^{-1} without inoculation). This may be due to that the high nitrogen level inhibits *Azospirillum* activity and increased mineral N loss from the soil by any way. Similar results were reported by Lopes, (2007).

Table 8: Effect of the interaction between N-levels and Azospirillum inoculation on N, P & K available in soil after harvest.

Treatment	P-available in soil (ppm)	N-available in soil (ppm)	K-available in soil (ppm)
N1C1	5.32	35.85	240.50
N1C2	5.53	42.63	369.81
N1C3	5.98	53.02	311.80
N2C1	5.91	42.16	454.14
N2C2	6.66	42.89	502.26
N2C3	8.55	45.26	370.19
N3C1	8.54	45.47	637.88
N3C2	5.28	40.62	412.68
N3C3	7.10	38.28	409.92

5- Azo. log. Viable (c.f.u. ml^{-1}), T.C.B. log. viable (c.f.u. ml^{-1}) and catalase activity (H_2O_2 g.soil $^{-1}$. 15 min $^{-1}$).

Data in Table 9 clearly show that, nitrogen fertilization levels high significantly affected Azo. log. viable (c.f.u. ml^{-1}), T.C.B. log. viable (c.f.u. ml^{-1}) and catalase activity (H_2O_2 g.soil $^{-1}$. 15 min $^{-1}$). The highest values (6.44, 8.61) and 124.81, respectively, were obtained with 100 kg N.fed^{-1}. No significant differences were detected between 100 and 120 kg N.fed^{-1} levels in catalase activity. On the other hand the lowest values (6.39, 8.46) and 80.11 respectively were recorded with 80 kg N.fed^{-1} . In respect to *Azospirillum* inoculation there were highly significant effect on *Azospirillum* log viable (c.f.u. ml^{-1}), T.C.B viable (c.f.u. ml^{-1}) and catalase activity. The highest values 189.58, 402.89 and 129.19 respectively were obtained with inoculation by strain 2. In the same trend spraying with strain 2 had the highest Azospirillum log viable C.F.U ml^{-1} of 223.47, T.C.B log viable CFU ml^{-1} of 392.58 and catalase activity H_2O_2 g^{-1} soil.15 minutes of 130.03. The lowest values of 89.44. 188.42 and 85.5 respectively were recorded with the treatment without spraying. These resulted agreed with those obtained by lopes, (2007) who reported that, the efficiency of biological fixation in *Azospirillum spp*. is rapidly reduced or even inhibited in the presence of higher concentrations of nitrogen compounds in the soil by inhibition of nitrogenase activity in bacteria.

Table 9: Effect of N-levels, Azospirillum inoculation and spraying of Azospirillum on azo log (cfu .ml^{-1}), TCB log (cfu.ml^{-1}) and catalase activity H_2O_2 g^{-1} soil. 15 min.

Treatment		Azo log viable (cfu..ml^{-1})	T.C.B log viable (cfu.ml^{-1})	Catalase activity (µ moles H_2O_2 g^{-1} soil.15 min^{-1})
A) nitrogen	80 N.fed^{-1}	6.39b	8.46a	80.11 b
	100 N.fed^{-1}	6.44a	8.61a	124.81 a
	120 N.fed^{-1}	6.40b	8.62b	124.08 a
F test L.S.D 0.05		* 0.03	** 0.01	** 4.5
B) inoculation	Without	6.41b	8.51b	98.00 c
	With S1	6.33c	8.48c	101.81 b
	With S2	6.49a	8.70a	129.19 a
F test L.S.D 0.05		** 0.03	** 0.02	** 2.45
C) spray	Without	6.23c	8.40c	85.50 c
	With S1	6.38b	8.59b	113.47 b
	With S2	6.62a	8.69a	130.03 a
F test L.S.D. 0.05		** 0.04	** 0.029	** 3.45
F test A*B L.S.D. 0.05		** 0.07	** 0.05	** 2.08
F test B*C L.S.D. 0.05		** 0.085	** 0.041	** 3.00
F test A*C L.S.D. 0.05		** 0.085	** 0.041	** 3.00
F test A*B*C L.S.D. 0.05		** 0.095	** 0.06	** 3.09

Data presented in Fig 6 and 7 show that, highly significant effects of the interaction between nitrogen fertilization levels and *Azospirillum* inoculation on log TCB, log *Azospirillum* CFU ml^{-1} and catalase activity were observed.

The highest values of log TCB (8.89), log *Azospirillum* 6.56 cfu. ml^{-1} and catalase activity (169.25) were obtained with N2C3 treatment (100 kg N fed^{-1} + inoculation with isolate 2 *Azospirillum)* Figs. (6 and 7). On the other hand the lowest values of log TCB (8.34) CFU ml^{-1}, catalase activity (74.25) H_2O_2. g soil. 15 minutes were detected with N1C1 treatment (80 kg N fed^{-1} + without inoculation) and the lowest value of log *Azospirillum* CFU ml^{-1} (6.30) was recorded with N1C2 treatment (80 kg N fed^{-1} + inoculation with isolate1). This mean that strain 2 of Azospirillum was the effective on maize and it needs up to 100 kg N fed^{-1} for obtain the best regards from maize. These results are in agreement with those obtained by Swedrzynska and Sawicka, (2000) and Creus *et al.* (2004).

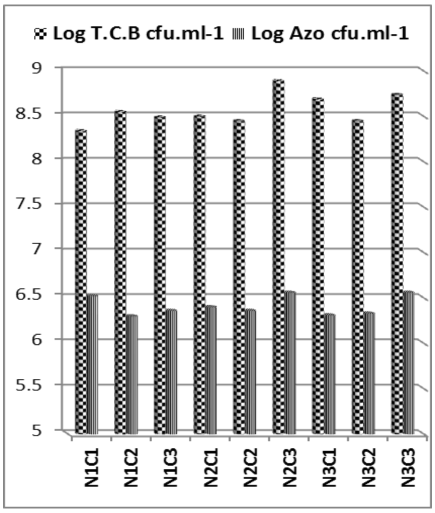

Fig 6: Effect of the interaction between N-levels and Azospirillum inoculation on log TCB, log Azospirillum (cfu. ml^{-1})

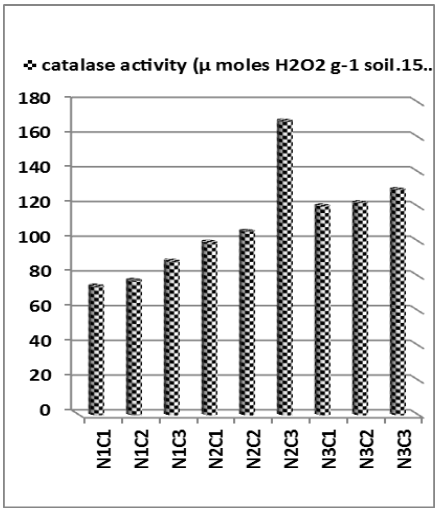

Fig 7 :Effect of the interaction between N- levels, Azospirillum inoculation on catalase activity

The interaction between grain maize inoculation and spraying with *Azospirillum* was highly significant for log TCB, log *Azospirillum* (cfu. ml^{-1}) (Fig. 8) and catalase activity H_2O_2. g. $soil^{-1}$.15 minutes (Fig 9). The highest values of logTCB (8.83), log *Azospirillum* (6.82) CFU ml^{-1} and catalase activity (159.83) H_2O_2 1g $soil^{-1}$. 15 minutes were obtained with inoculation with strain 2 Azospirillum + spraying with strain 2 *Azospirillum.* On the contrary the lowest values of TCB (8.36), log *Azospirillum* (6.23) and catalase activity (74.92) were recorded with neither inoculation and nor spraying treatment. Similar results were reported by Khaliq et al. (2004).

Data presented in Fig (10 and 11) show high significant effects of the interaction between nitrogen fertilization levels and *Azospirillum* spraying on log TCB, log *Azospirillum* (cfu. ml^{-1}) and catalase activity. The highest values (8.81, 6.64 and 157.0) respectively were obtained with 120 kg N .fed^{-1} + spraying with strain 2 *Azospirillum* treatment. On the other hand the lowest values were recorded with 80 kg N fed^{-1} without *Azospirillum* spraying. This may be due to *Azospirillum* spraying lead to the presence of the microorganism on the plant leaves and may penetrate the leaves which produce some phytohormones led to increase log TCB, log *Azospirillum* and catalase activity.

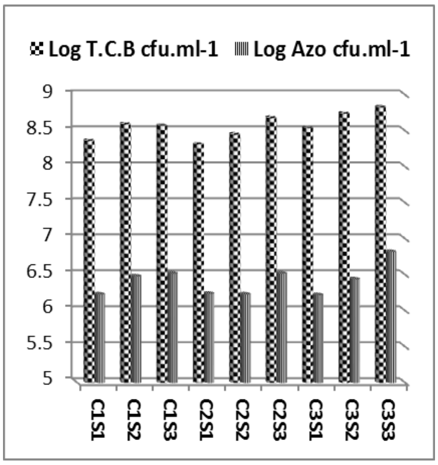

Fig-8 The interaction effect between Azospirillum inoculation and Azospirillum spraying on log TCB and log Azospirillum ml^{-1}

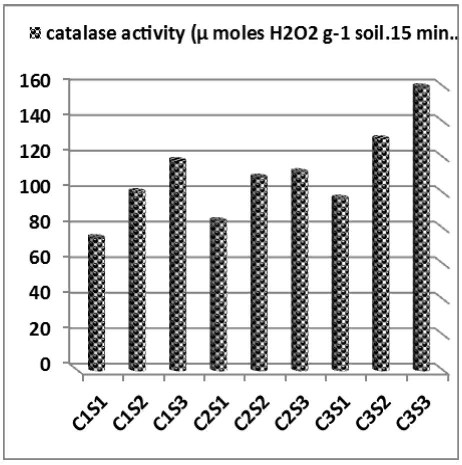

Fig-9 The interaction effect between Azospirillum inoculation and Azospirillum spraying on catalase activity

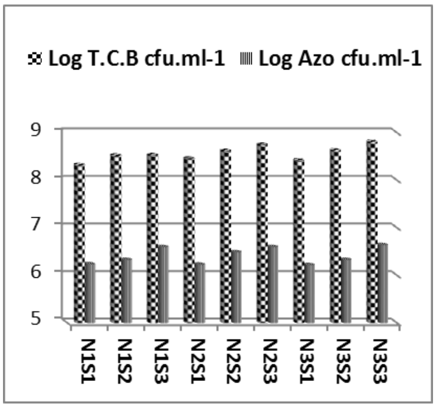

Fig-10:The interaction effect between N-levels and Azospirillum spraying on log TCB and log Azospirillum ml^{-1}

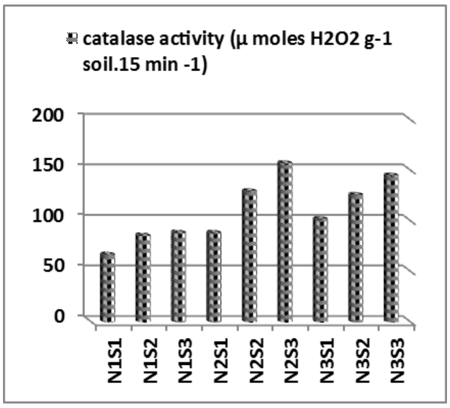

Fig-11:The interaction effect between N-levels and Azospirillum spraying on catalase activity

Data presented in Table 10 show that the interaction between nitrogen fertilization levels, *Azospirillum* inoculation and *Azospirillum* spraying highly significant effect on TCB, log *Azospirillum* and catalase activity. The highest values (810.25, 440 and 230) respectively were obtained with $N_2C_3S_3$ treatment (100 Kg N fed^{-1} + inoculation with strain 2 *Azospirillum* + spraying with strain 2 *Azospirillum.*

Table 10: Effect of the interaction between N- fertilization levels, Azospirillum inoculation and Azospirillum spraying on log TCB ,log Azospirillum cfu. ml^{-1} and catalase activity.

Treatment	Azo log viable (cfu..ml^{-1})	T.C.B log viable cfu..ml^{-1}	catalase activity (µ moles H_2O_2 g^{-1} soil.15 min $^{-1}$)
$N_1C_1S_1$	133.75	117.25	59.75
$N_1C_1S_2$	197.50	211.75	76.75
$N_1C_1S_3$	177.50	147.00	86.25
$N_1C_2S_1$	67.50	157.50	63.00
$N_1C_2S_2$	80.00	238.00	92.50
$N_1C_2S_3$	186.25	404.25	77.00
$N_1C_3S_1$	77.50	180.25	77.00
$N_1C_3S_2$	82.50	259.00	87.25
$N_1C_3S_3$	251.25	220.50	101.50
$N_2C_1S_1$	70.00	157.50	78.50
$N_2C_1S_2$	230.00	271.25	102.00
$N_2C_1S_3$	132.50	255.50	117.75
$N_2C_2S_1$	121.25	180.25	82.75
$N_2C_2S_2$	92.50	141.75	111.25
$N_2C_2S_3$	138.75	301.00	123.25
$N_2C_3S_1$	78.75	295.75	103.25
$N_2C_3S_2$	173.75	654.50	174.50
$N_2C_3S_3$	440.00	810.25	230.00
$N_3C_1S_1$	71.25	229.25	86.50
$N_3C_1S_2$	78.75	350.00	123.75
$N_3C_1S_3$	191.25	490.00	150.75
$N_3C_2S_1$	83.75	106.75	107.50
$N_3C_2S_2$	87.50	224.00	123.25
$N_3C_2S_3$	175.00	318.50	135.75
$N_3C_3S_1$	101.25	271.25	111.25
$N_3C_3S_2$	182.50	348.25	130.00
$N_3C_3S_3$	318.75	586.25	148.00
F test	**	**	**
L.S.D. 0.05	9.27	11.04	3.00

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تـأثير التسـميد النيتروجينـى واضـافة الازوسـبيريلليم علـى انتاجيـة محصـول الـذرة والاختبارات الحيوية بالارض

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تهدف الدراسة الى تقييم دور التلقيح بالازوسبيريلليم كلقاح حيوى على انتاجية الذرة الشامية وترشيد استحدام الاسمدة الكيماوية . اقيمت تجربتان حقليتان بمحطة البحوث الزراعية بالجميزة خلال الموسمين الصيفين 2012 و 2013 استخدم تصميم القطع المنشقة مرتين فى ثلاث مكرارات حيث شغلت القطع الرئيسية ثلاث معاملات لمستويات نيتروجين 80و 100و 120 كجم ن للفدان والقطع الشقية ثلاث معاملات تلقيح بالازوسبيريللم (بدون تلقيح و سلالة 1و سلالة 2) والقطع التحت شقية ثلاث معاملات رش بالازوسبيريللم (بدون رش و سلالة 1و سلالة 2)

وتتلخص النتائج فى الاتى:

- اعطى مستوى النيتروجينى 80 كجم ن للفدان تحت ظروف التجربة اعلى القيم لمحصول حبوب (32.26 اردب للفدان) و محصول العيدان (10.14 طن للفدان) و وزن 100 حبة (41.15 جم) و نسبة ن فى الحبوب (1.82%) و نسبة بروتين و محتوى بروتينى للفدان مقارنة بمستويات النيتروجين الاخرى كانت اقل قيم للحبوب والعيدان ووزن ال 100 حبة مع المستوى 120 كجم ن للفدان تحت ظروف التجربة
- اعطى التلقيح بالسلالة 2 ازوسبيريلليم اعلى محصول حبوب (32.53 اردب للفدان) وكانت اقل قيمة للحبوب مع المعاملة بدون تلقيح ولا يوجد تأثير معنوى على وزن ال 100 حبة او محصول العيدان كما اعطى التلقيح بالسلالة 2 اعلى القيم لنسبة النيتروجين بالحبوب (2 %) و نسبة فوسفور (0.52%) و نسبة بوتاسيوم (1.14%) فى الحبوب و نسبة محتوى بروتينى فى الحبوب و عدد كلى للبكتيريا فى الارض و عدد للازوسبيريلليم بالارض و نشاط لأنزيم الكتاليز بالارض مقارنة بمعاملات التلقيح الاخرى
- اعطى الرش بالسلالة 2 ازوسبيريللم اعلى محصول حبوب وعيدان ووزن ال 100 حبة واعلى نسبة بروتين ومحتوى بروتينى فى الحبوب واعلى قيم لعدد البكتيريا بالارض وعدد الازوسبيرييلم بالارض ونشاط انزيم الكتاليز مقارنة بمعاملات الرش الاخرى
- بينت نتائج التفاعل بين مستويات النيتروجين والتلقيح ان 80 وحدة ن للفدان والتلقيح بالسلالة 2 ازوسبيريللم اعطت افضل محصول حبوب وعيدان ووزن ال 100 حبة
- اعطت المعاملة 100 كجم ن للفدان اعلى فوسفور ميسر بالارض بعد الحصاد (7.04 جزء فى المليون) واعلى قيم للعد الكلى للبكتيريا بالارض وازوسبيريللم ونشاط انزيم الكتاليز واعلي نسبة فوسفور وبوتاسيوم بالحبوب
- كما اعطت المعاملة 120 كجم ن /فدان اعلي بوتاسيوم ميسر بالأرض بعد الحصاد ولا توجد فروق معنوية بين مستويات النيتروجين علي النيتروجين الميسر بالأرض بعد الحصاد