Response of barley to biofertilizer with N and P application under newly reclaimed areas in Egypt.

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ABSTRACT

Two field experiments were conducted in the 1995/1996 and 1996/1997 growing seasons in the new lands at Nubaria, which located at 46-km Southwest Alexandria, Egypt. Two experimental factors including mineral and bio-fertilizer in addition to different combination between the two factors were studied. Mineral fertilizer treatments were non-fertilizer (control), 140 kg N, 35 kg P_2O_5 and 140 kg N + 35 kg P_2O_5/ha. Biofertilizer treatments were non-inoculation (control), Microbin and Azottein. Biofertilizers (Microbin and Azottein) were constituted of mixture of different nitrogen fixers of the genera *Azospirillum, Azotobacter, Klebsiella and Bacillus*.... etc. Randomized complete block design was used with three replications. The plot size was 10.5 m^2 including 15 rows of 3.5 m long and 20 cm apart.

Results showed that grain yield reached about 2.81 and 3.60 ton/ha in the first and second season respectively due to application of 140 kg N + 35 kg P_2O_5/ha, with about 401.8 and 95.7% increase over the control. Microbin and Azottein caused significant increases in grain yield reached about 24.8 and 27.2% in the first season and 18.4 and 22.0% in the second season respectively compared to the un-inoculation. Application of 140 kg N + 35 kg P_2O_5/ha gave the highest straw yield, about 7.0 and 8.6 ton/ha with about 388.9 and 92.8% increase over the control in the first and second season respectively. Microbin and Azottein caused increases in straw yield reached about 15.6 and 7.8% in the first season and 4.6 and 4.7% in the second season respectively compared with the un-inoculated control. Application of 140 kg N + 35 kg P_2O_5/ha gave harvest index about 28.4 and 29.9 with about 0.0 and 0.7% increase over the control. Microbin and Azottein resulted in increases in harvest index by 1.1 and 6.3% in the first season and 10.0 and 13.1% in the second season respectively compared to the un-inoculated control. Highest number of tillers/m^2 (291.3 and 495.9) was obtained from the application of 140 kg N + 35 kg P_2O_5/ha, with about 97.6 and 87.5% increase over the control in the first and second season respectively. On the other hand, Microbin and Azottein caused increases in number of tillers/m^2 reached about 20.3 and 9.7% in the first season and 14.9 and 11.0% in the second season respectively over the un-inoculated control. Application of 140 kg N + 35 kg P_2O_5/ha resulted in spike length reached about 5.49 and 7.70 cm with about 35.6 and 102.1% increase compared to the control in the first and second season respectively. Inoculation of barley seeds with Microbin and Azottein resulted in spike length reached about 9.1 and 7.5% increase in the first season and 10.7 and 9.4% increase in the second season respectively compared to the un-inoculated control. The highest values of number of grains/spike 45.0 and 46.2, with about 25.7 and 24.9% increase over the control were obtained from the application of 140 kg N + 35 kg P_2O_5/ha. Microbin and Azottein gave increases in number of grains/spike reached about 10.2 and 11.8% in the first season and 10.6 and 7.3% in the second season respectively compared the un-inoculation. Application of 140 kg N + 35 kg P_2O_5/ha resulted in 1000-kernels weight reached about 48.3 and 50.9 with about 1.9 and 0.2% increase in the first and second season respectively compared to the control. Inoculation of barley with Microbin and Azottein caused increases in 1000-kernels weight reached about 1.1 and 4.5% in the first season and 1.6 and 8.8 in the second season respectively compared to the un-inoculation.

Key words: Barley, Biofertilizer, Microbin, Azottein, Nitrogen fertilizer, Phosphorous fertilizer, *Azotobacter, Azospirillum, Klebsiella, Bacillus.*

INTRODUCTION

The major use for barley (*Hordeum vulgare* L.) is for animal feeding, however, there is a recent interest in using the crop in human food. In Egypt, barley is the main cereal crop grown along the Northern Coast of Egypt under rainfed and new land conditions. Generally in a common agriculture nutrition is essential for plant life, therefore, nitrogen fertilization is a common agronomic practice that leads to improve productivity (Said 1998). The efficiency of the most of nitrogen fertilizers for the crops, potential pollution, losses of nitrogen fertilizer in the water of the drains, then, to the rivers and its destructive effects on the human and animals health. The nature of this problem requires intensive efforts to increase efficiency of biological nitrogen fixation, not only through conventional symbiotic Legume-Rhizobium associations but also through the non-symbiotic N_2-fixation regimes observed in grasses in order to prevent physiogenic disease caused by nitrogen deficiency (Madkour et al., 1987). On the other hand, Hassouna and Wareing (1964) established the asymbiotic relationship between rhizosphere N-fixers and plants. Several reports have affirm with positive plant growth responses after inoculation with different non-symbiotic N_2-fixing bacteria such as *Azotobacter* spp. (Madkour, 1972, Hassouna et al., 1973 and Hassan et al., 1985), *Azospirillum brasilens* (Baltensperger et al., 1978 and Nur et al., 1980) and *Klebsiella pneumniae* (Hassan et al., 1985). Also, nitrogen fertilization tends to increase barley yield (Paterson and Potts, 1985 and Muller and Beer, 1986). Nitrogen fertilization of barley must be very carefully managed because barley quality is not expected with increasing fertilization for maximum yield (Banasik and Power, 1986). At present we can satisfy N-requirements of plants grown in desert ecosystems, via mineral nitrogen fertilization or inoculation with N_2-fixing organisms (Fayez 1990).

MATERIAL AND METHODS

Two field experiments were conducted in the 1995/96 and 1996/97 growing seasons in the newly reclaimed lands at Nubaria area, which located at 46-km Southwest Alexandria, Egypt. The experimental sites were chosen to represent the newly reclaimed lands of Northwestern Egypt. A randomized complete block design was used with four replications. The plot size was 10.5 m^2 including 15 rows of 3.5 m long and 20 cm apart. Mineral and bio-fertilizer treatments were applied as below in addition to different combinations between mineral and bio-fertilizers:

(I) - Mineral fertilizer treatments

1 - Control (No fertilizers).
2 - 140 Kg N/ha.
3 - 35 Kg P_2O_5/ha.
4 - 140 Kg N + 35 Kg P_2O_5/ha.

(II) - Biofertilizer treatments

1 - Control (Non inoculation).
2 - Microbin.
3 - Azottein.

Phosphorous fertilizer was applied in one dose at sowing in the form of calcium super phosphate (15.5% P_2O_5), while nitrogen fertilizer was applied in three equal doses at sowing, tillering, and shooting stages. Nitrogen fertilizer was in the form of ammonium sulphate (20.6% N). Two biofertilizers were used in the present study, Microbin and Azottein. Microbin is a commercial multi-strains biofertilizer produced by the General Organization for Agricultural Equalization Fund, Ministry of Agriculture and Land Reclamation. It is constituted of a mixture of P-dissolving and N_2-fixing bacteria, e. g., *Klebsiella, Bacillus, Azotobacter,* and *Azospirillum*...etc. (Abou El-Naga, 1993 and Mitkees, *et al*., 1996). Azottein is composed of different nitrogen fixers of the genera *Klebsiella, Bacillus, Azotobacter,* and *Azospirillum*. This biofertilizer is prepared by adding equal amounts of these microorganisms to the carrier material. Azottein was kindly supplied by Soil Microbiology Research Department, Soil, Water and Environment Research Institute, Agricultural Research Center. Arabic gum was melted in amount of warm water and was added to the Azottein. Barley cultivar Giza 123 seeds were added to the mixture of Azottein and the gum and mixed carefully and spread over plastic sheet far from the direct sun effect for a short time before plantation. The other biofertilizer (Microbin) was used the same way, and then barley seeds were planted at a rate of 120 Kg/ha.

Representative soil samples were taken from the two experimental sites at Nubaria in 1995/96 and 1996/97 seasons before planting and were chemically and mechanically analyzed (Table 1). Standard analysis of variance using Least Significant Differences (LSD) was performed to estimate the significant differences among treatments (Steel and Torrie, 1980).

Table 1: Soil chemical and mechanical analyses of the two experimental sites at Nubaria before planting of 1995/1996 and 1996/1997 seasons.

	1995/1996	1996/1997
Chemical analysis		
N (ppm)	54.2	150
P (ppm)	2.6	14.4
K (ppm)	29.0	26.6
Na (ppm)	13.3	32.2
EC (dSm^{-1})	0.12	3.75
PH	8.8	8.13
Mechanical analysis		
Sand (%)	63.9	67.5
Silt (%)	24.6	10.0
Clay (%)	11.5	22.5
Texture	Sandy loam	Sandy clay loam
Total $CaCO_3$ (%)	22.8	20.0

RESULTS AND DISCUSSION

Data presented in Table 2 shows the effect of fertilizer treatments on grain yield, straw yield and harvest index of barley grown at Nubaria region during 1995/1996 and 1996/1997 growing seasons. Regarding to the grain yield, data indicated that both mineral and bio-fertilizer treatments had a significant effect in the two seasons of the study. Highest grain yield (2.81 and 3.60 ton/ha in the first and second season respectively) was obtained from the application of 140 kg N + 35 kg P_2O_5/ha, with about 401.8 and 95.7% increase over the control. On the other hand application of biofertilizers (Microbin and Azottein) caused significant increases reached about 24.8 and 27.2% in the first season and 18.4 and 22.0% in the second season respectively compared to the un-inoculated control.

For the straw yield, mineral fertilizer treatments were significant effect on the straw yield, while; the biofertilizer effects in the two seasons did not reach the significance level. Application of 140 kg N + 35 kg P_2O_5/ha gave the highest straw yield, about 7.0 and 8.6 ton/ha with about 388.9 and 92.8% increase over the control in the first and second season respectively. Biofertilzers (Microbin and Azottein) caused increases reached about 15.6 and 7.8% in the first season and 4.6 and 4.7% in the second season respectively compared with the un-inoculated control.

As for harvest index, results indicated that both mineral and bio-fertilizer treatments in the first season were insignificant effects, on the other hand both mineral and bio-fertilizer treatments had significant effects on the harvest index in the second season. Application of 140 kg N + 35 kg P_2O_5/ha gave about 28.4 and 29.9 with about 0.0 and 0.7% increase over the control. Biofertilizers (Microbin and Azottein) resulted in 1.1 and 6.3% increase in the first season and 10.0 and 13.1% in the second season respectively compared to the un-inoculated control.

Table 2: Effects of Mineral and Biofertilizers on Grain, Straw yields (ton/ha.) and Harvest index of barley grown at Nubaria region in 1995/96 and 1996/97 seasons.

Treatments	Grain yield (ton/ha)		Straw yield (ton/ha)		Harvest index %	
	1995/96	1996/97	1995/96	1996/97	1995/96	1996/97
Mineral fertilizer						
Control	0.56	1.84	1.44	4.44	28.4	29.7
35 kg P_2O_5/ha	0.61	1.99	1.73	5.32	27.2	27.2
	(8.93)*	(8.15)	(20.14)	(19.82)	(-4.2)	(-8.4)
140 kg N/ha	1.69	2.67	3.62	7.98	32.5	25.2
	(201.78)	(45.11)	(151.39)	(79.73)	(14.4)	(-15.2)
140 kg N/ha +	2.81	3.60	7.04	8.56	28.4	29.9
35 kg P_2O_5/ha	(401.78)	(95.65)	(388.89)	(92.79)	(0.0)	(0.7)
LSD $_{0.05}$	0.29	0.14	0.68	0.85	NS	3.0
Biofertilizer						
Non-inoculation	1.21	2.23	3.21	6.38	28.4	26.0
Microbin	1.51	2.64	3.71	6.67	28.7	28.6
	(24.80)	(18.40)	(15.60)	(4.60)	(1.1)	(10.0)
Azottein	1.54	2.72	3.46	6.68	30.2	29.4
	(27.20)	(22.00)	(7.80)	(4.70)	(6.3)	(13.1)
LSD $_{0.05}$	0.25	0.13	NS	NS	NS	1.6

***Values between parentheses are percentage increase of treated over the un-treated ones.**

Data presented in Table 3 shows the effect of mineral and bio-fertilizer treatments on number of tillers/m^2, Spike length (cm), number of grains/spike and 1000-kernels weight (g) of barley grown during 1995/1996 and 1996/1997 seasons. Regarding to the number of tillers/m^2, data indicated that both mineral and bio-fertilizer treatments had significant effects in the two seasons. Highest number of tillers/m^2 (291.3 and 495.9) was obtained from the application of 140 kg N + 35 kg P_2O_5/ha, with about 97.6 and 87.5% increase over the control in the first and second season respectively. On the other hand, biofertilizers (Microbin and Azottein) caused increases reached about 20.3 and 9.7% in the first season and 14.9 and 11.0% in the second season respectively compared to the un-inoculated control.

In respect to the spike length, mineral fertilizer treatments had significant effect in the two seasons; biofertilizer treatments in the first season did not reach the significance level, while in the second season it was significant effect. Application of 140 kg N + 35 kg P_2O_5/ha resulted in 5.49 and 7.70 cm with about 35.6 and 102.1% increase compared to the control in the first and second season respectively. Inoculation of barley seeds with Microbin and Azottein resulted in 9.1 and 7.5% increase in the first season and 10.7 and 9.4% increase in the second season respectively compared to the un-inoculated control.

For number of grains/spike, results revealed that both of mineral and bio-fertilizer treatments caused significant increases in the two seasons. The highest values of number of grains/spike 45.0 and 46.2, with about 25.7 and 24.9% increase over the control were obtained from the application of 140 kg N + 35 kg P_2O_5/ha. Microbin and Azottein gave increases in number of grains/spike reached about 10.2 and 11.8% in the first season and 10.6 and 7.3% in the second season respectively compared the un-inoculation.

Table 3: Effects of Mineral and Biofertilizers on Number of Tillers/m², Spike length (cm), Number Grains/spike and 1000-kernels weight (g) of barley grown at Nubaria region in 1995/96 and 1996/97 seasons.

Treatments	No. Tillers/m²		Spike length (cm)		No. Grains/spike		1000-kernels weight (g)	
	1995/96	1996/97	1995/96	1996/97	1995/96	1996/97	1995/96	1996/97
Mineral fertilizer								
Control	147.4	264.5	4.05	3.81	35.8	37.0	47.4	50.8
35 kg P_2O_5/ha	163.5	318.5	4.28	4.36	38.2	39.6	47.7	52.0
	(10.9)*	(20.4)	(5.68)	(14.43)	(6.7)	(7.0)	(0.63)	(2.36)
140 kg N/ha	227.2	402.9	5.20	6.83	44.6	45.0	46.1	49.2
	(54.1)	(52.3)	(28.39)	(79.26)	(24.6)	(21.6)	(-2.74)	(-3.15)
140 kg N/ha +	291.3	495.9	5.49	7.70	45.0	46.2	48.3	50.9
35 kg P_2O_5/ha	(97.6)	(87.5)	(35.55)	(102.1)	(25.7)	(24.9)	(1.90)	(0.20)
LSD $_{0.05}$	23.9	45.4	0.47	0.50	3.8	3.9	NS	0.9
Biofertilizer								
Non-inoculation	188.5	340.9	4.51	5.32	38.1	39.6	46.5	49.0
Microbin	226.7	391.8	4.92	5.89	42.0	43.8	47.0	49.8
	(20.3)	(14.9)	(9.10)	(10.70)	(10.2)	(10.6)	(1.1)	(1.6)
Azottein	206.8	378.7	4.85	5.82	42.6	42.5	48.6	53.3
	(9.7)	(11.0)	(7.50)	(9.40)	(11.8)	(7.3)	(4.5)	(8.8)
LSD $_{0.05}$	20.7	39.3	NS	0.43	3.3	3.3	1.7	0.8

***Values between parentheses are percentage increase of treated over the un-treated ones.**

Concerning to 1000-kernels weight, mineral fertilizer effect in the first season was insignificant, while it was significant effect in the second season. On the other hand biofertilizer treatments had significant in the two seasons of the study. Application of 140 kg N + 35 kg P_2O_5/ha resulted in 1000-kernels weight reached about 48.3 and 50.9 with about 1.9 and 0.2% increase in the first and second season respectively compared to the control. Inoculation of barley with Microbin and Azottein caused increases reached about 1.1 and 4.5% in the first season and 1.6 and 8.8 in the second season respectively compared to the un-inoculation.

Generally, the application of mineral and bio-fertilizers increased the different plant characteristics under the present study. Differences between the two biofertilizers (Microbin and Azottein) were insignificant, it means that these biofertilizers had the same performance under different fertilizer treatments in the new lands. In this respect, Okon (1982) stated that inoculated plants with biofertilizers exhibited about 30-50 % greater uptake of nitrate, phosphate and potassium than the un-inoculated plants. He suggested that associative nitrogen fixing bacteria enhance the mineral absorption of the cell cortex, which is reflected on the plant growth and yield increases. These increases in plant growth is most probably due to the principle mechanism that biofertilizer could benefit the plant growth through, a fixing molecular nitrogen and its transfer to the plant as direct effect on plant growth hormones Auxins, Gibbrelic Acids (GAs) and Cytokinones (CKs) that bacteria could release in the root media and affect its growth and extension positively. The resultant could be more absorption of nutrients which reflect more grow activity, nitrogenous compounds and enlargement, more forming of tissues and organs. Other wise, once roots emerge at seed germination and are colonized by N_2-fixing bacteria, energetic pathways such as glycolysis and conversion of conjugate Indole Acetic Acid (IAA) to active IAA are stimulated. Also, the nitrogen fixing-bacteria may increase the synthesis of the endogenous phytohormones i. e. IAA, GAs and CKs which plays an important role in formation of a big active root system, that allow more nutrients uptake and hence may promote photosynthesis and translocation as well as accumulation of dry matter within different plant parts. The plant growth could be considered as a resultant for that mentioned processes (El-Khawas 1990). It was concluded from this study that the application of biofertilizers (Microbin and Azottein) in the presence of mineral fertilizer could enhance plant growth and yield production of barley through increasing the soil-nutrients uptake by plants grown under new lands conditions.

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