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Effect of Application of Biofertilizer on the Yield and NPK Uptake of Some Wheat Genotypes as Affected by the Biological Properties of Soil

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Abstract: The effect of some wheat genotype grains inoculated with *Azosperillum brasilense* and *Azorhizobium caulinodanse* on wheat yield and NPK uptake was evaluated under different levels of liquid N-fertilization (0, 50 and 100% from the recommended N Kg/fed). Inoculation with any biofertilizer leads to considerable improvement in wheat grain and straw yield as compared with their respective control. However response differed according to the type of boifertilizers. The superiority related to *Azorhizobium* inoculation was clear on grain yield followed by inoculum containing a mixture of *Azorhizobium* and *Azosperillum*, although the use of *Azosperillum* gave the lowest response, but it was still higher than the uninoculated plants (control). Increasing levels of nitrogen caused increase in the wheat grain and straw yields, but the response was remarkably high at 100% N. The differences in grain yield became much lower when *Azosperillum* in combination with decreasing levels of N-fertilizer were used. The relative increase was 4.8% (when *Azorhizobium* + 100% N were used. At the same time the application of 100% N exceeded using both 100% N + *Azosperillum* (13.3%) and 100% N + mixture of inoculation (12.54%). Application of 100% N mixed with *Azorhizobium* + *Azosperillum* inoculum gave a positive effect and also affected the 1000-grains weight. This response varied according to the wheat varieties especially with Sakha 8 which gave the highest response. Differences in NPK uptake depend on wheat genotype and on different microbial treatments. The application of *Azorhizobium* + 100% N enhanced the mineral uptake followed by mixture of 100% N + (*Azosperillum* + *Azorhizobium*) inoculums.

Key words: Biofertilizer, *Azosperillum brasilense, Azorhizohium coulinodanse,* innoculation, wheat

Introduction

Wheat is the most important cereal crop in Egypt. Because of increasing human demand for food, attempts are made to cultivate more area with high yielding varieties, in addition to using the recommended cultural practices to maximize the wheat productivity to meet the national needs. Although nitrogen is an important nutrient in different soil types, its application has economic burdens and environmental risks.

Thus, use of biological nitrogen fixation by living N_2-fixers will help to minimize the amounts of chemical nitrogen fertilizer to be added and to improve plant growth to decrease the production cost and environmental risk (Aly *et al*., 1999; Bhattarai and Hess, 1998; El-Hawary, 1998). Factors controlling successful colonization by *Azospirillum* and *Azorhizobium* species are not yet fully understood, but it has been suggested that polar flagella and surface polysaccharides are involved in this process (Gallo and Fendrik, 1994). The mechanism for successful inoculation is still unknown, although some avenues have been suggested in the experiments (Okon, 1985). Therefore it is practically impossible to simulate the exact conditions and factors affecting field experiment results. Additionally, root colonization can not yet be enhanced by external application of beneficial bacteria (Dobbelaere *et al*., 1999), because the soil acts as a biological buffer against most of the nonresident bacteria (Bashan, 1986).

The present study aims to enhance the wheat growth by temporarily depressing soil microflora. The effect of inoculation on various wheat varieties was evaluated. The survivability and proliferation of isolated bacteria around root surfaces is reported. The beneficial effect of using N-biofertilizers as complementary source to mineral N-fertilization on grain yield, chemical composition of wheat cultivars Sakha 8 and Sids 1 was investigated.

Materials and Methods

The work was carried out at the Experimental Farm of Plant Pathology Dept., Fac. of Agric., Mansoura University during 2000/2001 to investigate the effect of application of two boifertilizers and three different levels of liquid fertilizer (32% N) as nitrate and ammonia, on the yield, chemical composition, yield components and NPK uptake on wheat cultivars Sakha 8 and Sids 1.

Azospirillum brasilense BKM.3B was obtained from Institute of Microbiology, Academy of Science, Moscow, USSR. *Azorhizobium caulinodans* IRG 46 was obtained from International Rice Research Institute IRRI, Manila, Philippines.

Stock cultures were maintained on nutrient agar, the medium for growth of *A. brasilense* was provided by Nelson and Knowles (1978) and *A. caulinodans* was cultured in TGYE medium (Ladha *et al*., 1989).

Inoculation of grains and soil: The wheat seeds were inoculated with *Azospirillum brasilense* (AZ) and *Azorhizobium caulinodans* (RH), Arabic gum solution (6%) was used as a sticking agent when wheat seeds were inoculated with *Azospirillum brasilense* or *Azorhizobium caulinodans*. Approx. 10^9 (cfu) ml^{-1} was added twice to each pot first at planting and the second was after one week. The total bacterial counts in the rhizosphere were preformed on young root, the solution was serially diluted, plotted on semisolid agar or solid supplemented with 0.05% yeast extract, incubated at 25° C for 72 h and counted by the most probable number technique as described by Sriskandarajah *et al*. (1993).

The nitrogen content analysis (Kjeldahl) was used to determine the total N content (Batten *et al*., 1991).

The soil physical and chemical analysis of the experimental pots is presented in Table 1.

Three levels of liquid N fertilizer i.e. 0, 50 and 100% of the recommended N level (75 N Unit/fed) were applied. The green house experiments were conducted in pots containing 20 kg of soil and the seeds were broadcasted.

Phosphate fertilizer was applied as super phosphate (15.5% P_2O_5) @ 15 kg/fed during preparation.

Soil mechanical analysis was done according to Piper (1974), pH, EC, available P as by Jackson (1967), O. M, available N and K and the available Fe, Mn, Zn and Cu by the methods of Lindsay and Norvell (1978).

Oven dried plant material (grains and straw) was used to determine N, P and K concentrations in the digestive extract of H_2SO_4 + HCl (1:1) according to Peterburgski (1968).

Table 1: Soil physical and chemical analysis of the soil used

character	Soil texture (% weight)			Water retention (% weight)						
Soil type	Clay	Silt	Sand	H.W.	S.P.	EC. (ds m^{-1})	pH	$CaCO_3$ (%)	O.M. (%)	Total N (%)
Alluvial	32.4	41.4	26.2	10.02	79.41	0.57	7.7	1.69	1.89	0.0382

Table 2: Number of viable *Azospirillum* spp, *Azorhizobium* spp and total numbers of bacteria in soil rhizosphere as affected by microbial inoculation

	Azospirillum brasilense			*Azhorhizobillum caulinodans*			Total bacteria		
Wheat cultivar	0 time	5 weeks	10 weeks	0 time	5 weeks	10 weeks	0 time	5 weeks	10 weeks
Sakha 8	$2.3x10^4$	$8.6x10^7$	$8.0x10^8$	$1.43x10^3$	$1.52x10^5$	$1.55x10^5$	$66.1x10^4$	$67.2x10^6$	$66.4x10^8$
Sids1	$2.5x10^4$	$8.8x10^8$	$9.0 x 10^8$	$1.65x10^3$	$1.41x10^5$	$1.04x10^6$	$64.7x10^4$	$69.9x10^6$	$68.4x10^8$

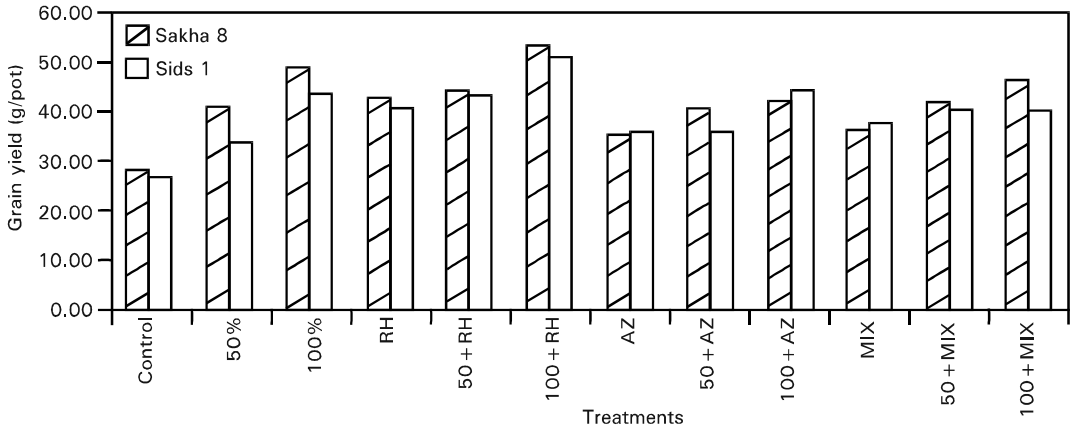

Fig. 1: Dry weight (g/pot) of the two wheat varieties grain yield as affected by biofertilizers

Pot experiments (96 pots per experiment) were conducted in a randomized complete block design (12 pots per block) in four replicates for two wheat genotypes. The data were statistically analyzed according to CoSTAT Program (1990) using LSD at 5% of variance analysis.

Results and Discussion

Effect of microbial inoculation on the viable number of *Azospirillum* and *Azorhizobium* in rhizospher soil: The organisms associated with wheat roots were isolated at zero time, 5 weeks and 10 weeks of growth on yeast extract peptone medium of *Azospirillum* and *Azorhizobium* cultured at yeast extract mannitol agar (Dobbelaere *et al.*, 1999).

The viable number of *Azospirilum* increased markedly due to inoculation with *Azospirilum* at both growth stages (after 5 and 10 weeks). Concerning the effect of inoculation treatments on the number of *Azorhizobium*, there was no marked increase in bacterial count observed between the number of bacteria during the two stages of growth and in the untreated inoculated plants. The rhizosphere bacterial population was constant, increasing slowly during the course of experiment (Table 2).

Effect of microbial inoculation, nitrogen application rate and their interactions on wheat

Grain yield (g/pot): Results of weight response to microbial inoculation, the nitrogen applications and their interactions on grain yield (g/pot) (Fig. 1) showed that inoculation of wheat seeds with *Azorhizobium, Azospirillum* and mixture of microbial inoculation significantly increased the grain yield. This response was 53.4, 27.9 and 29.6% in Sakha 8 variety while it was 55.6, 37.1 and 42.5% in Sids 1 variety respectively as compared with control.

The application of nitrogen alone (at 50 and 100%) increased the grain yield by 47.7 and 75.3% in Sakha 8 and 28.2 and 64.7% in Sids 1. On the other hand, incorporation of inoculation of microbial *Azorhizobium and Azospirilum* increased the grain yield significantly and the addition of (100% N + *Azorhizobium)* gave the highest grain yield 92.7% in Sakha 8 and 95.1% in Sids 1. This significant increase may be due to the production of growth promoting substances which had a pronounced effect on the development and morphology of the wheat root Amellal *et al.* (1998), Dobbelaere *et al.* (1999) and Hammouda *et al.* (2001).

Omar (1995) found that grain yield was increased by inoculation with *A. brasilense* and was significantly higher with 96 than 48 kg N. Omar *et al.* (1996) also found that the grain yield and protein content in pot experiment were increased with N application and inoculation of the variety Sakha 69.

Straw yield (g/pot): The inoculation of wheat seeds with *Azorhizobium* or *Azospirillum* and mixture of two significantly increased straw yield of wheat. The increase was 53.1, 25.8 and 29.2% in the variety Sakha 8 while it was 52.7, 34.8 and 39.9% in the variety Sids 1 respectively as compared with the control treatment.

On the other hand addition of nitrogen alone at 50 and 100% has contributed to the increase in straw yield (45.7 and 76.6% in Sakha 8 and 26.2 and 57.4% in Sids 1, respectively). While the incorporation of nitrogen with the inoculation of microbial *Azorhizobium* and *Azospirillum* increased the straw yield significantly and the addition of (100% N + *Azorhizobium*) gave the highest straw yield in Sakha 8 (92.0%) and in Sids 1 (94.2%). Our results are in accord with El-Kased *et al.* (1996) who found that using a mixture of *Azotobacter chroococcum* + *Azospirillum lipoferum* application to wheat cv. Sakha 69 doubled the grain yield in biofertilizer treatments as compared with the control. Also El-Din and Abdrabou (1995), found that grain and straw yields increased with increasing rates of N fertilizer and seed inoculation with *Azospirillum* sp. gave better results than *Azotobacter* sp.

1000-grain weight: The 1000-grains weight of the two wheat varieties were affected by microbial inoculation *(Azorhizobium, Azospirillum* and mix) and application of nitrogen at a rate of (50 and 100%) and their interactions (Fig. 3).

Microbial inoculation of seeds with (*Azorhizobium* and *Azospirillum* and mix.) of microbial inoculation significantly increased 1000-grains weight. This increase was 10.5, 8.5 and 11.1% in Sakha 8,

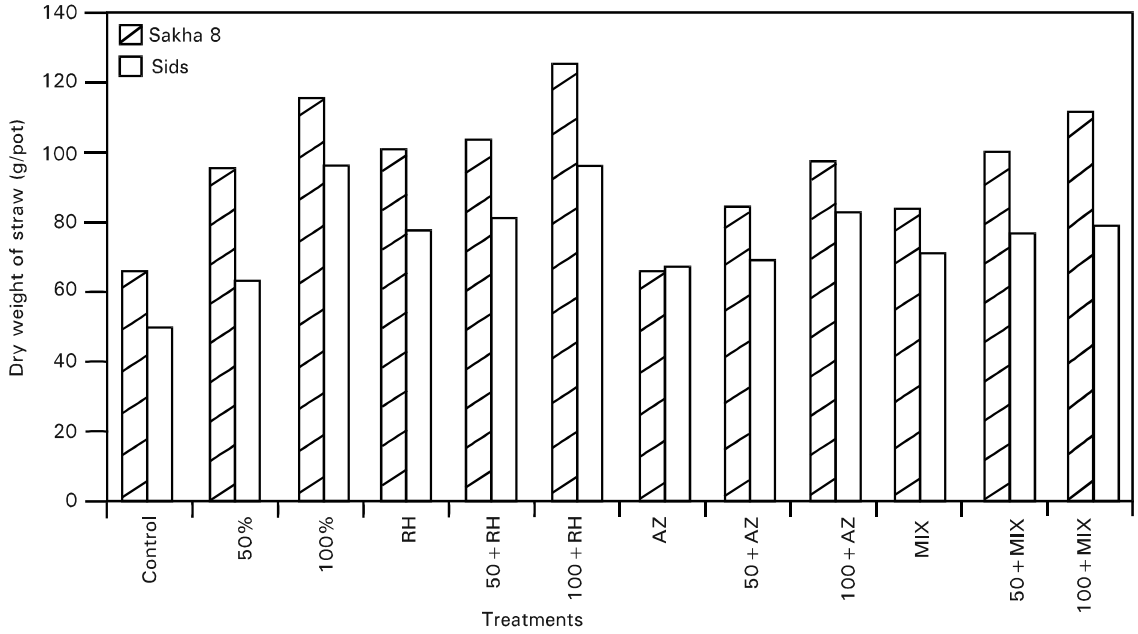

Fig. 2: Dry weight (g/pot) of straw yield as affected by biofertilizers in soil.

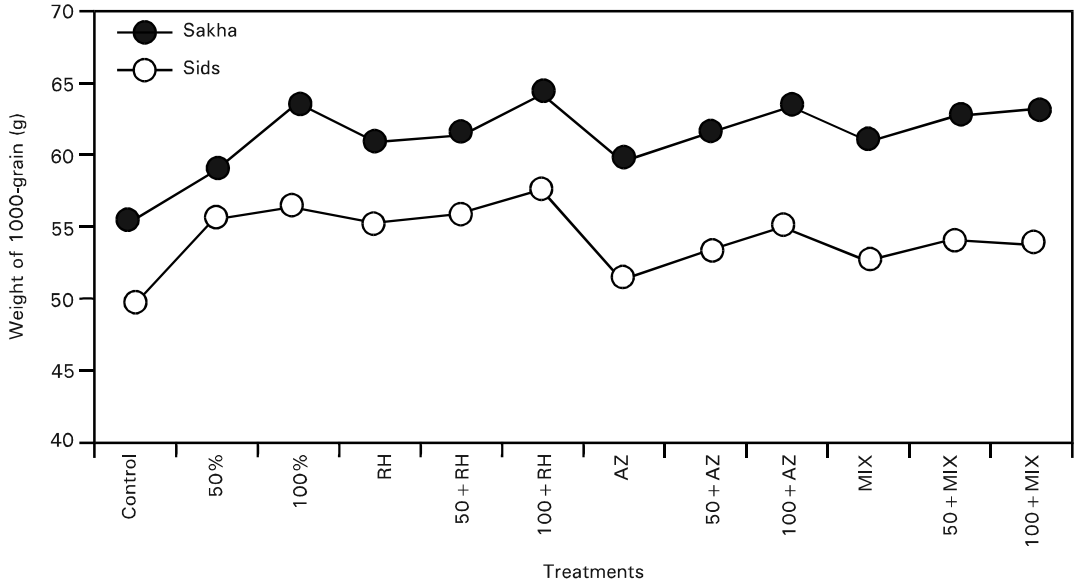

Fig. 3: Weight of 1000-grain as affected by biofertilizer application in soil.

while it was 13.7, 5.5 and 8.2% in Sids 1 against control.

However, it was found that the addition of nitrogen alone at 50 and 100% increased the 1000 grains weight 6.3 and 15.4% in Sakha 8 and 14.0 and 15.8% in Sids 1 respectively. While, incorporation with inoculation of microbial *Azorhizobium* and *Azospirillum* increased the 1000 grains weight significantly and the application of (100% N + *Azorhizobium*) gave the highest increase in weight of 1000 grains which was 16.8% in Sakha 8 and 18.5% in Sids 1 against the control. Sharaan and El-Samie (1999), found that increasing N treatments to the inoculated seeds as 75 kg N/fed increased 1000-grains weight, grain yield and biological yield. Sabry *et al.* (1998), found that grain yield and 1000-grains weight differed significantly between different sites with increasing N fertilizer rate up to 75%. The grain inoculation treatments had significant effect on grain yield and yield components at any level of N fertilizer application.

Effect of microbial inoculation, application rate of nitrogen and their interactions on NPK uptake in two wheat varieties

NPK uptake in grains: Significantly increased NPK uptake was observed at all treatments in Sakha 8 and Sids 1 varieties compared with the control and the increasing NPK uptake was more pronounced with Sakh 8 followed by Sids 1 (Table 3).

Generally, the interaction between N biofertilizer and mineral fertilizer (100% N+ *Azorhizobium*) gave the best results in grain NPK uptake among other treatments in both wheat varieties. It was followed by (100N+ *Azospirillum*) without significant difference.

Omar *et al.* (1991) used Sakha 61 and Giza 163 wheat varieties seeds inoculated with *Azospirillum brasilense* or *Bacillus polymexa* (isolated from wheat rhizosphere) produced. They found that inoculation with *B. polymexa* and *A. brasilense* increased the N content in grains by 6.26 and 6.62%, respectively.

Table 3: NPK uptake (g/pot) of the two wheat varieties grains as affected by biofertilizers in soil

	Grain uptake of Sakha 8			Grain uptake of Sids 1		
Treatments	N	P	K	N	P	K
Control	0.46	0.06	0.31	0.44	0.06	0.30
50%	0.73	0.09	0.50	0.57	0.08	0.44
100%	1.03	0.12	0.65	0.77	0.11	0.59
RH	0.96	0.09	0.52	0.75	0.09	0.47
50%+ RH	1.11	0.10	0.64	0.87	0.10	0.60
100%+ RH	1.49	0.14	0.82	1.06	0.13	0.74
AZ	0.80	0.08	0.48	0.75	0.08	0.48
50%+ AZ	0.96	0.10	0.63	0.78	0.09	0.54
100%+ AZ	1.10	0.12	0.67	1.00	0.11	0.68
MIX	0.82	0.08	0.48	0.77	0.08	0.48
50%+ MIX	1.05	0.12	0.63	0.88	0.10	0.61
100%+ MIX	1.24	0.15	0.77	0.99	0.12	0.67
LSD 0.05	0.2489	0.0249	0.1442	0.2138	0.0275	0.1523
0.01	0.3373	0.0337	0.1954	0.2898	0.0372	0.2064

Table 4: NPK Uptake (g/pot) of wheat variety straw affected by biofertilizers in soil

	Sakha 8 straw uptake			Sids 1 straw uptake		
Treatments	N	P	K	N	P	K
Control	0.73	0.07	1.05	0.56	0.05	0.82
50%	1.16	0.12	1.59	0.79	0.07	1.10
100%	1.49	0.15	1.97	1.04	0.12	1.39
RH	1.17	0.12	1.64	0.93	0.09	1.32
50%+ RH	1.30	0.13	1.73	1.04	0.12	1.42
100%+ RH	1.75	0.19	2.13	1.37	0.15	1.74
AZ	0.91	0.10	1.25	0.82	0.09	1.17
50%+ AZ	1.05	0.12	1.44	0.87	0.11	1.24
100%+ AZ	1.33	0.16	1.71	1.17	0.14	1.57
MIX	1.04	0.12	1.40	0.87	0.10	1.21
50%+ MIX	1.26	0.15	1.73	1.01	0.12	1.38
100%+ MIX	1.54	0.18	1.98	1.10	0.13	1.41
LSD 0.05	0.3977	0.0427	0.5405	0.2533	0.0279	0.3448
0.01	0.5389	0.0579	0.7324	0.3433	0.0379	0.4672

RH (*Azorhizobium*) AZ (*Azospirillum*), MIX (*Azorhizobium* + *Azospirillum*)

Uptake in straw: NPK uptake was significantly increased in all treatments for Sakha 8 and Sids 1 varieties against the control (Table 4).

The 100% N+ *Azorhizobium* among all treatments gave the highest increase in NPK uptake in both wheat varieties. It was followed by 100% N+ *Azospirillum* without significant difference. Reynders and Viassak (1982) and Zaghloul *et al.* (1996), found that plant NPK concentrations were higher with *A. brasilense* + 45 kg N inorganic fertilizer than inorganic fertilizers alone.

El-Sersawy *et al.* (1997) conducted field experiment at Wadisudr, South Sinai, using organic manures alone and in combination with three levels of N chemical fertilizer. The variety Sakha 8 showed corresponding improvements in microbial counts, *Azotobacter*, *Azospirillum* and phosphate dissolving bacteria, as well as NPK uptake, which were reflected on high grain and straw yields.

It is concluded that inoculation with *Azorhizobium* increased the yield of wheat, grown in pots with high levels of liquid organic nitrogen fertilizer. These results showed the possibility of inoculation of *Azorhizobium* in combination with nitrogen fertilizer to improve the yield of wheat especially the variety Sakha 8, it may be due to enhancing the development of root in the early growth stages, which may be one of the factors responsible for the yield increases, these results are in conformity with those obtained by Bhattarai and Hess (1998), Webster *et al.* (1998) and Soliman *et al.* (1999).

Finally we can recommend that, half of the applied N fertilizer could be saved if wheat seeds were inoculated with non-symbiotic fixing bacteria especially *Azorhizobium*, without affecting the yield. Furthermore, the liquid N fertilizers originated from organic sources were much effective and the variety Sakha 8 was more responsive to this application.

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