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PHYSIOLOGICAL EFFECTS OF SOME BIOFERTILIZERS AND THEIR RELATION TO MINERAL NUTRITION ON GROWTH, YIELD AND CHEMICAL CONSTITUENTS OF WHEAT (*Triticum aestivum* L.)

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ABSTRACT

In two pot experiments, wheat (*Triticum aestivum* L., var Sakha 8) plants were fertlized with the NPK levels of 25, 50 and 100% as the main level. Under each NPK level, the biofertlizers nitrobien, phosphorine and phosphorine + nitrobien treatments were applied as the sub-main level. As the NPK level was increased, shoot length, number of tillers/plant, number of leaves/plant and the dry weight of shoots, roots and whole plant dry weights of wheat plant were significantly increased in both sampling dates. All the previously mentioned characters were significantly increased as a result of the all applied b iofertilizers t reatments i ncluding n itrobien, phosphorine a nd p hosphoorine when compared to non-treated control treatment at both sampling dates. Grain yield/plant significantly increased as the NPK level was incraesed. Also, grain yield and yield components were increased due to the application of biofertilizers as compared to the non-treated control treatment. However, the highest values for grain yield/plant was obtained when treated with the biofertilizer phosphorine + nitrobien treatment. Application of NPK fertilization tended to increase mineral concentartions including total nitrigen, phosphorus, potassium and calcium as well as sugars in both shoots and roots of wheat plant at both sampling dates. Also, biofertilizer treatments tended to increase the same attributes when compared to the non-treated control treatment. Similar trend was obtained for total nitrogen, phosphorus, potassium and calcium as well as sugars concentrations in wheat grains at harvest. It might be concluded that applying NPK with biofertlizers treatments might improve wheat p lant n utrtional s tatus w hich l eads t o h igher p lant productivity.

INTRODUCTION

Wheat (*T. aestivum* L.) is the most important cereal crop as the main food stable for the Egyptian public. Improving the productivity of this crop is a main task due to its short supply which mandated importing about 50% of the needed wheat grains from outside the country.

Recent attention has been given to less pollution practices in modern agriculture. One of the ways to reduce soil pollution is the use of biofertilizers which have been recommended by several investigators to substitute chemical fertilizers partially (Saber, 1993 and El-Agory *et al.,* 1996). In addition, the use of biofertilizers may have an additional benefits such as nitrogen fixation, mobilizing phosphate and micronutrients through the production of organic acids and lowering soil pH (Saber, 1993). Besides, microorganisms such as *Pseudomonas, Azotobacter, Azospirillium* and mycorrhyzae can secrete growth promoting factors, e.g., gibberellins, cytokinins like substances and auxins (Brown, 1972 and Hartmann *et al.,* 1983). The following biofertilizers were used in this study: nitrobien and phophorine under different levels of NPK fertilization. The previously mentioned treatments were evaluated for their effects on wheat plant growth, yield and chemical constituents. Usually biofertilizers contain one or more of the following; symbiotic and/or non-symbiotic N-fixing bacteria or phosphorus dissolving bacteria such as *Bacillus megatherium*.

MATERIALS AND METHODS

Two pot experiments were conducted at the Plant Physiology Section greenhouse, Faculty of Agriculture, Cairo University at Giza, during 2000/2001 and 2001/2002 seasons. Wheat (*Triticum aestivum* L.) seeds var. Sakha 8 were planted on November 15, 2000 and 2001 in a mixture of 1 clay: 2 sand soil. Plastic pots of 30 cm in diameter and 30 cm in depth were used in both experiments. Fertilization (mineral fertilizer) was carried out according to the recommended dose by the ministry of Agriculture. Three levels of mineral nutrition (25, 50 and 100%) were used as the main plot. Each subplot has the treatments non-treated control, nitrobien, phosphorine and nitrobien + phosphorine. The 100% leve of mineral nutrition consisted of 2.2g of Calcium superphosphate (15.5% $p2O_5$), 1.1g potassium sulphate (48% k_2O) and 2.0g ammonium sulphate (20.5%N) before planting as well as more 2.0g ammonium sulphate 30 days after planting. Wheat grain were planted in 5 groups/pot and each group consists of 2 grains (seeds). Grains were coated with the biofertilizers nitrobien, phosphorine and nitrobien + phosphorine in addition to the non-treated control treatment. Coating of wheat grains was conducted as recommended by the Ministry of Agriculture, Giza, Egypt. Emerging plants were thinned leaving only 5 plants/pot after 2 weeks. Two vegetative samples were taken at 45 and 105 days after planting (DAP). Another sample were harvested at 140 DAP for yield. In the first two samples, the plants were divided into shoots and roots and the following measurements were recorded: shoot height (cm), root length (cm), number of tillers / plant, number of spikes/plant and dry weight of shoots, roots and spikes/plant (g).

In the third sample yield and yield components including wheat grain weight/plant, spikes weight and number of spikes/ plant as well as 1000-grain weight were estimated at harvest; 140 DAP. In order to obtain dry weight, plant materials were chopped into small pieces and weighed then were kept in an electric fan oven at 70 °C for 48 hr. after that dry materials were ground into fine powder using an electric Wily Mill grinder, mixed thoroughly and packed in air tight glass containers and were kept for chemical analysis.

Determinations of total nitrogen, phosphorus, potassium and calcium were carried out on the ground dry material. The samples were digested in a mixture of sulfuric acid, salicylic acid and hydrogen peroxide according to Linder (1944). For the determination of total N, the modified "Microkjeldahl apparatus of Parnas and Wagner as described by Pregl (1945) was used. Phosphorus was determined colorimetrically using the chlorostannus reduced molybdophosphoric blue color method in sulfuric acid system as described by Jackson (1967). Potassium was determined using the flamephotometer ELE). Calcium was determined by the Atomic Absorption spectrophotometer (Spectro AA220).

Hot ethanol extract of fresh rocket leaves was used for the determination of reducing, non-reducing and total sugars. Briefly, 0.2 gm of dry plant material was accurately weighed and extracted with 20 ml of 80 % ethyl alcohol by heating on boiling water bath under reflex condenser. After filtration the alcohol was evaporated from the extract under reduced pressure and the residue was transferred into 50 ml volumetric flask then made up to the volume with distilled water.

Reducing, non-reducing and total sugars were determined using the phosphomolybdic acid method (A.O.A.C., 1975). All chemical analysis were conducted in both growing seasons.

The data were statistically analyzed using split plot design according to Snedecor and Cochran (1980). Data were subjected to the analysis of variance and treatments means were compared to the control treatment using the least significant difference (L.S.D. 0.05). Four replications were used for each treatment and all data are shown as a combined analysis for both seasons.

RESULTS AND DISCUSSION

Growth:

All discussed data are the mean values of the two seasons. As the NPK fertilization level was increased, shoot length of wheat plant was significantly increased reaching its maximum value at 100% NPK fertilization in both samples (Table 1). These increases were in the order of 20.5 and 41.8% as a result of the NPK levels of 50 and 100%, respectively when compared to the 25% NPK level in the first sample. The respective values in the second sample were 25.5 and 54.0%.

Table 1: Shoot height and root length (cm) as affected by biofertilizers treatments under different NPK fertilization levels on wheat plants.

Treatment	First sample				Second sample			
	% NPK			Mean	% NPK			Mean
	25	50	100		25	50	100	
Shoot length								
Control	23.51	31.51	46.30	33.62	28.55	38.74	71.25	46.10
Nitrobien	37.58	45.09	50.70	44.46	49.90	67.73	74.66	64.18
Phosphorine	36.72	44.31	49.00	43.34	27.56	67.50	73.90	63.99
Phophorin + Nitrobien	43.43	48.53	58.70	48.55	69.76	76.16	87.08	77.67
Mean	35.19	42.36	49.90		49.75	62.54	76.72	
	L.S.D. (0.05):				L.S.D. (0.05):			
NPK	1.68				0.81			
Biofertilizers (B)	1.93				0.93			
NPK x B	2.87				1.45			
Root length								
Control	11.33	26.84	24.72	17.82	16.01	21.00	31.02	22.68
Nitrobien	20.01	22.62	26.22	22.97	24.16	28.71	35.11	29.34
Phosphorine	19.67	22.59	25.91	22.76	24.85	29.16	35.26	29.75
Phophorin + Nitrobien	22.41	26.20	35.54	28.01	30.89	34.79	41.20	35.63
Mean	18.37	22.30	28.00		23.98	28.43	35.65	
	L.S.D. (0.05):				L.S.D. (0.05):			
NPK	0.673				0.660			
Biofertilizers (B)	0.778				0.762			
NPK x B	1.346				1.311			

Data represent the mean values of two successive growing seasons.

All biofertilizers treatments were significantly higher in shoot length as compared to the non-treated control in both samples. However, the treatment phosphorin + nitrobien was significantly excelled the other biofertilizer treatments in both samples.
Similar trend was obtained as regard to root length in both samples. The highest value was obtained at 100% NPK level and the biofertilizer treatment phosphorin + nitrobien.

Number of tillers were increased as the NPK levels increased in both samples and this increased was significant then treated with the NPK level except for the level 50% in the first samples (Table 2). Biofertilizers treatment significantly increased number of tillers/plant as compared to the non-treated control treatment in both samples. These increased in number of tillers/plant were in the order of 32.2, 29.0 and 86.2% when treated with nitrobien, phosphorine and nitrobine + phosphorine, respectively in the first sample. The respective values for these increase in the second sample were 28.3, 26.7 and 98.3%, respectively.

Table 2: Number of tillers and leaves/plant as affected by biofertilizers treatments under different NPK fertilization levels on wheat plants.

Treatment	First sample				Second sample			
	% NPK			Mean	% NPK			Mean
	25	50	100		25	50	100	
Number of tillers/plant								
Control	2.00	2.35	3.35	2.55	2.00	3.00	4.00	3.00
Nitrobien	2.85	3.15	4.15	3.37	3.00	3.85	4.70	3.85
Phosphorine	2.50	3.15	4.20	3.29	3.00	3.85	4.50	3.80
Phophorin + Nitrobien	3.70	4.50	6.00	4.75	4.65	6.00	7.00	5.95
Mean	2.78	3.30	4.45		3.25	4.20	5.05	
	L.S.D. (0.05):				L.S.D. (0.05):			
NPK	0.75				0.23			
Biofertilizers (B)	0.717				0.26			
NPK x B	N.S.				N.S.			
Number of leaves/plant								
Control	12.15	15.35	23.65	17.05	16.00	25.35	33.15	24.50
Nitrobien	23.50	28.65	30.15	27.40	24.15	30.95	38.00	31.00
Phosphorine	21.15	27.00	29.00	25.70	24.70	30.85	37.20	30.90
Phophorin + Nitrobien	26.85	31.00	50.15	36.00	38.85	49.00	57.15	48.35
Mean	20.90	25.00	33.25		25.90	33.75	41.35	
	L.S.D. (0.05):				L.S.D. (0.05):			
NPK	2.77				1.34			
Biofertilizers (B)	3.20				1.55			
NPK x B	5.54				2.30			

Data represent the mean values of two successive growing seasons.

The same trend was obtained for the number of leaves/plant in wheat plant (Table 2). Significant increases in the number of leaves/plant were recorded at 100% NPK level as compared to 25% NPK level as well as the biofertilizer treatment phosphorine + nitrobien when compared to the non-treated control treatment.

Shoot, dry weight root and whole plant were significantly increased as the NPK level fertilization increased in both samples (Table 3). Whole plant dry weights were increased by 33.7 and 61.7% in the first sample and 24.4 and 68.0% in the second sample when treated with 50 and 100% NPL level as compared to 25% NPK level, respectively.

Table 3: Shoot, root and whole plant dry weight (g) as affected by biofertilizers treatments under different NPK fertilization levels on wheat plants.

Treatment	First sample				Second sample			
	% NPK			Mean	% NPK			Mean
	25	50	100		25	50	100	
Shoot dry weight								
Control	0.45	0.71	1.05	0.74	2.58	3.72	6.64	4.31
Nitrobien	0.92	1.13	1.21	1.09	4.80	5.94	7.75	6.16
Phosphorine	0.88	1.10	1.20	1.06	4.80	6.09	7.63	6.17
Phophorin + Nitrobien	1.05	1.25	1.42	1.24	7.10	8.14	9.99	8.41
Mean	0.83	1.06	1.22		4.82	5.98	8.01	
	L.S.D. (0.05):				L.S.D. (0.05):			
NPK	0.105				0.18			
Biofertilizers (B)	0.121				0.20			
NPK x B	N.S.				0.35			
Root dry weight								
Control	0.14	0.24	0.38	0.25	0.41	0.62	0.98	0.67
Nitrobien	0.25	0.33	0.49	0.36	0.62	0.78	1.20	0.87
Phosphorine	0.23	0.38	0.48	0.36	0.64	0.80	1.18	0.45
Phophorin + Nitrobien	0.388	0.57	0.68	0.52	1.09	1.31	1.59	0.83
Mean	0.24	0.37	0.51		0.69	0.88	1.24	
	L.S.D. (0.05):				L.S.D. (0.05):			
NPK	0.02				0.04			
Biofertilizers (B)	0.03				0.05			
NPK x B	0.04				N.S.			
Whole plant dry weight								
Control	0.59	0.95	1.42	0.99	2.99	4.34	7.62	4.98
Nitrobien	1.17	1.45	1.71	1.44	5.41	6.72	8.95	7.03
Phosphorine	1.11	1.48	1.68	1.42	5.44	6.89	8.83	7.05
Phophorin + Nitrobien	1.43	1.83	2.35	1.79	8.19	9.45	11.58	9.74
Mean	1.07	1.43	1.73		5.50	6.84	9.24	
	L.S.D. (0.05):				L.S.D. (0.05):			
NPK	0.06				0.20			
Biofertilizers (B)	0.07				0.23			
NPK x B	0.10				0.41			

Data represent the mean values of two successive growing seasons.

Treatments of biofertilizaers, generally increased shoot, root and whole plant dry weight of wheat plant in both samples. The highest values of these increases were obtained when wheat plants were treated with nitrobien + phosphorine in both samples.

Yield:

All yield components (number of spikes/plant, spikes weight/plant), weight of 1000-grain and grain yield of wheat plant were significantly increased as the NPK level was increased (Table 4). Grain yield was significantly increased by 98.8 and 219.7% as a result of the NPK levels of 50 and 100%, respectively as compared 25% NPK level.

All biofertilizers treatments caused significant increases in wheat plant grain yield as compared to the non-treated control treatment. Grain yield was increased by 69.2, 66.7 and 169.7% when treated with nitrobien, phosphorine and nitrobien + phosphorine, respectively as compared to the non-treated control treatment.

Table 4: Number of spikes/plant, spikes weight/plant, number of grains/plant and grain yield/plant (g) as affected by biofertilizers treatments under different NPK fertilization levels on wheat plants.

Treatment	Number of spikes/plant				Spikes weight/plant			
	% NPK			Mean	% NPK			Mean
	25	50	100		25	50	100	
Shoot dry weight								
Control	1.15	2.85	4.30	2.80	0.74	2.29	4.61	2.55
Nitrobien	2.30	3.85	5.00	3.80	2.11	4.01	6.17	4.09
Phosphorine	2.50	3.85	4.85	3.75	1.78	3.97	6.17	3.98
Phophorin + Nitrobien	4.50	5.65	6.70	5.60	4.77	5.92	8.20	6.09
Mean	2.65	4.05	5.25		2.20	4.04	6.29	
	L.S.D. (0.05):				L.S.D. (0.05):			
NPK	0.37				0.45			
Biofertilizers (B)	0.43				0.52			
NPK x B	N.S.				N.S.			
	Weight of 1000 grains				Grain yield/plant			
Control	8.29	13.59	19.85	13.91	0.44	1.72	3.88	2.01
Nitrobien	14.71	19.71	24.39	19.60	1.65	3.44	5.37	3.49
Phosphorine	14.17	19.47	23.74	19.12	1.28	3.34	5.42	3.35
Phophorin + Nitrobien	19.19	23.66	28.64	23.83	3.56	5.26	6.09	5.42
Mean	14.09	19.10	24.16		1.73	3.44	5.53	
	L.S.D. (0.05):				L.S.D. (0.05):			
NPK	1.24				0.46			
Biofertilizers (B)	1.38				0.53			
NPK x B	1.193				N.S.			

Data represent the mean values of two successive growing seasons.

Nitrogen is an important factor for plant growth and is involved in many metabolic processes, including carbohydrates metabolism, protein biosynthesis, assimilates translocation and conformation of enzymes. Kandeel *et al.* (1992) found that increasing nitrogen level to rosemary plants significantly increased plant height, total number of branches/plant, fresh and dry weights of herb/plant and final yield as well as increasing volatile oil percentage. Also, nitrogen, phosphorus and potassium concentrations were increased in rosemary as nitrogen fertilization increased. Singh *et al.* (1983) studied *C. winterianus* plants as affected by N at 40, 80 & 120, P_2O_5 at 40 & 80 and K_2O at 40 kg/ha. The plants responded best to N at the highest rate with an essential oil yield of 283.9-317.4 kg/ha. Yadav *et al.* (1984) found that *C. winterianus* plants at each harvest herbage yield, essential oil yield and total N uptake increased with increasing rates of N (0-180 kg/ha/year) at all spacing (45x30 to 60x60 cm). Essential oil yield was greatest at 60x30 cm (294 kg/ha) and with 180 kg N (303 kg/ha). The proportion of the applied N recovered by the plants decreased with increasing N rate and with wider spacing. Munsi and Mukherjee (1982) studied 4 levels of N and P which were applied to *Mentha, C. winterianus* and *C. martinii* in a field experiment. They concluded that the the optimum rates giving the highest oil yields, were 100, 120 and 60 kg N/ha, respectively and 60 kg P/ha for all 3 crops. The quality of oil in fertilized crops was better than in unfertilized controls.

Singh (2000) found that the application of 200 kg N/ha to *Pelargonium graveolense* plants resulted in higher herbage and oil yields than application of 0 or 100 kg N/ha. However, the concentration and quality of essential oil were not affected. Singh and Sharma (2001) reported that increasing nitrogen fertilization rate to *C. martini* plants increased both biomass and essential oil yields up to 200 kg N/ha. Similar results were reported by Lenardis *et al.* (2000) on coriander plants.

The present data are in agreement with those reported by Talaat (1995) on lettuce plants. Inthis connection, Abd Alla *et al.* (1994), Indegit and Dakshini (1997) and Totey *et al.* (1997) working on wheat found that inoculation with a cyanobacteria as biofertilizers improved plant growth. Moreover, El-Akabawy *et al.* (2000) mentioned that cotton seed yield increased significantly through the use of the biofertilizer nitrobien. This treatment excelled the remaining treatments including rhizobactrien, phosphorine as well as control. They also mentioned that nitrobien produced higher plant dry weight and N uptake. Also, Yousry *et al.* (1978) working on pea plants, pointed out that inoculation with *Bacillus megatherium* increased plant dry matter by 10.9%. Shahaby (1981) found that tomato plants inoculated with *Azospirillium* and *Azotobacter* increased dry matter by 44% and 55.1%, respectively during the summer season. Hamam (1986) working on soybean, reported that the best results in plant height, number of leaves/plant was obtained by the addition of 80 kg N/fed. or the addition of 20-40 kg N/fed. plus inoculation of seeds with strains of *Bacillus sp.* Bashan *et al.* (1989a) working on pepper and Bashan *et al.* (1989b) working on eggplant reported that inoculation of seedlings with *Azospirillium brasilense* stimulated plant growth. Cohen (1980) found that both dry weight and nitrogen contents in corn increased by 50-100% when corn seeds were inoculated with *Azospirillium* before planting. The same results were obtained by Mohandas (1987). The same author found that inoculating seedlings of tomato with *Azotobacter* resulted in high increase in leaf area, dry weight, nitrogen & phosphorus contents and yield. Similar results were obtained by Sundaravelu and Muthukrishinan (1993) on radish plants. In this respect, Schank *et al.* (1981) and Okon (1984) mentioned that N_2 fixation was not the sole cause of growth response in diazotrophs-inoculated plants. Thus, it has been suggested by many workers that other factors may also contribute to growth enhancement and yield production such as: a- formation of growth-promoting substances, e.g. auxins, gibberellins and cytokinins

(Brown, 1972 and Hartmann *et al.*, 1983), b- synthesis of some vitamins, e.g. B_{12} (Mishustin and Shilnikova, 1969 and Okon, 1984), c- increasing amino acids content (Schank *et al.*, 1981), d- increasing water and mineral uptake from the soil (Lin *et al.*, 1983 and Sarig *et al.*, 1984). These effects could be ascribed to the increases in root surface area, root hairs and root elongation as affected by *azotobacter* as mentioned by Sundaravelu and Muthukrishinan, (1993), e- increasing the ability to convert N_2 to NH_4 and thus make it available to plant, and f- enhancing the production of biologically active fungistatical substances which may change the microflora in the rhizosphere and affect the `balance between harmful and beneficial organisms (Apte and Shende, 1981). Similar results and suggestions were reported by Hanafy Ahmed *et al.* (1997) on jew's mallow and radish plants.

In this regard, Moshtohry *et al.* (1995) and El-Bana and Gomaa (2000) obtained similar data where grain yield significantly increased by raising nitrogen levels but there was no significant differences between 100 or 120 kg N/fed. In another study, Abd El-Razik and Ghoneim (1999) found that significant increases in maize yield and its components were corresponded to progressive increments of N level up to 105 kg/fed. However, highest levels (70 and 105 kg/fed.) were not significantly different in their effects on grain yield. Recently, El-Metwally *et al.* (2001) observed that maize grain yield markedly increased by raising nitrogen levels up to 120 kg/fed. Millet and Feldman (1984) studied the yield response of wheat to inoculation with *Azospirillum brasilense* was studied in pots, at four levels of N fertilization. Plant yield increased due to inoculation only at medium and high levels of N fertilization, with a maximum yield increase of about 8% at the highest level (approximately 1.0 g of pure N per plant). Yield increase was mostly due to an increase in the number of grains per spike, and, at the highest level of fertilization, to a higher number of spikes per plant. At all N levels, inoculation caused an increase of 0.5-1.4% in the number of fertile spikelets per main spike. Grain protein percentage was unaffected by inoculation, though significantly increased by fertilization. It is concluded that the contribution of *Azospirillum brasilense* to wheat yield is not through N_2-fixation. In a field experiment on a medium black soil in India, Zambre *et al.* (1984) stated that tiller numbers, dry matter production, grain yield and grain protein content of wheat (*Triticum durum*) were increased by nitrogen fertilizer applications (up to 120 kg N/ha) and by inoculation of seed with *Azotobacter chroococcum* and *Azospirillum brasilense*. Response to inoculation was observed at all fertilizer levels. In this connection, Reynders and Vlassak conducted a field experiment ten cv. of winter wheat and 4 of spring wheat were grown in field experiments during 1979 and 1980. N was applied at 10, 80 or 160 kg/ha, and *A. brasilense* was applied by overhead spraying. Significant yield increased that were independent of cv. and N rate were obtained in winter wheat, and tillering was increased in both spring and winter wheat. In a greenhouse experiment, *A. brasilense* caused a decrease in root mass; the yield increases thus may be due to the increased tillering and improved nutrient uptake capacity. Also, Pandey, and Kumar (1989) stated that the application of *Azotobacter* and *Azospirillum* to wheat, maize, sorghum, rice, millets, vegetables, potatoes, cotton and sugarcane grown under both irrigated and barani [rainfed] conditions, with and without application of NPK, increased yields of these crops. The beneficial effects of *Azotobacter* and *Azospirillum* are related not only to their N-fixing proficiency but also with their ability to produce antibacterial and antifungal compounds, growth regulators and siderophores. In field trials with wheat, Saha *et al.* (1990) reported that seed inoculation with *A. lipoferum* increased grain yield and N uptake by 42 and 46%, respectively. These parameters were not affected by inoculation in the presence of 10-20 kg P or 30-50 kg N/ha. Inoculation increased the bacterial populations on the root surface and in root tissue especially in the presence of applied P, but had no significant effect on bacterial population in rhizosphere soil. Swedrzynska (2000) mentioned that inoculation with *Azospirillum brasilense* contributed to yield increases of up to 27% in wheat and 6% in oats.

Chemical analyses:

Total nitrogen, phosphorus, potassium and calcium showed increases in their concentrations in both the shoots and roots of wheat plant as the NPK fertilization level increased reaching their maximum values at 100% NPK (Tables 5 and 6). Generally the concentrations of these elements were higher in the second sample as compared to the first sample.

Table 5: Total nitrogen and phosphorus concentrations (mg/g D.W.) as affected by biofertilizers treatments under different NPK fertilization levels on wheat plants.

Treatment	First sample				Second sample			
	% NPK			Mean	% NPK			Mean
	25	50	100		25	50	100	
Shoot total nitrogen (mg/g D.W.)								
Control	7.1	11.4	20.9	13.1	10.2	17.2	29.6	19.0
Nitrobien	10.1	15.3	25.6	17.0	13.5	22.9	33.2	23.2
Phosphorine	9.2	13.8	24.4	15.8	14.4	20.2	32.2	22.3
Phophorin + Nitrobien	11.4	18.8	29.1	19.8	16.9	25.6	34.2	25.6
Mean	9.5	14.8	25.0		13.8	21.5	32.3	
Root total nitrogen (mg/g D.W.)								
Control	3.2	6.6	14.9	8.2	5.4	9.2	18.0	10.8
Nitrobien	5.7	9.4	17.5	10.9	8.3	14.9	23.4	15.5
Phosphorine	5.0	8.0	16.4	9.8	7.9	13.2	22.5	14.5
Phophorin + Nitrobien	7.2	12.7	20.6	13.5	9.6	16.9	25.0	16.5
Mean	5.3	9.2	17.4		7.8	13.6	22.2	
Shoot phosphorus (mg/g)								
Control	0.6	1.2	2.7	1.5	0.9	1.8	3.3	2.0
Nitrobien	0.8	1.5	2.9	1.7	1.2	2.4	5.0	2.9
Phosphorine	0.9	1.6	3.0	1.8	1.4	2.6	5.5	3.2
Phophorin + Nitrobien	1.1	2.2	3.4	2.2	1.6	3.0	6.2	3.6
Mean	0.9	1.6	3.0		1.3	2.5	5.0	
Root phosphorus (mg/g)								
Control	0.4	1.1	2.3	1.3	2.7	1.5	0.7	1.6
Nitrobien	0.6	1.3	2.7	1.5	2.9	1.7	0.8	1.8
Phosphorine	0.7	1.4	2.8	1.6	3.1	1.9	0.9	2.0
Phophorin + Nitrobien	0.9	2.0	3.1	2.0	3.4	2.4	1.2	2.3
Mean	0.7	1.5	2.7		3.0	1.9	0.9	

Biofertilizers treatments increased the concentrations of total nitrogen, phosphorus, potassium and calcium in the shoots and roots of wheat plant in both samples. The highest concentrations of these elements were obtained from the treatment phosphorine + nitrobien.

Table 6: Potassium and calcium concentrations (mg/g D.W.) as affected by biofertilizers treatments under different NPK fertilization levels on wheat plants.

Treatment	First sample				Second sample			
	% NPK			Mean	% NPK			Mean
	25	50	100		25	50	100	
Shoot potassium (mg/g D.W.)								
Control	6.3	13.3	30.2	19.6	10.3	22.5	38.5	23.8
Nitrobien	10.8	21.1	37.6	23.2	15.4	28.5	45.4	29.8
Phosphorine	9.9	22.3	38.1	23.4	16.0	29.5	45.8	30.4
Phophorin + Nitrobien	12.5	27.4	40.0	26.6	19.4	35.5	47.8	34.2
Mean	9.9	21.0	36.5		15.3	29.0	44.4	
Root potassium (mg/g D.W.)								
Control	4.0	9.0	19.6	10.9	7.2	12.9	26.3	15.5
Nitrobien	7.2	15.2	28.5	17.0	9.4	17.3	33.9	20.2
Phosphorine	7.0	14.9	28.3	16.7	9.7	16.9	34.1	20.2
Phophorin + Nitrobien	8.4	18.5	29.7	18.9	11.3	20.3	35.5	22.4
Mean	6.7	14.4	26.5		9.4	16.9	32.5	
Shoot calcium (mg/g)								
Control	4.5	2.1	0.9	2.5	6.7	3.0	1.1	3.6
Nitrobien	5.9	2.8	1.0	3.2	7.9	4.2	1.2	4.4
Phosphorine	5.9	2.7	1.1	3.2	7.9	4.2	1.2	4.4
Phophorin + Nitrobien	6.0	3.0	1.2	3.4	8.0	4.8	1.5	4.8
Mean	5.6	2.7	1.1		7.6	4.1	1.3	
Root calcium (mg/g)								
Control	2.2	1.3	0.6	3.2	4.5	2.1	0.9	2.5
Nitrobine	3.8	1.7	0.9	2.1	5.6	3.1	1.1	3.3
Phosphorine	3.7	1.7	0.8	2.1	5.6	3.1	1.1	3.3
Phophorin + Nitrobine	4.0	2.1	0.9	2.3	6.2	4.1	1.3	3.9
Mean	3.4	1.7	0.8		5.5	3.1	1.1	

Similar trend was obtained for the concentrations of total nitrogen, phosphorus, potassium and calcium in wheat grains at harvest (Table 7).

As for nitrogen level fertilization, these results are in harmony with those obtained by Moursy *et al.* (1979) who found that increasing nitrogen fertilization resulted in increasing photosynthetic pigments (chlorophyll a, b and carotenoids). Moreover, Tollenaar *et al.* (1994) noted that leaf chlorophyll content was significantly higher at high nitrogen level (120 kg/ha) than low level (20 kg/ha) at 3 or 6 weeks after silking. Mahmoud (2000) found that higher nitrogen levels up to 120 kg/fed., significantly increased chlorophyll a, b and carotenoids in leaf ears at 60 days after planting.

Significant increases in protein content in maize grains with higher nitrogen fertilization levels were reported by Perry and Olson (1975); Pierre *et al.* (1977); Rendig and Broadbent (1979) and Salem *et al.* (1982). Moreover, Sairam *et al.* (1991) observed that increasing nitrogen rates (from 0 to 60 and 120 kg/ha) increased protein content in maize leaves at 20 and 60 DAS.

Also, Silva *et al*., (1993) reported that application of 120 kg N resulted in greater protein content in maize grains (9.6%) than no nitrogen (8.3%). This trend was generally confirmed by Hammam (1995) who observed that increasing N level significantly increased protein content in maize grains during 1991 and 1992 seasons. He added that protein percentage increased by 16 and 17.9% as a result of increasing N level from 15 to 105 kg N/fed. In addition, Selim and El-Sergany (1995) found that grain protein percentage gradually increased with increasing N rate from 75 up to 125 kg N/fed. Recently, Abd El- Megeid (2001) reported that there was a direct relationship between protein content in maize grains and applied nitrogen. Protein content increased by raising N rate from 60 to 120 or 180 kg/fed. by 8.9 and 18.8 %, respectively. A lso, increasing n itrogen l evels u p t o 1 20 kg N/fed., increased protein percentage in grains (9.63 and 9.67 %) in 1999 and 2000, respectively(El-Metwally *et al*., 2001). In this connection, Mosolow and Pylneva (1964) found that increased synthesis of amino acids and alanine contents in maize plants with the application of urea fertilization. Total free amino acids content in maize grains were increased by higher rates of added nitrogen (Abd El-Latif, 1979). Similarly, Mahmoud (2000) found that amino acids contents in maize grains were increased with higher nitrogen fertilization levels up to 120 kg/fed. in 1998. However, Abd El-Megeid (2001) showed that m aize l eaves a nalysis a t 4 5 D AS r evealed t hat s oluble a mino acids content was reduced by increasing nitrogen rate up to 120 kg/ fed.

Table 7: Total nitrogen, phosphorus, potassium and calcium concentrations (mg/g D.W.) as affected by biofertilizers treatments under different NPK fertilization levels in wheat grains at harvest.

Treatment	Total nitrogen				Phosphorus			
	% NPK			Mean	% NPK			Mean
	25	50	100		25	50	100	
Control	9.3	14.3	20.5	14.7	1.2	2.1	2.6	2.0
Nitrobien	13.0	19.4	30.1	20.8	1.5	2.3	3.1	2.3
Phosphorine	12.8	18.1	27.8	19.6	1.6	2.3	3.1	2.3
Phophorin + Nitrobien	13.9	21.4	31.6	22.3	2.0	2.6	3.2	2.6
Mean	12.3	18.3	27.5		1.6	2.3	3.0	
	Potassium				Calcium			
Control	26.4	20.1	10.1	18.9	5.2	3.2	2.1	3.5
Nitrobien	32.1	24.8	12.2	23.0	6.3	3.9	2.2	4.1
Phosphorine	32.3	25.7	12.0	23.3	6.3	4.0	2.3	4.2
Phophorin + Nitrobien	33.8	27.0	13.4	24.7	7.0	5.0	2.4	4.8
Mean	31.2	24.4	11.9		6.2	4.0	2.3	

Reducing, non-reducing and total sugars concentrations in the shoots and roots of wheat plant were increased as the NPK level was increased in both the first and second sample (Table 8) as well as in the grains at harvest (Table 9).

All biofertilizer treatments increased Reducing, non-reducing and total sugars concentrations in the shoots and roots of wheat plant were increased as the NPK level was increased in both the first and second sample as well as their concentrations in the grains at harvest.

Table 8: Reducing, non-reducing and total sugars concentrations (mg/g D.W.) as affected by biofertilizers treatments u nder d ifferent NPK fertilization levels on wheat plants.

Treatment	First sample				Second sample			
	% NPK			Mean	% NPK			Mean
	25	50	100		25	50	100	
Shoot reducing sugars								
Control	27.3	14.4	6.9	16.2	41.3	24.8	13.3	26.5
Nitrobine	38.5	17.3	9.9	21.9	46.3	29.9	19.4	31.9
Phosphorine	37.4	18.4	10.0	21.9	47.1	31.5	18.2	32.3
Phophorin + Nitrobine	39.8	24.6	13.4	25.9	50.0	36.0	22.1	36.0
Mean	35.8	18.7	10.1		46.2	30.6	18.3	
Shoot non-reducing sugars								
Control	28.4	16.2	7.8	17.5	48.5	27.2	15.6	30.4
Nitrobine	39.2	18.4	10.8	22.8	60.3	36.7	21.7	39.6
Phosphorine	40.1	19.3	11.1	23.5	61.1	37.5	22.0	40.2
Phophorin + Nitrobine	42.2	25.3	14.8	27.4	63.2	40.1	28.3	43.9
Mean	37.5	19.8	11.1		58.3	35.4	21.9	
Shoot total sugars								
Control	55.7	30.6	14.7	25.3	89.8	52.0	28.9	56.9
Nitrobine	77.7	35.7	20.7	44.7	106.6	66.6	41.1	71.4
Phosphorine	77.5	37.7	21.1	45.4	108.2	69.0	40.2	72.5
Phophorin + Nitrobine	82.0	49.9	28.2	53.4	113.2	76.1	50.4	79.9
Mean	73.2	38.5	21.2		104.5	65.9	40.2	
Root reducing sugars								
Control	6.4	3.1	1.5	3.8	11.4	6.4	3.5	7.1
Nitrobine	9.2	5.8	2.7	5.9	18.7	8.2	4.7	10.5
Phosphorine	9.5	6.1	2.7	6.1	18.4	8.3	4.7	10.5
Phophorin + Nitrobine	10.3	6.4	2.9	6.5	19.6	11.2	6.0	12.3
Mean	8.9	5.4	2.5		17.0	8.5	4.7	
Root non-reducing sugars								
Control	9.6	5.2	2.5	5.8	13.1	8.2	5.2	8.8
Nitrobine	18.1	8.4	3.4	10.0	30.8	12.4	7.2	16.8
Phosphorine	18.2	8.5	3.4	10.0	31.2	13.0	6.9	17.0
Phophorin + Nitrobine	19.4	9.3	4.7	11.1	33.1	14.2	7.3	18.2
Mean	16.3	7.9	3.5		27.1	12.0	6.7	
Root total sugars								
Control	16.1	8.3	4.0	9.5	24.5	14.6	8.7	15.9
Nitrobine	27.3	14.2	6.1	15.9	49.5	20.6	11.9	27.3
Phosphorine	27.7	14.6	6.1	16.1	49.6	21.3	11.6	27.5
Phophorin + Nitrobine	29.7	15.7	7.6	17.7	52.7	25.4	13.3	30.5
Mean	25.2	13.2	6.0		44.1	20.5	11.4	

Table 9: Reducing, non-reducing and total sugars concentrations (mg/g D.W.) as affected by biofertilizers treatments u nder d ifferent NPK fertilization levels in wheat grains plants at harvest.

Treatment	Reducing sugars				Non-reducing sugars				Total sugars			
	% NPK			Mean	% NPK			Mean	% NPK			Mean
	25	50	100		25	50	100		25	50	100	
Control	5.3	13.6	28.1	15.7	7.8	15.8	36.4	29.0	13.1	29.4	64.5	35.7
Nitrobien	8.1	17.2	36.6	20.6	10.2	20.1	38.7	23.0	18.3	37.3	75.3	43.6
Phosphorine	7.3	16.8	35.9	19.8	9.9	20.0	39.2	23.0	17.2	36.8	75.1	43.0
Phophorin + Nitrobien	11.4	19.8	40.2	23.8	13.5	22.4	43.1	26.3	24.9	42 2	83.4	50.0
Mean	8.0	16.9	35.2		10.4	19.6	39.4		18.4	36.4	73.1	

Thus, increasing wheat yield could be achieved through rationalization of the use of mineral fertilizers in combination with the biofertilizers containing nitrogen fixing bacteria, phosphorus and potassium dissolving bacteria. Such practice could lead to less polluted environment and obtaining an economical yield of wheat.

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التأثيرات الفسيولوجية لبعض المخصبات الحيوية وعلاقتها بالتغذية المعدنية على النمو وصفات المحصول والمكونات الكيماوية فى القمح

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فى تجربتي اصص ، تم زراعة القمح صنف سخا ٨ وتم التسميد بمستويات ٢٥ و ٥٠ و ١٠٠% NPK. تحت كل مستوى تمت المعاملة بالمخصبات الحيوية نتروبين وفوسفورين وفوسفورين + نتروبين. تشير النتائج الى ان زيادة مستوى تسميد NPK يؤدى الى زيادة معنوية فى كل من طول النبات وعدد الاشطاء وعدد الاوراق وكذلك الوزن الجاف للافرع الهوائية والجذور والوزن الجاف الكلى للنبات فى كل من العينتين الاولى والثانية. زادت كل من الصفات السابق ذكرها زياده معنوية عند المعاملة بالمخصبات الحيوية وذلك مقارنة بالكونترل الغير معامل. زاد محصول الحبوب للنبات بزيادة معدل تسميد NPK زيادة معنوية. زاد محصول الحبوب للنبات وكذلك مكوناتة عند المعاملة بمعاملات المخصبات الحيوية. وكانت اعلى زيادة فى المحصول ناتجة من المعاملة فوسفورين + نتروبين. ادت زيادة مستوى تسميد NPK الى زيادة تركيز العناصر المعدنية شاملة النيتروجين والفوسفور والبوتاسيوم والكالسيوم وكذلك تركيز السكريات فى كل من العينتين الاولى والثانية. تم الحصول على قيم مشابهة فى عينة المحصول. ومن ثم فانة يمكن استنتاج ان مستوى تسميد NPK مع استخدام المخصبات الحيوية ربما يؤدى الى تحسين الحالة الغذائية لنبات القمح والتى تؤدى بدورها الى زيادة انتاجية النبات.