J. Agric. Sci. Mansoura Univ., 30 (5): 2895 - 2908, 2005

EFFECT OF *Azotobacter chroococcum* AND PHOSPHATE SOLUBILIZING BACTERIA ON WHEAT GROWTH, PRODUCTIVITY AND NUTRIENT AVAILABILITY

El-Maddah, E.I.*; M. EL-D. El-Sodany* and E.A. Koreish**

*** Department of Improvement and Conservation of Cultivated Soils, Soil, Water and Environment Res. Inst., A. R. C., Giza, Egypt.**

**** Soil and Water Sciences Department, Faculty of Agriculture, University of Alexandria, Egypt.**

ABSTRACT

Two field experiments were carried out during two winter seasons of 2002/2003 and 2003/2004 at El-Nubaria (highly calcareous soil) and El-Gemmeiza Agricultural Research Station (Nile Delta soil), to evaluate two inocula to be used as biofertilizers. These inocula are made of *Azotobacter chroococcum* and phosphate-solubilizing bacteria (PSB). The inocula may be able to solubilize sources of insoluble phosphate and improve the use of nitrogen in wheat fields under different rates of N and P mineral fertilizers. Wheat growth, yield, N, P-uptake and soil N and P available were recorded.

Wheat plant growth was promoted by bacteria combined with mineral fertilizers. Wheat dry weight and plant height were significantly increased by the two inocula in both locations. The nitrogen content and uptake were significantly increased in wheat plants inoculated with *Azotobacter chroococcum* combined with mineral nitrogen fertilizer with respect to the uninoculated plants in both sites. Similar trend was observed for the phosphorus content and uptake in wheat plants inoculated with PSB inoculum. Common treatment without bacterial inoculation gave very low N or P contents and uptake in plants under low level of mineral fertilization treatments. Wheat yield and yield components were also affected significantly by *Azotobacter chroococcum* and for PSB inoculation combined with mineral fertilization with respect to the uninoculated plants in both sites. The grain yield of 2432 and 2670 kg fed.$^{-1}$ recorded with 100 kg N fed.$^{-1}$, were statistically similar to 2379 and 2510 kg fed.$^{-1}$ obtained from *Azotobacter chroococcum* treatment combined with 80 kg N fed.$^{-1}$ for El-Nubaria and El-Gemmeiza, respectively. The grain yield of 2688 and 2595 kg fed.$^{-1}$ recorded with 30 kg P_2O_5 fed.$^{-1}$, were statistically similar to 2678 and 2636 kg fed.$^{-1}$ obtained from PSB treatment combined with 20 kg P_2O_5 fed.$^{-1}$ for the two sites, respectively. The same trend was observed for biological and straw yield for both inocula and the two locations. These results suggest that *Azotobacter chroococcum* and PSB are able to fix and mobilize more N and P to the plants and improve plant growth.

Keywords: *Azotobacter chroococcum*, phosphate solubilizing bacteria, wheat, nutrient availability.

INTRODUCTION

Fertilizers applied to field crops basically include elements such as nitrogen and phosphorus. Frequently, these nutrients are present within agricultural soils, but are less available for plant without the presence of microorganisms that are able to solubilize, mobilize and/or biologically fix such elements. These microorganisms are a part of the soil native microflora and fall under the *Pseudomonas*, *Enterobacter*, *Bacillus* and *Micrococcus* genera, among others. They have the ability to solubilize phosphates into assimilable compound (Fawaz *et al.*, 1980), allowing the plant to absorb such nutrients. On the other hand, there are native free-moving bacteria populations, such as the *Azotobacter* genus, which can produce growth promoting substances that enhance the plant ability to grow.

Many soils throughout the world are P-deficient because the free phosphorus concentration even in fertile soils is, generally, not higher than 10 μM even at pH 6.5 where it is most soluble (Arnou, 1953). Soluble forms of P fertilizers applied to the soil are easily precipitated as insoluble forms. This often leads to an excess application of P fertilizer to cultivated land. This unmanaged excess may be both an environmental and economic problem. The positive interaction between plants and rhizosphere microorganisms can improve plant nutrition, nitrogen fixation, plant tolerance to environmental stresses and biologically control pathogens. These would reduce chemical fertilizers and pesticides need. It is often assumed that enhancement of plant growth after inoculation is a direct response to the introduced microorganisms. Improvement in germination, plant growth or yield after soil or seed inoculation have been attributed solely to N-fixation (Mishustin, 1970). There has been much interest in obtaining an effective nitrogen-fixing system for non-leguminous field crops such as wheat, barley and corn.

Phosphate solubilizing bacteria (PSB) play a key role in the plant metabolism and crop productivity. It had been reported to increase the availability and uptake of native soil P in different crops by converting insoluble phosphates in the soil to soluble forms by producing various organic acids (Fawaz *et al.*, 1980 and Kucey, 1987 and Raced, 1994).

Therefore, this work intends to evaluate two inocula to be used as bio-fertilizers, which are made up of phosphate-solubilizing bacteria and *Azotobacter chroococcum*, and that may be able to solubilize sources of insoluble phosphate and improve the efficiency of nitrogen in fields cultivated with wheat under different rates of P and N mineral fertilizers.

MATERIALS AND METHODS

Two field experiments were conducted during two winter 2002/03 and 2003/04 seasons in two locations at El-Nubaria and El-Gemmeiza Agricultural Research Station Farms. The two selected locations varied in total calcium carbonate percentage and organic matter contents. The soil physical, chemical and fertility indices are presented in Table (1). The experiments were arranged in a split plot design with three replications. The main plots were for the mineral fertilization levels and the sub-plots were for the inoculation treatments. The area of experimental plot was 5 m^2, with 8 rows, 2.5 m long and 25 cm apart. Cultivar bread wheat (*Triticum vulgaris* L.) variety Gemmeza-7 was planted at the rate of 70 kg seeds $fed.^{-1}$ during the first week of December and harvested on the first week of May.

Experimental procedures

Azotobacter chroococcum experiment

- Obtaining isolates

Free living N_2-fixing bacteria, 32 *Azotobacter* isolates were separated from rhizosphere samples of wheat plants grown in the Nubaria and

Gemmeiza Agricultural Research Stations (16 for each). Isolates were purified and identified as *Azotobacter chroococcum* according to the classification of Bergey (Holt and Krieg, 1984). The abilities of these isolates to fix nitrogen were tested by modified Kjeldahl method after Chapman and Pratt (1961). The highest isolate activity No. 8 was used for *Azotobacter chroococcum* for Nubaria and No. 11 for El-Gemmeiza. Pure cultures were prepared just before seed inoculation to reach the final density of 10^8 cells ml^{-1}.

Table (1): Main soil characteristics of El-Nubaria and El-Gemmeiza Agricultural Research Station Farms.

Soil characteristics \ Soil depth (cm)	El-Nubaria		El-Gemmeiza	
	0-20	**20-40**	**0-20**	**20-40**
Soil pH, 1:2.5 (suspension)	8.26	8.24	7.92	7.88
Soil EC, $dS\ m^{-1}$	1.92	1.98	1.16	1.25
Soluble cations ($meq\ l^{-1}$)				
Ca^{++}	6.92	7.13	2.91	3.12
Mg^{++}	1.87	2.11	3.21	3.29
Na^{+}	7.59	6.62	5.11	5.76
K^{+}	2.82	3.94	0.37	0.33
Soluble anions ($meq\ l^{-1}$)				
CO_3^{--}	-	-	-	-
HCO_3^{-}	4.86	3.97	3.36	3.47
Cl^{-}	10.14	11.28	5.81	6.12
SO_4^{--}	4.20	4.55	2.43	2.91
Sand, %	55.91	59.53	19.28	19.16
Silt, %	24.82	21.26	43.38	41.71
Clay, %	19.27	21.21	37.34	39.13
Soil texture	SL	SCL	SiCL	SiCL
$CaCO_3$, %	22.62	25.31	3.27	3.18
O.M, %	0.43	0.27	2.21	1.67
Av. N, ppm	37.91	28.41	48.72	32.16
Av. P, ppm	4.16	2.94	9.37	7.19
Av. K, ppm	227	168	368	357

SL = Sandy loam. SCL = Sandy clay loam. SiCL = Silty clay loam.

- Mineral nitrogen fertilizer application and rates

Mineral nitrogen fertilizer in the form of ammonium nitrate (33.5% N), was added at three equal doses, at planting, tillering and heading stages at the rates of zero, 60, 80 and 100 kg N $fed.^{-1}$. Phosphorus fertilizer in the form of mono-superphosphate (15.5% P_2O_5) was added at land preparation at the rate of 30 kg P_2O_5 $fed.^{-1}$. Potassium sulphate (48% K_2O) fertilizer was added at the rate of 48 kg K_2O $fed.^{-1}$ with the second dose of N fertilizer.

Phosphate solubilizing bacteria (PSB) experiment

- Obtaining isolates

Bacterial strains of *Pseudomonas* sp., *Arthrobacter* sp. and *Rhizobium meliloti* were used for the experiment. Glycerin-peptone-agar medium was used for isolation of bacterial strains (Hirte, 1961). For isolation of rhizosphere bacteria, 1 g of wheat roots was washed, macerated and shacked with 10 ml sterile water. The resulting suspensions were evaluated for colony forming units (cfu) according to the dilution-plate method in glycerine-peptone-agar medium. After incubation for 7 days at 28°C. The reisolated strains were identified. The identification of strains relied on standard biochemical and physiological tests according to the classification of Bergey (Holt and Krieg, 1984). Gram stain, morphology, spore formation, motility and gas production from glucose were determined according to methods for LAB described by Gerhardt (1981).

Inoculation of wheat seeds was run by immersing in appropriate bacterial suspension for 3 h, then drying before using in plantation. This cell suspension containing a mixture of PSB of the genera *Pseudomonas*, *Arthrobacter* and *Rhizobium* which was used as a bio-fertilizer.

- Mineral phosphorus fertilizer application and rates

Mineral phosphorus fertilizer in the form of mono-superphosphate (15.5% P_2O_5) at the rates of zero, 10, 20 and 30 kg P_2O_5 fed.$^{-1}$ was added at land preparation. Nitrogen fertilizer in the form of ammonium nitrate (33.5% N) was added at three equal doses; at planting, tillering and heading stages at the rate of 100 kg N fed.$^{-1}$. Potassium sulphate (48% K_2O) fertilizer was added at the rate of 48 kg K_2O fed.$^{-1}$ with the second dose of N fertilizer.

Sampling and analytical procedure

Composite initial soil samples were obtained at 30 cm depth. Also, soil and plant samples for the three replications were collected from all treatments at harvest time. Total yield for each plot was determined for grain and straw and related to kg fed.$^{-1}$. Soil samples were air-dried, ground, sieved through 2 mm sieve and analyzed for available nitrogen and phosphorus (Chapman and Pratt, 1961). Fresh plant material was washed, weighted and dried at 65°C for 48 h. Wet ashing by concentrated sulphuric acid and H_2O_2 (FAO, 1980) was used to determine total N and P content and calculate their uptake.

The collected data were statistically analyzed according to procedures outlined by Snedecor and Cochran (1981) using Costat Software (1985).

RESULTS AND DISCUSSION

Initial state analysis of the two experimental site soils (Table 1) indicate that the soil surface layers have a light texture of sandy loam to sandy clay loam with high content of total $CaCO_3$ (from 22 to 25%) for El-Nubaria site and silty clay loam with low content of total $CaCO_3$ (about 3%) for El-Gemmeiza site. Soil pH values are around basicity for the two sites. Total soluble salts content was about 2 and 1.2 dS m^{-1} for El-Nubaria and El-Gemmeiza sites, respectively. The available NPK and organic matter content are low and moderate for El-Nubaria and El-Gemmeiza sites, respectively, accordingly, the two soils under consideration are deficient in fertility status.

Response of wheat plants to *Azotobacter chroococcum* inoculation
- Plant growth and biomass

Data presented in Tables (2 and 3) show that the inoculation and nitrogen mineral fertilization resulted in several increases in wheat dry weight and plant height in the two sites. The magnitude of increases depended on the inoculation and fertilizer rates. Concerning the inoculation, the increase percentages in dry weight and plant height were 4.67 and 2% at El-Nubaria site and 7.75 and 4% at El-Gemmeiza site, respectively. Also, the fertilization under inoculation and uninoculation treatments significantly increased the same above mentioned tested variables. Increasing rates of nitrogen fertilizer significantly and progressively increased the dry weight and plant height, the maximum values (21.65 g/10 plants and 90.29 cm plant^{-1}) were recorded with inoculation in combination with the third rate of mineral fertilizer (100 kg N fed.$^{-1}$), with increment rates of 5 and 2.3% over the control (uninoculated plants), respectively at El-Nubaria site. The dry matter and plant height were increased with increasing N fertilizer level from N_0 to N_3 by 5.0, 4.3, 4.2 and 4.9% and by 1.3, 1.9, 2.5 and 2.3%, respectively as affected by inoculation (Table 2). It could be seen that both dry matter weight and plant height tended to increase by higher increment rate in El-Gemmeiza site than El-Nubaria site as affected with inoculation and/or N fertilizer level. The increment rates over the control were 20.5, 4.0, 2.4 and 7.3% for dry matter and 1.0, 4.6, 3.0 and 7.4% for plant height with increasing N fertilizer levels from N_0 to N_3, respectively. The obtained results revealed that mineral fertilizers applied to wheat field basically include elements such as nitrogen, phosphorus and potassium. Frequently, these nutrients are added to agricultural soils, but are less available for plants without the presence of microorganisms that are able to solubilize and biologically fix such elements. Therefore, inoculation of wheat seeds with *Azotobacter chroococcum* in addition to presence of native free-moving bacteria populations, such as the *Azotobacter* genus which can produce growth-promoting substances, enhance the plant ability to grow. Also, results indicated that the *Azotobacter chroococcum* inoculum improved the use of nitrogen in wheat fields.

- Wheat yield and yield components

Tables (2 and 3) showed that conjunction of biofertilization and various N-fertilizer rates significantly increased the 1000-seed weight and the biological yield of wheat (grain and straw). The increases were linked to the application rates of N-fertilizer. The 1000-seed weight (g), generally, tended to increase significantly by *Azotobacter* inoculant, the increment rates due to the inoculation were 3.7 and 2.1% over the control for El-Nubaria and El-Gemmeiza sites, respectively. In view of the various N levels, the maximum increment rates reached 23.6 and 19.8% at the rate of 100 kg N fed.$^{-1}$ under inoculation treatment for both sites, respectively. It could be seen that the 1000-seed weight tended to increase as N fertilizer level increased with or without inoculation in both sites. The maximum 1000-seed weight was at N_3 level followed by N_2 for inoculated plants with significant differences between them.

Table (2): Effect of *Azotobacter chroococcum* and mineral nitrogen fertilizer levels on growth parameters, yield and yield components and N concentration in dry matter of wheat plants at Nubaria (average of two seasons).

Treatments		Dry weight/ 10 plants (g)	Plant height (cm)	1000-seed weight (g)	N concentration in dry matter (%)		Biological yield (kg fed.$^{-1}$)	Yield (kg fed.$^{-1}$)		Harvest index
					Grain	Straw		Grain	Straw	
Uninoculated	N_0	12.83 d	71.82 d	46.29 d	1.73 d	0.68 d	2861 e	1074 e	1787 e	0.375
	N_1	16.89 c	78.63 c	49.87 c	2.06 c	0.77 bc	3894 d	1468 d	2426 d	0.377
	N_2	18.14 bc	85.14 b	54.91 b	2.43 b	0.87 a	5853 c	2215 c	3638 c	0.378
	N_3	20.63 ab	88.26 a	55.26 ab	2.62 ab	0.87 a	6387 b	2432 b	3955 b	0.381
Inoculated	N_0	13.48 d	72.73 d	47.11 d	1.73 d	0.73 cd	3120 e	1173 e	1947 e	0.376
	N_1	17.62 c	80.14 c	51.26 c	2.14 c	0.81 ab	4159 d	1568 d	2591 d	0.377
	N_2	18.91 bc	87.25 a	57.42 a	2.71 a	0.88 a	6264 bc	2379 bc	3885 b	0.380
	N_3	21.65 a	90.29 a	58.21 a	2.73 a	0.89 a	7001 a	2682 a	4319 a	0.383
Inoculation	Un (-)	17.12 b	80.96 b	51.58 b	2.21 b	0.798 b	4498.8 b	1797.3 b	2951.5 b	0.378
	In (+)	17.92 a	82.60 a	53.50 a	2.33 a	0.818 a	5136.0 a	1950.5 a	3185.5 a	0.379
Interaction Inoculation x N		*	*	**	**	*	**	**	**	-

Table (3): Effect of *Azotobacter chroococcum* and mineral nitrogen fertilizer levels on growth parameters, yield and yield components and N concentration in dry matter of wheat plants at El-Gemmeiza (average of two seasons).

Treatments		Dry weight/ 10 plants (g)	Plant height (cm)	1000-seed weight (g)	N concentration in dry matter (%)		Biological yield (kg fed.$^{-1}$)	Yield (kg fed.$^{-1}$)		Harvest index
					Grain	Straw		Grain	Straw	
Uninoculated	N_0	14.62 e	74.60 d	49.11 d	1.87 d	0.74 c	3120 d	1140 e	1980 d	0.365
	N_1	19.18 c	81.41 cd	53.30 c	2.14 c	0.79 bc	4850 c	1770 d	3080 c	0.365
	N_2	19.94 c	87.52 bc	56.26 b	2.39 b	0.84 b	6370 b	2360 c	4010 b	0.370
	N_3	21.58 ab	92.13 ab	57.51 ab	2.58 ab	0.94 a	7080 a	2670 ab	4410 a	0.377
Inoculated	N_0	17.62 d	75.36 d	49.62 d	1.94 d	0.76 c	3430 d	1260 e	2170 d	0.367
	N_1	19.95 c	85.13 c	54.15 c	2.47 b	0.83 b	5190 c	1920 d	3270 c	0.370
	N_2	20.42 bc	90.25 b	58.23 ab	2.63 a	0.87 ab	6640 b	2510 b	4130 b	0.378
	N_3	23.16 a	98.94 a	59.46 a	2.68 a	0.98 a	7390 a	2820 a	4570 a	0.382
Inoculation	Un (-)	18.83 b	83.92 b	44.05 b	2.15 b	0.83 a	5355 b	1985 b	3370 b	0.371
	In (+)	20.29 a	87.42 a	44.99 a	2.39 a	0.86 a	5660 a	2125 a	3535 a	0.375
Interaction Inoculation x N		*	*	*	*	N.S	**	**	**	-

The same trend was observed for the biological yield. It is clear that inoculation with *Azotobacter* increased biological and grain yields by 14.0 and 8.5% at El-Nubaria and by 5.7 and 7.0% at El-Gemmeiza, respectively. In view of the various N doses, the maximum biological and grain yields were 124 and 115%, and 129 and 124% over the control at the rate of 100 kg N fed.$^{-1}$ for the inoculated plants followed by the rate of 80 kg N fed.$^{-1}$ (100 and 94%, and 103 and 99%) for the two sites, respectively. The same trend was observed for straw yield in the two sites. In addition, the results clearly showed that the harvest index was enhanced with either inoculation or fertilization for the two sites. These results confirm that the soil at El-Gemmeiza is more fertile than the Nubaria soil (Table 1).

- Nitrogen concentration and uptake

Nitrogen concentration and uptake by wheat plants were significantly increased by inoculation and nitrogen fertilization in the two sites (Tables 2 and 3 and Figs 1 and 2). Nitrogen concentration in wheat grain and straw and total uptake were significantly increased by 5.4 and 2.5%, and 9.6% at El-Nubaria and by 11.1 and 3.6%, and 14.3% at El-Gemmmeiza site, respectively as affected by inoculation treatment. The maximum values of N concentration in wheat grain and straw were obtained when N_3 and N_2 were applied to inoculated plants with insignificant difference between them for the two sites.

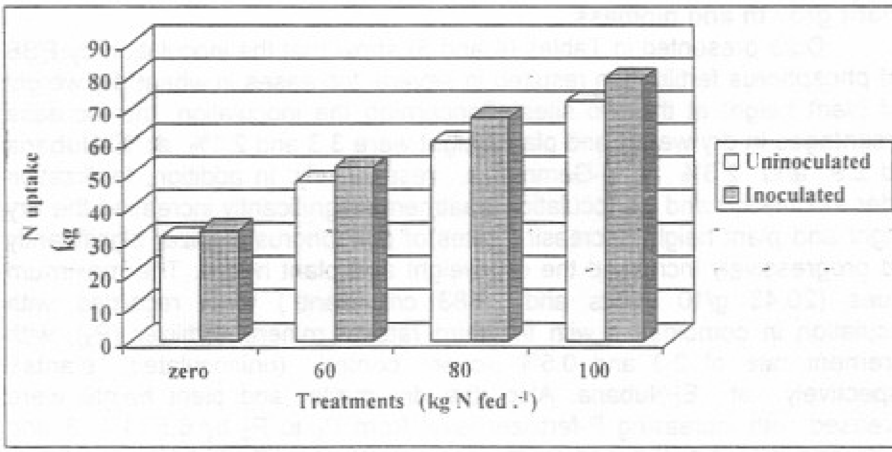

Fig. (1): The effect of inoculation with *Azotobacter chroococcum* with or without N fertilizer on nitrogen uptake by wheat plants at El-Nubaria site.

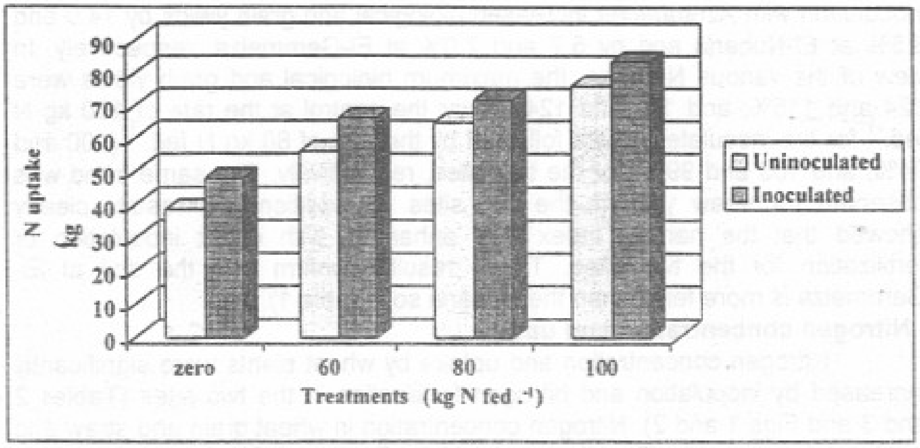

Fig. (2): The effect of inoculation with *Azotobacter chroococcum* with or without N fertilizer on nitrogen uptake by wheat plants at El-Gemmeiza site.

Response of wheat plants to phosphate solubilizing bacteria (PSB) inoculation

- Plant growth and biomass

Data presented in Tables (4 and 5) show that the inoculation by PSB and phosphorus fertilization resulted in several increases in wheat dry weight and plant height at the two sites. Concerning the inoculation, the increase percentages in dry weight and plant height were 3.3 and 2.1% at El-Nubaria and 2.9 and 2.3% at El-Gemmeiza, respectively. In addition, fertilization under inoculation and uninoculation treatments significantly increased the dry weight and plant height. Increasing rates of phosphorus fertilizer significantly and progressively increased the dry weight and plant height. The maximum values (20.43 g/10 plants and 89.83 cm plant^{-1}) were recorded with inoculation in combination with the third rate of mineral fertilizer (P_3), with increment rate of 2.3 and 0.5% over control (uninoculated plants), respectively at El-Nubaria. Also, the dry matter and plant height were increased with increasing P-fertilizer level from P_0 to P_3 by 6.5, 1.4, 3 and 2.3%, and 1.5, 4.4, 2.3 and 0.5%, respectively as affected by inoculation (Table 4). It can be seen that both dry matter weight and plant height tended to increase by inoculation and/or P-fertilization, the increment rates, over the control, were 6.6, 0.7, 3.0 and 1.5% for dry matter and 3.7, 1.8, 2.2 and 1.7% for plant height with increasing P-fertilizer levels from P_0 to P_3, respectively at El-Gemmeiza. Fawaz *et al.*(1980) and Banik and Dey (1981) reported that the phosphate solubilizing bacteria solubilize insoluble P by producing various organic acids. Also, Bonner (1961) concluded that the positive effects of PSB on dry and fresh weights was probably due to the presence of inoculated bacteria that support absorption, production of vitamins and growth promoting substances and solubilization of phosphates. This process has a direct impact on production of ATP and enzymes in charge of stimulating the growth.

- Wheat yield and yield components

Tables (4 and 5) showed that biofertilization and various P-fertilizer levels significantly increased the 1000-seed weight and biological yield of wheat. The increases were linked to the application rates of P-fertilizer with or without inoculation. The 1000-seed weight tended to increase significantly by PSB inoculant, the increment rate due to inoculation was 1.9 and 1.2% over the control for El-Nubaria and El-Gemmeiza, respectively. In view of the various P-levels, the maximum values of the 1000-seed weight were obtained at P_3 and P_2 for inoculated plants with insignificant difference between them. The increment rates were 5.4 and 2.8%, and 6.5 and 4.3% for El-Nubaria and El-Gemmeiza, respectively. It is clear that inoculation with PSB significantly increased biological and grain yield by 4.5 and 4.8% at El-Nubaria and 2.2 and 2.3% at El-Gemmeiza, respectively. The obtained results revealed that both the biological and grain yield tended to increase by twice increment rate in El-Nubaria site (highly calcareous soil) than El-Gemmeiza site as affected by PSB inoculant. This may be related to the low availability of P at El-Nubaria site due to high content of $CaCO_3$ (from 23 to 25%) and the basic reaction of soil (pH 8.24 to 8.26). This highly positive effect of PSB inoculant in case of El-Nubaria site than El-Gemmeiza may be related to that the PSB which release several organic acids including oxalic, citric, butyric, malonic, lactic, succinic, malic, gluconic, acetic, glyconic, fumaric, adipic and 2-ketogluconic acid that solubilize insoluble P, this available P is taken up by plants (El-Gibaly *et al.*, 1977; El-Attar *et al.*, 1979 and Leyval and Berthelin, 1989).

Data, also, revealed that the maximum biological and grain yield reached 19 and 20%, and 7.3 and 8.5% at the rate of P_3 over the control for the inoculated plants followed by P_2 (reached 10 and 10.9%, and 4.6 and 5.0%) for the two sites, respectively. The same trend was observed for straw yield at both sites. In addition, the results showed that the harvest index was enhanced by either inoculation or fertilization treatments at both sites.

- Phosphorus concentration and uptake

Phosphorus concentration in wheat plants (grain and straw) was insignificantly affected by inoculation for the two sites (Tables 4 and 5), while P-uptake (Figs. 3 and 4) was significantly increased with inoculation. The total P-uptake was increased by 30 and 17% as affected by inoculation for the two sites, respectively. The maximum values of P-concentration in wheat grain and straw were obtained when P_3 and P_2 were applied to inoculated plants with insignificant difference between them for the two sites.

Table (4): Effect of phosphate solubilizing bacteria (PSB) and mineral phosphorus fertilizer levels on growth parameters, yield and yield components and P concentration in dry matter of wheat plants at Nubaria (average of two seasons).

Treatments		Dry weight/ 10 plants (g)	Plant height (cm)	1000-seed weight (g)	P concentration in dry matter (%)		Biological yield (kg fed.$^{-1}$)	Yield (kg fed.$^{-1}$)		Harvest index
					Grain	Straw		Grain	Straw	
Uninoculated	P_0	17.45 c	79.91 c	54.36 d	0.48 d	0.30 c	6378 e	2398 f	3980 e	0.376
	P_1	18.61 bc	80.13 c	54.81 cd	0.50 cd	0.33 bc	6568 de	2480 ef	4088 de	0.378
	P_2	18.97 b	85.74 a	55.72 cd	0.55 ab	0.33 bc	6686 cd	2527 de	4159 cd	0.378
	P_3	19.98 ab	89.36 a	57.76 ab	0.57 ab	0.36 ab	7092 b	2688 bc	4404 b	0.379
Inoculated	P_0	18.59 bc	81.14 c	55.26 cd	0.52 cd	0.31 c	6406 e	2415 f	3991 d	0.377
	P_1	18.87 b	83.62 b	56.43 bc	0.54 bc	0.34 ab	6833 c	2583 cd	4250 c	0.378
	P_2	19.56 ab	87.71 a	56.81 ab	0.57 ab	0.35 ab	7066 b	2678 b	4388 bc	0.379
	P_3	20.43 a	89.83 a	58.26 a	0.59 a	0.38 a	7624 a	2897 a	4727 a	0.380
Inoculation	Un (-)	18.75 b	83.79 b	55.66 b	0.53 a	0.33 a	6681.1 b	2523.3 b	4157.8 b	0.378
	In (+)	19.36 a	85.58 a	56.69 a	0.56 a	0.35 a	6982.3 a	2643.3 a	4339.0 a	0.379
Interaction Inoculation x P		N.S	*	*	N.S	N.S	*	*	*	-

Table (5): Effect of phosphate solubilizing bacteria (PSB) and mineral phosphorus fertilizer levels on growth parameters, yield and yield components and P concentration in dry matter of wheat plants at El-Gemmeiza (average of two seasons).

Treatments		Dry weight/ 10 plants (g)	Plant height (cm)	1000-seed weight (g)	P concentration in dry matter (%)		Biological yield (kg fed.$^{-1}$)	Yield (kg fed.$^{-1}$)		Harvest index
					Grain	Straw		Grain	Straw	
Uninoculated	P_0	18.36 c	81.49 d	55.63 d	0.52 e	0.29 d	6748 d	2490 d	4258 d	0.369
	P_1	19.67 bc	88.36 b	57.11 c	0.57 cde	0.31 c	6790 d	2519 d	4271 cd	0.371
	P_2	20.11 abc	90.11 b	58.42 b	0.59 bcd	0.31 c	6879 cd	2566 bc	4313 bc	0.373
	P_3	21.98 ab	91.26 ab	59.66 a	0.67 ab	0.38 b	6957 bc	2595 bc	4362 b	0.373
Inoculated	P_0	19.58 bc	84.52 c	56.46 c	0.56 de	0.33 c	6776 d	2507 d	4269 cd	0.370
	P_1	19.82 abc	89.91 b	58.13 b	0.58 cde	0.37 b	6868 cd	2541 cd	4327 bc	0.370
	P_2	20.74 abc	92.13 a	58.91 a	0.63 abc	0.40 ab	7067 b	2636 b	4431 a	0.373
	P_3	22.31 a	92.78 a	60.14 a	0.69 a	0.42 a	7273 a	2720 a	4553 a	0.374
Inoculation	Un (-)	20.03 b	87.81 b	57.71 b	0.59 a	0.32 a	6843.5 b	2542.5 b	4301.0 b	0.372
	In (+)	20.61 a	89.84 a	58.41 a	0.62 a	0.38 a	6996.0 a	2601.0 a	4395.0 a	0.372
Interaction Inoculation x P		N.S	*	*	N.S	N.S	*	*	*	-

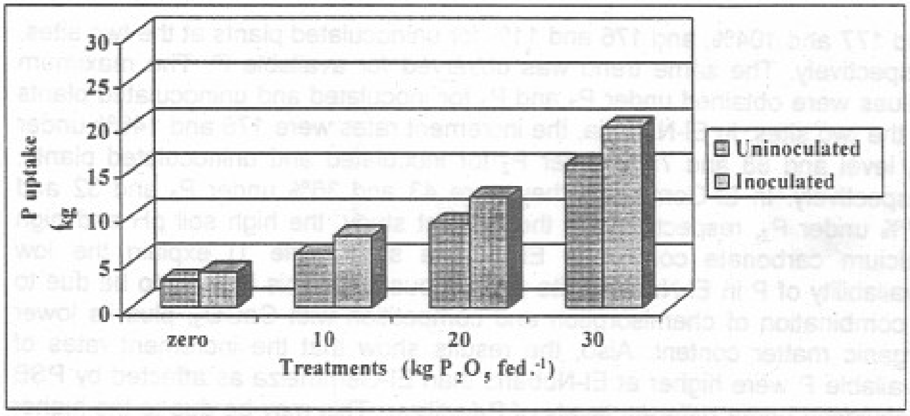

Fig. (3): The effect of inoculation with *Azotobacter chroococcum* with or without P fertilizer on phosphorus uptake by wheat plants at El-Nubaria site.

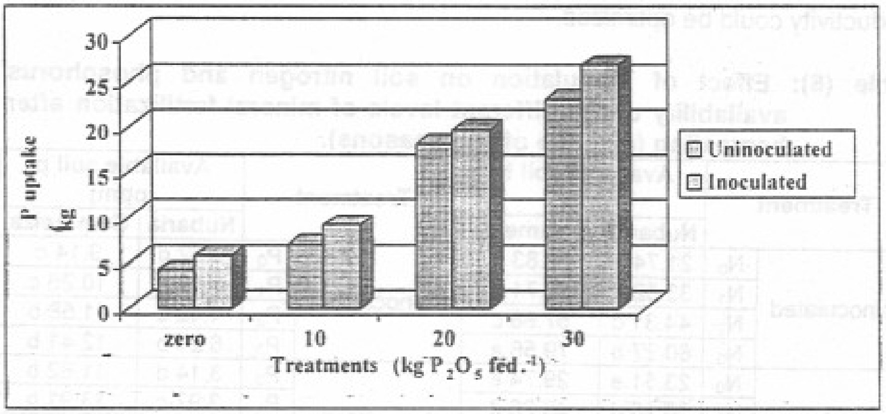

Fig. (4): The effect of inoculation with *Azotobacter chroococcum* with or without P fertilizer on phosphorus uptake by wheat plants at El-Gemmeiza site.

Soil nitrogen and phosphorus availability after harvest as affected by inoculation

Data presented in Table (6) show significant increases in the amounts of available N and P in both soils after harvest as affected with inoculation and mineral fertilization. Concerning the effect of biofertilization on N and P availability, data revealed that soil N-availability was increased by 11.3 and 8.6% for El-Nubaria and El-Gemmeiza sites, respectively, while P-availability was increased by 24.2 and 31.7% for both sites, respectively. As for the effect of mineral fertilizers on available N and P (Table 6), it is shown that, under all treatments, the soil content of available N and P was affected by mineral fertilizer application rates. The maximum values of available N were obtained under N_3 level followed by N_2 with or without inoculation, by increment rates of 190 and 112%, and 197 and 143% for inoculated plants and 177 and 104%, and 176 and 11% for uninoculated plants at the two sites, respectively. The same trend was observed for available P. The maximum values were obtained under P_3 and P_2 for inoculated and uninoculated plants at the two sites. In El-Nubaria, the increment rates were 176 and 146% under P_3 level and 88 and 75% under P_2 for inoculated and uninoculated plants, respectively. In El-Gemmeiza they were 43 and 36% under P_3 and 32 and 27% under P_2, respectively. In the present study, the high soil pH and high calcium carbonate content in El-Nubaria soil (Table 1) explain the low availability of P in El-Nubaria site (calcareous soil). This is likely to be due to a combination of chemisorption and competition with $CaCO_3$, plus its lower organic matter content. Also, the results show that the increment rates of available P were higher at El-Nubaria than El-Gemmeiza as affected by PSB inoculation under different levels of P-fertilizer. This may be due to the higher effect of PSB on solubilization of natural insoluble phosphate in case of highly calcareous soil. Therefore, the use of chemical fertilizers with PSB inoculation could reduce the use of chemical fertilizers, reduce pollution impact and crop productivity could be optimized.

Table (6): Effect of inoculation on soil nitrogen and phosphorus availability under different levels of mineral fertilization after harvesting (average of two seasons).

Treatment		Available soil N (ppm)		Treatment		Available soil p (ppm)	
		Nubaria	Gemmeiza			Nubaria	Gemmeiza
Uninoculated	N_0	21.74 e	28.83 e	Uninoculated	P_0	2.52 d	9.14 c
	N_1	33.52 d	46.71 d		P_1	3.19 d	10.26 c
	N_2	44.31 c	57.68 c		P_2	4.42 c	11.58 b
	N_3	60.27 b	79.56 a		P_3	6.21 b	12.41 b
Inoculated	N_0	23.51 e	29.74 e	Inoculated	P_0	3.14 d	11.82 b
	N_1	36.18 d	43.26 d		P_1	3.97 c	13.91 b
	N_2	49.82 c	69.72 b		P_2	5.91 b	15.68 a
	N_3	68.31 a	88.34 a		P_3	8.68 a	16.94 a
Inoculation	Un-	39.96 a	53.19 b	Inoculation	Un-	4.09 b	11.08 b
	In+	44.46 a	57.77 a		In+	5.08 a	14.59 a

In conclusion, soil and seed inoculation might be recommended as a soil cultivation management to increase the microorganisms population especially in low fertile soils in newly reclaimed highly calcareous soils such as El-Nubaria soils.

REFERENCES

Arnou, D.I. (1953). In: Soil and Fertilizer Phosphorus in Crop Nutrition (IV). Ed. W.H. Pierse, Noramn, A.G. Acad. Press, NY.

Banik, S. and B.K. Dey (1981). Phosphate solubilizing microorganisms of a lateritic soil. III. Effect of inoculation of some tricalcium phosphate-solubilizing microorganisms on available content of rhizosphere soils of rice plants and their uptake of phosphorus. Zeitralbl Bakteriol Abt, 136: 493-501.

Bonner, J. (1961). On the mechanics of auxin-induced growth. In: Plant Growth Regulation. Fourth International Conference, The Iowa State University Press, Ames, Iowa, USA, pp. 307.

Chapman, H.D. and P.F. Pratt (1961). Methods of Analysis for Soils, Plants and Water. University of California, Division of Agricultural Science.

Costat Software (1985). User's Manual, Version 3, Co. Hort., Tusson, Arizona, USA.

El-Attar, H.A.; Kareman M. Fawaz; A.S. Abdel-Ghaffar and Nabila Hassan (1979). A study of solubilization of mineral phosphate by soil microorganisms. S. Soc. Appl. Microbiol., Ann. Meet., Cairo, 249.

El-Gibaly, M.H.; F.M. El-Reweiny; M. Abdel-Nasser and T.A. El-Dahtory (1977). Studies on phosphate-solubilizing bacteria in soil and rhizosphere of different plants. 1. Occurrence of bacteria, acid producers and phosphate dissolves. Zeitralbl Bakteriol Abt, 132: 233-239.

FAO (1980). Soil and plant as a basis of fertilizer recommendations. Soils Bulletin No. 38 (2), FAO, Rome.

Fawaz, K.M.; H.A. El-Attar; A.S. Abdel-Ghaffar and Nabila A. Hassan (1980). Inoculation of Egyptian soils by phosphate dissolving microorganisms. Alex. J. Agric. Res., 28: 787.

Gerhardt, P. (1981). Manual of Methods for General Bacteriology. American Society of Microbiology, Washington, DC.

Hirte, W. (1961). Glyzerin-pepton-agar, ein vorteilhafter nahrboden fur bodenbakteriologische arbeiten Zbi. Bakteriology, 114: 141-146.

Holt, J.G. and N.R. Krieg (1984). Bergey's Manual of Systematic Bacteriology. Wiliams and Wilkins, Baltimore.

Kucey, R.M.N. (1987). Increased phosphorus uptake by wheat and beans inoculated with a phosphorus solubilizing *Penicillum bilagi* strain and with vesicular arbuscular mycorrhizal fungi. Appl. Environ. Microbiol., 52: 2699-2703.

Leyval, C. and J. Berthelin (1989). Interactions between *Laccaria laccata*, *Agrobacterium radiobacter* and beech roots: influence on P, K, Mg and Fe mobilization from minerals and plant growth. Plant and Soil, 117: 103-110.

Mishustin, E.N. (1970). The importance of non-symbiotic nitrogen fixing microorganisms in agricultural soil. Plant and Soil, 32: 545-554.

Raced, A. (1994). Phosphorus use efficiency in soils of Pakistan. Proc. 4th National Cong. Soil Sci., Islamabad, pp. 115-127.

Snedecor, G.W. and W.G. Cochran (1981). Statistical Methods. 7th Ed., Iowa State Univ. Press, Ames, Iowa, U.S.A, pp. 305-310.

تأثير الأزوتوباكتر كروكوكم والبكتيريا المذيبة للفوسفات على نمو وإنتاجية القمح وصلاحية العناصر

الحسينى ابراهيم المداح* ، منصور الدسوقى السودانى* ، عصام عبدالرحمن قريش**
* معهد بحوث الأراضى والمياه والبيئة – مركز البحوث الزراعية – الجيزة – مصر.
** قسم علوم الأراضى والمياه – كلية الزراعة (الشاطبى) – جامعة الاسكندرية – مصر.

أجريت تجربتين حقليتين على نبات القمح خلال الموسمين الشتويين ٢٠٠٢/٢٠٠٣ و ٢٠٠٣/٢٠٠٤ بالمزرعة البحثية لمحطة البحوث الزراعية بالنوبارية (أرض جيرية) والمزرعة البحثية لمحطة البحوث الزراعية بالجميزة بغرض تقييم الملقحين الحيويين الأزوتوباكتر كروكوكم والبكتريا المذيبة للفوسفات تحت مستويات من التسميد الأزوتى والتسميد الفوسفاتى على نمو وإنتاجية القمح ومدى قدرتهما على إمداد النباتات بعنصرى النيتروجين والفوسفور وصلاحيتهما بالتربة. وقد أظهرت النتائج الآتى:

- أدى التلقيح بكل من الملقحين وتحت المستويات المختلفة من التسميد المعدنى إلى زيادة معنوية فى كل من الوزن الجاف وطول النباتات مقارنة بالنباتات الغير ملقحه لكل من موقعى التجربة.
- زاد محتوى النباتات من عنصر النيتروجين وامتصاص النبات له زيادة معنوية مع التلقيح بالأزوتوباكتر كروكوكم والتسميد الأزوتى مقارنة بالنباتات الغير ملقحة بموقعى التجربة وكانت النتائج أكثر وضوحا بالجميزة عنها بالنوبارية.
- زاد محتوى النباتات من عنصر الفوسفور وامتصاص النبات له زيادة معنوية مع التلقيح باليكتريا المذيبة للفوسفات والتسميد الفوسفاتى مقارنة بالنباتات الغير ملقحة بموقعى التجربة وكانت النتائج أكثر وضوحا بالنوبارية عنها بالجميزة.
- أقل قيم لمحتوى النباتات من النيتروجين أو الفوسفور وامتصاص النباتات لهما كانت بالنباتات الغير ملقحة وتحت المستويات المنخفضة من التسميد المعدنى.
- زاد محصول القمح ومكوناته زيادة معنوية مع التلقيح والتسميد المعدنى مقارنة بالنباتات الغير ملقحة لكل من الملقحين وبموقعى التجربة.
- بلغ محصول الحبوب ٢٤٣٢ و ٢٦٧٠ كيلوجرام/فدان عند مستوى ١٠٠ وحدة أزوت مع عدم التلقيح بينما بلغ ٢٣٧٩ و ٢٥١٠ كيلوجرام/فدان عند مستوى ٨٠ وحدة أزوت مع التلقيح بالأزوتوباكتر كروكوكم لموقعى التجربة على الترتيب دون وجود فارق معنوى بينهما.
- بلغ محصول الحبوب ٢٦٨٨ و ٢٥٩٥ كيلوجرام/فدان عند مستوى ٣٠ وحدة فو٢أ٥ مع عدم التلقيح بينما بلغ ٢٦٧٨ و ٢٦٣٦ كيلوجرام/فدان عند مستوى ٢٠ وحدة فو٢أ٥ مع التلقيح بالبكتيريا المذيبة للفوسفات لموقعى التجربة على الترتيب دون وجود فارق معنوى بينهما.
- أوضحت النتائج أن المحصول البيولوجى للقمح ومحصول القش له نفس إتجاه النتائج السابقة لكل من الملقحين وموقعى التجربة.

مما سبق وبصفة عامة تحت ظروف التجربة فإن كل من الملقحين لهما القدرة على إمداد النباتات بعنصرى النيتروجين والفوسفور مع زيادة صلاحيتهما بالتربة مما كان له المردود الإيجابى على نمو النباتات وزيادة المحصول تحت مستويات أقل من التسميد المعدنى (٨٠ وحدة أزوت، ٢٠ وحدة فوسفور للفدان) مقارنة بالنباتات الغير ملقحة والتى يلزم تسميدها بمعدلات ١٠٠ وحدة أزوت و٣٠ وحدة فوسفور للفدان للحصول على نفس المحصول دون وجود فارق معنوى بينهما.