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EFFECT OF PHOSPHORUS FERTILIZATION AND GRAINS INOCULATION WITH PHOSPHATE DISSOLVING BACTERIA ON MICROBIOLOGY OF RHIZOSPHERE, WHEAT YIELD AND YIELD COMPONENTS

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ABSTRACT

Two field experiments were carried out at Sakha Agricultural Research Station In this investigation inoculated wheat grain cultivars Sakha 63, *Triticum aestivum* L., were cultivated in clayey soils during the two successive seasons of 1998/1999 and 1999/2000. The inoculation was done using phosphate dissolving bacterial strains namely *Bacillus megatherium var.phosphaticum* to study the effect of inoculation with different levels of superphosphate on microbiology of wheat rhizospherre, NPK uptake, and the plant growth as well as yield and yield components.

Obtained data showed increase in five bacterial groups examined in wheat rhizosphere. These groups are P-dissolving bacteria, total viable bacteria, *Rhizobium spp., Azotobacter spp. and Azospirillum spp.*

Regarding the growth characters, the values of plant height and number of tillers were significantly increased as results of bacterial inoculation as well as P fertilization.

For yield and yield components, the obtained values of the spike weight, 1000-grain weight, grain yield and straw yield also increased as a result of bacterial inoculation with P-dissolving bacteria. After the harvesting, the plant analysis showed increases in the content of nitrogen, phosphorus and potassium either in grains or in wheat straw as well.

This can also take part in reducing the pollution of the soil from the chemicals added every year. Furthermore, this also increased the yield of wheat and the nutritive value as well.

INTRODUCTION

Wheat is the most important food crop for many of the world population especially in the developing countries, such as Egypt. There are several factors that affect the production of wheat plant and the most important one of these factors is the fertilization. The intensive use of expensive mineral fertilizers in recent years, which results in environmental pollution problems has focused the attention of research on the possibility of using biofertilizers as an alternative or complementary for mineral fertilization (Raju *et al.*, 1997). Besides the other plant essential nutrients, phosphorus comes next to nitrogen as a vital nutrient for plants and microorganisms. The inorganic forms of the element in soil are compounds of calcium, iron, aluminum and fluorine. The organic forms are compounds of phytins, phospholipids and nucleic acids, which come mainly by way of decaying vegetation (Subba-Rao, 1988). Besides the other important functions of P in plants, it plays as an agent of energy transfer and deficiency of available P is more likely to limit crop production than any other material except water. Recently, emphasis has been paid to the possibility of great utilization of unavailable P-forms such as rock-phosphate by the action of P-solubilizing microorganisms. Published data demonstrated that the significant increases in the yield of different crops were possible when inoculated with P-dissolvers (El-Faramawi, 1994). Biofertilizers denote preparations containing living micro-organisms, such as bacteria; *Rhizobia, Azotobacter, Azospirillum* and phosphate dissolvers, such as; *Bacillus megatherium*. These microorganisms can improve the soil fertility by changing unavailable sources of atmospheric nitrogen and soil phosphorus into available form for growing crops. Bio-fertilizers are considered to be cheap way to recycle the elements to conserve natural resources and to act as protection factor against increasing pollution due to the extensive use of mineral fertilizers (Zaghloul *et al.*, 1996 and Neeru *et al.*, 2000). In the developed countries, chemically produced water-soluble P fertilizers are routinely applied to crops. The P in these fertilizers is initially available for plant use, then rapidly reacts with soil components and becomes progressively less available for plant uptake. In the developing countries, where chemical fertilizers are not available or are too expensive, ground rock P offers a less costly option (Nahas, 1996). This form of P is much less available to plants than standard fertilizers, except in acidic soils. Many soil microorganisms are able to solubilize otherwise unavailable forms of bound P. These organisms are found in most soils but the numbers and types vary from soil to soil. The ability of microorganisms to solubilize P in soil, and make it available for plant use was demonstrated. In the 50 years since this discovery, researchers world-wide have worked to isolate P-solubilizing organisms, study their characteristics, and use them in inoculants to make P more readily available in commercial agriculture (Krishan *et al.*, 1996 and Singh and Kapoor, 1999).

This work was carried out to examine the inoculation of wheat grains with *Bacillus megatherium* var. *phosphaticum* together with different level of superphosphate on microbiology of wheat rhizosphere. The yield and yield components, wheat growth and NPK uptake were also undertaken in this investigation.

MATERIALS AND METHODS

This investigation was carried out at Sakha Agricultural Research Station. In this investigation, wheat grains cultivars Sakha 69 were used. Field experiments were conducted during the two successive seasons of 1998/1999 and 1999/2000.

Soil samples:

Soil samples of experimental fields were collected from Sakha Agricultural Research Station before setting up the trial. Twenty surface samples were taken at ten different locations at 0-20 cm depth. Mixed sample was obtained and saved in plastic bags. Mechanical analysis was carried out using the method described by Piper (1950). The organic carbon content of soil samples was determined according to Jackson (1958). The soluble cations and anions were determined according the method described by Richards (1954). The electrical conductivity was determined according to the method described by Richards (1954). Their analysis either physical or chemical are presented in Table (1).

Table (1): Some physical and chemical properties of the experimental clayey soil during 1998/1999 and 1999/2000 seasons

Soil properties	Season of cultivation	
	1st (1998/1999)	2nd (1999/2000)
Clay (%)	49.19	49.95
Silt (%)	25.74	24.95
Sand (%)	24.28	23.92
$CaCO_3$ (%)	2.10	2.15
Organic matter (%)	0.96	1.05
pH (1:2.5 soil water suspension)	8.10	8.00
EC dSm^{-1} (Soil paste extract at 25°C)	2.10	2 30
Total phosphorus (ppm)	1200	1250
Avaialble macronutrients (ppm):		
N	22.00	35.00
P	8.30	8.50
K	825.5	914.50
Avalaible micronutrients (ppm):		
Zn	0.98	1.10
Fe	7.85	8.30
Mn	7.50	8.20
Soluble cations meq/liter:		
Ca^{++}	5.55	5.35
Mg^{++}	4.63	4.00
Na^{+}	10.55	11.11
K^{+}	0.40	0.36
Soluble anions meq/liter:		
$CO^{=}_3$	–	–
HCO^{-}_3	2.50	2.00
Cl^{-}	10.86	11.17
$SO^{=}_4$	7.77	7.65

Bacterial strain:

An active phosphate dissolving bacterial strain namely *Bacillus megatherium* var. *phosphaticum* was provided from Microbiology Department, Agric. Res. Center, Ministry of Agriculture, Egypt. This strain was maintained on nutrient agar slant at 5°C till used. Strain of *Bacillus megatherium* var. *phosphaticum* was grown on a liquid medium of modified Bunt and Rovira (Abdel-Hafez, 1966) using 250 ml Erlenmeyer flasks containing 100 ml of the medium. The flasks were incubated at 28°C for 7 days. The bacterial growth was washed and suspended in a physiological mineral solution. Grains of wheat plants were washed and soaked for 30 minutes in the physiological mineral solution contained about 10^6 cells/ml. An adhesive agent Arabic gum was used to obtain coated grains. The coated grains were then air dried in the shade for 30 minutes and sown immediately.

The two field experiments. were designed in a split split design. The arrangement of the field experiment involves two factors. The first is the microbial inoculation with phosphate dissolving bacteria as main plots, two treatments were conducted for wheat included uninoculated and inoculated one. The second factor is P-fertilization using superphosphate (15.5% P_2O_5) included five treatments, i.e., 0, 5, 10, 15 and 20 kg P_2O_5/fed. The treatments were replicated 4 times,and the plot area was 3 x 3 = 9 m^2.

Wheat grains were sown on 18th November, 1998 in the 1st season and on 21st November, 1999 in the 2nd season. All treatments received nitrogen fertilizer (Ammonium nitrate 33% N) at the rate of 75 kg N/fed in 3 doses, the 1st dose represented 20% of nitrogen applied rate and added during sowing as activator dose, the 2nd dose represented 40% of the nitrogen applied rate and applied before the 1st irrigation (3 weeks from planting) and 3rd dose was 40% of the nitrogen applied rate which broadcasted before the 2nd irrigation (3 weeks from the 1st irrigation). Phosphorus fertilizer was added in one dose before the 1st irrigation.

Harvesting was in 30th May, 1999 and 5th June, 2000 in the 1st and 2nd season, respectively.

Before harvesting, 10 plants from wheat were randomly collected, put in paper bags, then immediately carried to the laboratory. These plants were then used to study the behaviour of plant growth as affected by addition of P_2O_5 and bacterial inoculation with phosphate dissolving bacteria as well. The subjected examination were plant height (cm), and number of tillers.

Yield and yield components:

The grain yield of wheat was determined by threshing the harvested plants and collecting the grains of the area of 9 m^2 (plot), separated from straw, weighed and determined per plot and the obtained results were expressed as ton/fed. Straw yield was obtained by subtracting the values of grain yield from the values of total yield of accumulated weight (grain + straw). The obtained results were expressed as ton/fed.

Plant analysis:

For determination of nitrogen content, samples of shoots after 60 days from planting, grain, as well as straw at maturity from treatments were ground in a mill, portions of each grain and straw (0.2 g) were digested according to Chapman and Pratt (1963). The digested samples were distilled by micro-kjeldahl procedure. The nitrogen content (%) of distillate was determined by titration according to Black *et al.* (1965). Phosphorus content in the digested samples was colorimetrically measured according to the method described by Snell and Snell (1967). Potassium content was measured by the method described in AOAC (1980) using the Perkin Elmer flame photometer.

Microbiological measurements:

The count of total viable content was determined in rhizosphere soil samples according to Vincent (1970) using the soil extract agar medium

(Allen, 1959). By using Bunt and Rovira medium modified by Abdel-Hafez (1966), phosphate dissolving bacteria were counted by the decimal plate count technique. Symbiotic N-fixers expressed as *Rhizobium spp.* were determined by yeast extract-mannitol agar (YMA) medium (Allen, 1959). Non-symbiotic N-fixers experiment as both *Azotobacter spp.* and *Azospirillum spp.* by using the technique of most probable number (MPN) as described by Vincent (1970), the total number of *Azotobacter spp.* were counted in rhizosphere samples. The medium of Ashby modified by Abdel-Malek and Ishac (1968) was used. The positive tubes were distinguished by the presence of the pellicle and by examining stained preparation. By using the semi-solid malate medium of D bereiner (1978), the total number of viable *Azospirillum spp.* were counted in rhizosphere samples. The technique of most probable number (MPN) described by Vincent (1970) was used. The number of viable bacteria were calculated using Cochran's tables (Cochran, 1950) and related to oven dry weight sample.

Statistical analysis:

The obtained results were subjected for statistical analysis according to the procedure outlined by Gomes and Gomes (1984).

RESULTS AND DISCUSSION

Microbiological value of wheat rhizosphere:

Data in Table (2) clearly show that the counts of P-dissolving bacteria in rhizosphere of wheat plant increased with time to reach the maximum values after the 2nd month of sowing under all phosphatic and inoculation treatments.

Table (2): Changes in counts of phosphate dissolving bacteria in rhizosphere of wheat as affected by different rates of P_2O_5 and P-dissolving bacterial inoculation.

P_2O_5 (kg/fed)	Uninoculated					Inoculated				
	Months				Mean	Months				Mean
	1	2	3	4		1	2	3	4	
	P-dissolving bacteria x 10^3/cells/g soil					P-dissolving bacteria x 10^4/cells/gv				
0	30	55	35	30	37.50	30	65	45	30	42.5
5	30	60	40	31	40.25	30	70	45	31	44.0
10	30	62	43	31	41.50	30	75	50	32	46.8
15	31	65	45	32	43.25	30	80	52	33	48.8
20	32	70	50	33	46.25	33	85	55	35	52.0
Mean	30.6	62.4	42.6	31 4	41.75	30.6	75.0	49.4	32.2	46.8
	P	T	I							
F test	**	**	**							
LSD 0.05	22	7.57	9.85							
0.01	30	10.3	10.63							

The inoculation with P-dissolving bacteria high significantly increased the count of bacteria, under all P fertilization treatment compared to uninoculated ones. The highest mean count value is 75.0 x 10^4 cfu/g soil under the inoculated treatment compared to 62.4 x 10^2 cfu/g soil for uninoculated treatment.

P-fertilization high significantly affected the count of P-dissolving bacteria where the count increased with increasing the level of added P. Under the inoculated treatment the mean count of P-dissolving bacterial increased from 37.5 x 10^2 cfu/g soil (control) to 40.25, 41.50, 43.25 and 46.25 x 10^2 cfu/g soil as a result of applying 5, 10, 15 and 20 kg P_2O_5/fed, respectively. In the case of inoculated treatment the mean count of P-dissolving bacteria increased from 42.5 x 10^4 cfu/g soil (control) to 44.0, 46.8, 48.8 and 52.0 x 10^4 cfu/g soil for the above mentioned P level, respectively.

Data in Table (2) show also that the inoculation gave several fold increases in the count of P-dissolving bacteria under P-applied levels compared to uninoculation treatments. For example after 2 months from sowing, the count of P-dissolving bacteria was 85.0 x 10^4 cfu/g soil for the treatment of inoculation and 20 kg P_2O_5/fed compared to 70.0 x 10^5 cfu/g soil for uninoculated + 20 kg P_2O_5/fed treatment, the increase represents 121.4 fold.

The count of P-dissolving bacteria dropped sharply after the 3rd and 4th month from planting. The count after the 4th month is nearly equal the count after the 1st month which means that the increase in the count of P-dissolving bacteria is related to the activity of plant growth.

Data in Table (3) showed that the count of total viable bacteria in the rhizosphere of wheat plant which took the trend of P-dissolving bacteria. Raising the rate of P_2O_5/fed accompanied with an increase in the count of total viable bacteria but the increase under the inoculation is higher than the increase under the uninoculation state. The mean count increased from 37.5 x 10^4 cfu/g soil to 43.5 x 10^4 cfu/g soil under uninoculated treatment, while the mean increase was from 38.8 x 10^5 cfu/soil to 45.0 x 10^5 cfu/g soil under inoculated treatment due to raising the rate of P_2O_5 from zero to 20 kg/fed. Maximum increase was attained after 2 months from sowing. The count after 2 months and with 20 kg P_2O_5/fed was to 65.0 x 10^4 and to 70.0 x 10^5 cfu/g soil for uninoculated and inoculated treatment.

As shown in Table (4), data proved the proliferation of the count of *Rhizobium spp.* in wheat rhizosphere with the inoculation with P-dissolving bacteria. In case of uninoculation, the count reach its maximum value after the 2nd month of planting with 20 kg of P_2O_5/fed. This value was found to be 65 x 10^2 cfu/g soil. The counter part number in case of inoculation experiments was 80 x 10^4 under the same conditions. The mean value of *Rhizobium spp.* changed from 26.5 x 10^2 cfu/g without P fertilization to 35.8 x 10^2/g soil fertilized with 20 kg of P_2O_5/fed in case of unioculation. These figures became 41.3 x 10^4 cfu/g and of unfertilized soil which changed to 50.0 x 10^4 cfu/g of 20 kg P_2O_5/fed of fertilized soil as can be seen in the same table. The increasing of such bacteria in wheat rhizosphere means more N-fixation and increasing the amount of growth promoting substances. Therefore, all parameters of the plant growth and yield would be increased as found by Ahmed (1995).

Table (3): Changes in counts of the total viable bacteria in rhizosphere of wheat plant as affected by different rates of P_2O_5 and P-dissolving bacterial inoculation.

P_2O_5 (kg/fed)	Uninoculated					Inoculated				
	Months				Mean	Months				Mean
	1	2	3	4		1	2	3	4	
	Total viable bacteria x 10^4/cells/g soil					Total viable bacteria x 10^5/cells/g soil				
0	30	55	35	30	37.5	30	60	35	30	38.8
5	30	60	40	30	40.0	30	65	38	32	41.3
10	30	62	43	31	41.5	30	65	40	32	41.8
15	31	63	45	31	42.5	30	68	42	31	42.8
20	32	65	45	32	43.5	33	70	45	32	45.0
Mean	30.6	61.0	41.6	30.8	41.0	30.6	65.6	40.0	31.4	41.9
	P	T	I							
F test	**	**	**							
LSD 0.05	0.1	0.5	0.07							
0.01	0.2	0.7	0.08							

Table (4): Changes in counts of *Rhizobium spp.* in rhizosphere of wheat plant as affected by different rates of P_2O_5 and P-dissolving bacterial inoculation.

P_2O_5 (kg/fed)	Uninoculated					Inoculated				
	Months				Mean	Months				Mean
	1	2	3	4		1	2	3	4	
	Rhizobium spp. x 10^2/cell/g soil					*Rhizobium spp.* x 10^4/cell/g soil				
0	15	50	30	11	26.5	30	65	45	25	41.3
5	15	52	33	12	28.0	30	70	50	30	45.0
10	17	55	35	12	29.8	32	75	50	30	46.8
15	16	60	40	13	32.3	31	78	55	30	48.5
20	18	65	45	15	35.8	31	80	60	30	50.3
Mean	16.2	56.4	36.6	12.6	30.5	30.8	73.6	52.0	29.0	46.4
	P	T	I							
F test	**	**	**							
LSD 0.05	2.23	1.94	7.97							
0.01	3.08	2.64	13.78							

Data recorded in Table (5) reveal that the counts of *Azotobacter spp.* in rhizosphere of wheat increased to reach the maximum value of 83 x 10^3 cfu/g soil fertilized with 15 kg P_2O_5/fed under inoculation condition after the 2nd month. The lowest counts was found to be 10 x 10^2 cfu/g of not fertilized soil under uninoculated treatments after the 1st month. The mean value of *Azotobacter spp.* count was increased from 20.3 x 10^2 cfu/g soil without P fertilization to reach 22.3 x 10^2 cfu/g soil with 20 kg P_2O_5/fed and from 37.0 x 10^3 cfu/g soil without P to 47.0 x 10^3 cfu/g soil with 20 kg P_2O_5/fed in case of uninoculated and inoculated treatments, respectively. This may enhance the N-fixation and consequently encouraged plant growth and nutrient uptake by wheat plants.

Table (5): Changes in counts of *Azotobacter spp.* In rhizosphere of wheat plant as affected by different rates of P_2O_5 and P-dissolving bacterial inoculation.

P_2O_5 (kg/fed)	Uninoculated					Inoculated				
	Months				Mean	Months				Mean
	1	2	3	4		1	2	3	4	
	Azotobacter spp. x 10^2/cells/g soil					*Azotobacter spp.* x 10^3/cells/g soil				
0	10	35	24	12	20.3	21	74	38	15	37 0
5	12	37	25	10	21.0	35	80	50	30	48.8
10	14	37	25	12	22.0	42	81	52	32	51.8
15	12	40	24	10	21.5	40	83	54	33	52.5
20	14	40	25	10	22.3	38	81	51	18	47.0
Mean	12.4	37.8	24.6	10.8	21.4	35.2	79.8	49.0	25.6	47.4
	P	T	I							
F test	**	**	**							
LSD 0.05	2.31	0.21	1.07							
0.01	3.29	0.29	1.48							

The recorded data in Table (6) reveal that the inoculation of wheat grains with *Bacillus megatherium* resulted in an increase in the densities of *Azospirillum spp.* var. *phosphaticum* colonized the rhizosphere region. The mean increases under zero P_2O_5 were found to be from 17.5 x 10^2 cfu/g of uninoculated soil to 34.3 x 10^3 cfu/g of inoculated soil. The maximum value of *Azospirillium spp.* densities was 78 x 10^3 cfu/g soil, which was observed after the 2^nd^ month of inoculation and 10 kg P_2O_5/fed. At the next months, the bacterial counts were sharply dropped and reached their lowest values being 10 x 10^2 cfu/g soil after the 4^th^ month without addition P and uninoculated treatments as shown in Table (10). This results clearly show an interesting relation between phosphate dissolving bacteria and the number of *Azospirillum spp.* in rhizosphere. These results came in harmony with those obtained by Zaghloul *et al.* (1996). They found that the highest count of *Azospirillum* was activated in soil inoculated with *Bacillus megatherium*.

Wheat growth:

Data in Table (7) show the effect of phosphate fertilization level and grain inoculation of wheat plant on some growth parameters in 1998/99 and 1999/2000 seasons in the field experiments. The data reveal that increasing phosphorous (P_2O_5/fed) resulted in high significant increases in plant height and number of tillers / plant under inoculated and uninoculated treatments. Increasing the rate of P_2O_5/fed increased plant height in case of uninoculation to reach the maximum values to be 101.3 and 101.25 cm in 1st and 2nd seasons, respectively. On the other hand, in case of inoculation the increase of P_2O_5 leads to increase the height of wheat plant to the maximum value to be 104.75 cm for both cultivated seasons. This could be attributed to the role of microorganisms by supplying wheat roots with their fixed nitrogen and improving the vegetative growth. The mean values of plant height under phosphate fertilization were 98.50 and 103.60 cm in the 1st season, while they were 99.00 and 103.30 cm in the 2nd season under uninoculated and inoculated treatments, respectively.

Table (6): Changes in counts of *Azospirillum spp.* in rhizosphere of wheat plant as affected by different rates of P_2O_5 and P-dissolving bacterial inoculation.

P_2O_5 (kg/fed)	Uninoculated					Inoculated				
	Months				Mean	Months				Mean
	1	2	3	4		1	2	3	4	
	Azospirillum spp. x 10^2/cell/g soil					*Azospirillum spp.* x 10^3/cell/g soil				
0	10	30	20	10	17.5	19	71	33	14	34.3
5	11	32	22	10	19.0	34	75	42	16	41.8
10	12	35	22	11	19.8	38	78	45	20	45.3
15	12	40	25	10	21.8	35	74	44	20	43 3
20	14	45	30	10	24.8	21	70	30	12	33.3
Mean	11.8	36.4	23.8	10 2	20.6	29.4	73.6	38.8	16.4	39.6
	P	T	I							
F test	NS	**	**							
LSD 0.05	–	1.05	1.18							
0.01	--	1.42	1.45							

Table (7): Effect of phosphorus fertilization and bacterial inoculation with *Bacillus megatherium* var *phosphaticum* on wheat growth.

P_2O_5 (kg/fed)	First season		Second season	
	Plant Height (cm)	No. of tillers / plant	Plant Height(cm)	No. of tillers / plant
	Uninoculated			
0	95.60	3.95	96.180	3.98
5	97.20	3.90	97.75	4.07
10	98.90	4.05	99.25	4.13
15	99.50	4.43	100.63	4.53
20	101.30	4.30	101.25	4.82
Mean	98.50	4.10	99.00	4.30
F test	**	NS	**	**
LSD 0.05	1.36	--	1.73	0.30
0.01	1.55	--	2.93	0.41
	Inoculated			
0	102.40	4.88	102.50	4.50
5	103.00	4.75	102.25	4.95
10	103.75	4.85	103.25	5.05
15	104.00	4.95	103.50	5.08
20	104.75	5.15	104.75	5.60
Mean	103.60	4.90	103.30	5.00
F test	**	**	**	**
LSD 0.05	1.88	0.24	1.50	0.20
0.01	2.18	0.33	2.01	0.27

Concerning the number of tillers / plant, it could be noticed that the same trend with plant height was realized. Under the uninoculated treatments, the number of tillers per plant was significantly increased in 1st season and high significantly increased in the 2nd season, the highest value was achieved with applying 20 kg P_2O_5/fed (4.82 tillers/plant). In case of bacterial inoculation, raising the rate of P_2O_5/fed induced high significantly increases in the number of tillers in both seasons. Maximum values were attained due to applying 20 kg P_2O_5/fed (5.15 and 5.60 tillers/plant, in the 1st and 2nd season, respectively). The increase in plant height may be due to the role of fertilization in stimulation of plant cell division and internode elongation (Peng and Li, 1991). The positive effect of phosphorous may be due to the role of phosphorous in photosynthesis and respiration in addition to its role in cell division and development of meristematic tissues as reported by Ashour (1998).

Inoculation of wheat grains by phosphate soluabilizing bacteria increased the mean values of tillers per plant in both growing seasons. The mean values of tillers / plant were 4.10 and 4.30 in case of uninoculation, while they were 4.90 and 5.00 in case of bacterial inoculation for the first and the second seasons, respectively. This increase may be due to the amount of biological nitrogen fixed by the three different bacterial groups found in plant rhizosphere. This in addition to vitamins and auxin-like growth substances and the considerable amount of ascorbic acid, which usually liberated by rhizosphere microbes. Wheat plant can assimilate these materials and reflected their beneficial effect on all parts of the plant (Hegazy *et al.*, 1995).

Yield and yield components:

Data in Table (8) show the values of spike length (cm), spike weight (g), 1000-grain weight (g), grain and straw yield (ton/fed) as affected by phosphate fertilization levels and grain inoculation by phosphate dissolving bacteria in 1998/99 and 1999/2000 cultivation seasons. In case of the uninoculated treatment, in the first season the data clearly show that increasing phosphate levels significantly increase spike length to reach the maximum length of 9.7 cm when using 20 kg P_2O_5/fed. Spike weight also increased to reach 1.94g when using 20 kg P_2O_5/fed. For the 1000-grain weight, the same trend was observed, so the weight of 1000 grain was 42.35 g under the highest P_2O_5 level (20 kg/fed)..

Similar effect of P_2O_5 was found with the grain yield as well as straw yield in ton/fed. The values were 2.51 and 5.14 ton/fed for grain and straw yield, respectively. Similar trend was found in the 2nd season, where all studied parameters were high significantly increased as the rate of P_2O_5/fed increased giving the highest values of 10.1 cm, 2.02 g, 42.56 g, 2.52 ton/fed and 5.13 ton/fed for spike length, spike weight, 1000-grain weight, grain yield and straw yield, respectively at the highest P_2O_5 rate (20 kg P_2O_5/fed)..

The bacterial inoculation of wheat grain with phosphate dissolving bacteria high significantly increased the spike length, giving the maximum value in the 1st season of 9.88 cm when using 15 kg P_2O_5/fed. The obtained value of the spike weight is 2.34g when using 20 kg of P_2O_5/fed. The other values obtained in the 1st season are 43.41, 5.53 and 5.29 for 1000-grain weight (g), grain yield (ton/fed) and straw yield (ton/fed) by using 20 kg of P_2O_5/fed, respectively. The increase in spike length due to adding P_2O_5 together with *Bacillus megatherium* var. *phosphaticum* leads to increase in cell elongation and cell division. This is due to increase in photosynthesis and nitrogen fixed by bacteria present in wheat rhizosphere. These explanation is in accordance with that of Ishac (1988), Sharief *et al.* (1998) and El-Naggar (1999). Also Singaram and Katharaman (1991) revealed that using *Bacillus megatherium* increased grain and straw yield of maize.

Regarding the values obtained in the 2nd season, it could be seen that using of 20 kg P_2O_5/fed achieved the highest value for each examined parameters as shown in Table (8). These values are 10.30 cm, 2.29g, 43.75g, 2.55 ton/fed and 5.56 ton/fed for spike length, spike weight, 1000-grain weight, grain yield and straw yield, respectively. The increase in straw yield may be mainly due to the increase in plant height and number of tillers per plant.

Table (8): Effect of phosphorus fertilization and bacterial inoculation with *Bacillus megatherium* var. *Phosphaticum* on wheat yield and yield components in field experiments.

P_2O_5 (kg/fed)	First season					Second season				
	Spike Length cm)	Spike Weight (g)	1000-grain weight (g)	Grain yield (ton/ fed)	Straw yield (ton/ fed)	Spike Length(cm)	Spike Weight (g)	1000-grain weight (g)	Grain yield (ton/ fed)	Straw yield (ton/ fed)
	Uninoculated									
0	8.85	1.83	39.86	2 24	4.18	8.97	1.83	40.01	2 24	4.14
5	9.00	1.87	40.61	2.31	4.36	9.10	1.90	40.68	2.31	4.37
10	9 03	1.88	41.11	2.38	4.90	9.15	1.94	41.18	2.38	4.88
15	9.00	1.92	41.94	2.49	5 04	9.80	1.95	42.21	2.49	5.21
20	9.70	1.94	42.35	2.51	5.14	10.10	2.02	42.56	2.52	5.13
Mean	9.12	1.89	41.18	2.38	4.72	9.42	1.93	41.33	2 39	4.74
F test	**	**	**	**	**	**	*	**	**	**
LSD 0.05	0 46	0.09	0.22	0.02	0.26	0.45	0.03	0.20	0.03	0.13
0.01	0.63	0.15	0.29	0.03	0.35	0.64	--	0.28	--	0.18
	Inoculated									
0	8 97	1.90	41.03	2.30	4.32	9.05	1.91	41.01	2.30	4.35
5	9.25	1.94	41.61	2.35	4.49	9.30	1.93	41.69	2 36	4.51
10	9.63	2.06	42.10	2.40	4.95	9.50	2.09	42.28	2.41	5.24
15	9.88	2.16	42.83	2.52	5.28	9.90	2.08	43.15	2.54	5.74
20	9.73	2.34	43.41	2.53	5.29	10.30	2.29	43.75	2.55	5.56
Mean	9.45	2.08	42.19	2.42	4.87	10.35	2.06	42.37	2.43	5.08
F test	**	**	**	**	**	*	*	**	*	**
LSD 0 05	0.18	0.13	0.24	0.01	0.11	0.20	0.17	0.24	0.02	0 10
0.01	0.24	0 21	0.33	0.01	0.14	--	--	0.33	--	0.14
P x I	NS	S	NS	NS	NS	NS	NS	NS	NS	NS

N, P and K content:

Data presented in Table (9) show that the N content of wheat organs increased due to raising the P_2O_5 so that the maximum values in case of uninoculation were obtained when using 20 kg P_2O_5 / fed. These values are 1.93, 2.38; 2.04, 2.29 and 0.380, 0.343% in the 1st and 2nd season for shoots at boating stage, grain and straw at maturity, respectively.

Table (9): Effect of phosphorus fertilization and bacterial inoculation with *Bacillus megatherium* var. *Phosphaticum* on N content (%) of wheat plant.

P_2O_5 (kg/fed)	First season			Second season		
	Boating stage	Grain at maturity	Straw at maturity	Boating stage	Grain at maturity	Straw at maturity
	Uninoculated					
0	1.81	1.86	0.285	1.93	1.99	0.293
5	1.84	1.90	0.298	2.08	2.09	0.305
10	1.87	1.93	0.315	2.24	2.19.	0.323
15	1.89	1.94	0.340	2.29	2.25	0.335
20	1.93	2.04	0.380	2.38	2.29	0.343
Mean	1.87	1.93	0.324	2.18	2.16	0.320
F test	**	**	**	**	**	**
LSD 0.05	0.02	0.07	0.010	0.04	0.037	0.01
0.01	0.03	0.10	0.014	0.06	0.051	0.02
	Inoculated					
0	1.86	1.93	0.298	2.04	2.04	0.305
5	1.92	2.01	0.313	2.18	2.12	0.333
10	1.93	2.11	0.325	2.30	2.25	0.343
15	1.94	2.14	0.345	2.32	2.29	0.368
20	1.97	2.16	0.400	2.42	2.35	0.388
Mean	1.92	2.07	0.336	2.25	2.21	0.347
F test	**	**	**	**	**	**
LSD 0.05	0.02	0.06	0.018	0.04	0.03	0.019
0.01	0.03	0.09	0.017	0.06	0.05	0.021
P x I	NS	NS	NS	S	NS	NS

On the other hand, in case of inoculated treatment, tabulated data prove also that the use of 20 kg P_2O_5 / fed was the best for all tested parts of plants. The obtained values were 1.97, 2.42; 2.16, 2.35 and 0.400, 0.388% for shoots at boating stage, grain and straw at maturity in the 1st and 2nd season, respectively. Furthermore, data in Table (9) show clearly that the inoculation with P-dissolving bacteria magnified the nitrogen content of wheat plant organs as compared with uninoculated treatment under the phosphorus application treatments. For example, the mean values of N% of grain, under uninoculated treatment are 1.93 and 2.16% for the 1st and 2nd season. This values are 2.07 and 2.21% under inoculation treatments.

Data presented in Table (10) clearly show that the increasing of P-fertilization (P_2O_5) levels resulted in significant increases in P-content (%) in wheat shoot at boating stage after 60 days of planting. The contents of phosphorus were also increased in wheat grains and straw at maturity either in case of uninoculated or inoculated treatments. The maximum values of P-contents were noticed under the treatments of 20 kg P_2O_5 / fed either in case of uninoculation or inoculation treatment.

Regarding the data of non inoculated treatments, the values of P% were 0.365, 0.428; 0.350, 0.400 and 0.118, 1.43 in the 1st and 2nd season for wheat shoots at boating stage, grain and straw at maturity, respectively. These values, on the other hand, become 0.398, 0.435; 0.355, 0.418 and

0.125, 0.153 in the 1st and 2nd season for shoots at boating stage, grain and straw at maturity, respectively, when wheat grains were inoculated before planting by P-dissolving bacteria.

The increase of P content in different wheat plant organs due to phosphorus fertilization may be attributed to the increase in soil available P and consequently the high efficiency of the roots in absorbing various nutrients including phosphorus. Neeru *et al.* (2000) reported that the higher levels of phosphorus in soil were associated with increase in N and P content of plant. The observed increase was definitely due to the synergesting effect between the examined bacterial groups in plant rhizosphere These finding could be attributed to that phosphorus dissolving bacteria increased the soluble phosphorus in root zone, so the plant absorb more P under the inoculation treatments. These results are similar to those obtained by Saber *et al.* (1981) and Singh and Kopoor (1999).

Table (10): Effect of phosphorus fertilization and bacterial inoculation with *Bacillus megatherium* var. *Phosphaticum* on P content (%) of wheat plant.

P_2O_5 (kg/fed)	First season			Second season		
	Boating stage	Grain at maturity	Straw at maturity	Boating stage	Grain at maturity	Straw at maturity
	Uninoculated					
0	0.300	0.295	0.095	0.308	0.320	0.105
5	0.310	0.305	0.103	0.325	0.343	0.118
10	0.323	0.320	0.108	0.340	0.350	0.123
15	0.345	0.338	0.115	0.367	0.378	0.128
20	0.365	0.350	0.118	0.428	0.400	0.143
Mean	0.329	0.372	0.108	0.356	0.358	0.123
F test	**	**	**	**	**	**
LSD 0.05	0.012	0.011	0.007	0.015	0.015	0.010
0.01	0.017	0.015	0.010	0.021	0.020	0.014
	Inoculated					
0	0.310	0.305	0.105	0.315	0.338	0.113
5	0.345	0.318	0.108	0.340	0.343	0.125
10	0.353	0.328	0.115	0.345	0.358	0.135
15	0.385	0.343	0.123	0.405	0.395	0.140
20	0.398	0.355	0.125	0.435	0.418	0.153
Mean	0.358	0.329	0.115	0.368	0.370	0.133
F test	**	**	**	**	**	**
LSD 0.05	0.016	0.012	0.008	0.015	0.014	0.009
0.01	0.022	0.017	0.012	0.021	0.020	0.013
P x I	S	NS	NS	NS	NS	NS

Data presented in Table (11) reveal that the phosphorus fertilization used for wheat plant resulted in significant increases of K% content in wheat shoots at booting stage, grain and straw at maturity under uninoculated and inoculated treatments. So the highest K% was achieved when P_2O_5 added at the rate of 20 kg P_2O_5 / fed however the wheat grains before planting inoculated or not but the values of K% was higher under inoculated treatments. The obtained values in case of uninoculated treatment are 2.01, 2.25; 0.755, 0.875 and 1.16, 1.42 in the 1st and 2nd season for shoots at boating stage, grain and straw at maturity, respectively. These values were increased due to inoculation with P-dissolving bacteria to be 2.18, 2.33; 0.823, 0.893 and 1.23, 1.46 in the 1st and 2nd season for shoots at boating stage, grain and straw at maturity, respectively. It is known that increasing soluble P in the root zone well increase the root system, so absorb more nutrients. These results are in agreement with those obtained by Neeru *et al.* (2000).

Table (11): **Effect of phosphorus fertilization and bacterial inoculation with *Bacillus megatherium* var. *Phosphaticum* on K content (%) of wheat plant.**

P_2O_5 (kg/fed)	First season			Second season		
	Boating stage	Grain at maturity	Straw at maturity	Boating Stage	Grain at maturity	Straw at maturity
	Uninoculated					
0	1.82	0.680	0.900	1.99	0.715	1.23
5	1.88	0.698	0.953	2.09	0.752	1.33
10	1.89	0.728	1.01	2.14	0.798	1.35
15	1.92	0.737	1.11	2.19	0.850	1.39
20	2.01	0.755	1.16	2.25	0.875	1.42
Mean	1.90	0.719	1.03	2.13	0.798	1.34
F test	**	**	**	**	**	**
LSD 0.05	0.08	0.027	0.050.07	0.06	0.032	0.020.03
0.01	0.11	0.037		0.09	0.044	
	Inoculated					
0	1.92	0.665	0.982	2.11	0.760	1.28
5	2.06	0.702	1.08	2.18	0.808	1.34
10	2.12	0.750	1.14	2.22	0.838	1.35
15	2.12	0.792	1.18	2.28	0.863	1.42
20	2.18	0.823	1.23	2.33	0.893	1.46
Mean	2.08	0.746	1.12	2.22	0.830	1.37
F test	**	**	**	**	**	**
LSD 0.05	0.08	0.025	0.070.09	0.07	0.027	0.03
0.01	0.11	0.033		0.09	0.038	0.04
P x I	NS	S	NS	NS	NS	NS

Concerning the combination of the three elements, nitrogen, phosphorus and potassium, many researchers in different countries revealed that NPK have active role to increase grain yield and yield components. NPK have also supreme effect on plant growth and hence affect grain yield, yield components and grain quality as well. Nitrogen, phosphorus and potassium are essential for wheat plants as well as they are basic for nucleic acid, NAD, NADP, co-enzymes, ATP, meristemic cells of plants. nucleic proteins and they are share through ATP in active amino acids to protein synthesis.

On the light of the obtained results. it can be, generally, concluded that the inoculating wheat plants *Triticum aestivum* L variety Sakha 69 cultivated in clayey soil with an active bacterial strain as phosphate dissolver namely *Bacillus megatherium* var. *phosphaticum* is of great importance. This lead to significant increases in the biofertility of soil as well as the yield of the plant growth. The biofertility of soil expressed in increasing the number of different bacterial groups in rhizosphere area. These groups are the phosphate dissolving bacteria, the total viable bacteria, *Rhizobium spp., Azotobacter spp.* and *Azospirillum spp.* These groups have active role in releasing phosphorus in addition to the N_2-fixation process and the degradation of the organic materials by the enzymatic systems they have.

This can also take part in reducing the pollution of the soil from the chemicals added every year. Furthermore, this also increased the yield of wheat and the nutritive value as well.

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تأثير التسميد الفوسفاتى وتلقيح حبوب القمح بالبكتيريا المذيبة للفوسفات على ميكروبيولوجيا الريزوسفير ومحصول القمح الناتج ومكوناته

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تم دراسة تأثير تلقيح حبوب القمح (سخا ٦٩) بالبكتيريا المذيبة للفوسفات وذلك على ميكروبيولوجيا ريزوسفير النبات وكذلك على محصول القمح الناتج ومكوناته وذلك خلال التجارب الحقلية.

اجريت تجربتان حقليتان بمحطة بحوث سخا بكفر الشيخ فى اراضى طميية خلال موسمى الزراعة ١٩٩٨ / ١٩٩٩ ، ١٩٩٩/٢٠٠٠ حيث تم زراعة حبوب القمح صنف سخا ٦٩ والتى تم تلقيحها بالبكتيريا المذيبة للفوسفات متمثلة فى ميكروب *Bacillus megatherium var.phosphaticum*

بينت النتائج المتحصل عليها زيادة تعداد البكتيريا المذيبة للفوسفات فى منطقة الريزوسفير مقارنة بالكنترول هذا علاوة على زيادة بعض المجاميع البكتيرية الأخرى مثل التعداد الكلى للبكتيريا الحية وتعداد ميكروبات الريزوبيم وكذلك بكتيريا الأزوتوباكتر والأزوسبيريلم.

وبالنسبة لقياسات النبات فقد إزدادت أطوال النباتات مع التلقيح البكتيرى جنباالى جنب مع زيادة للنبات تحت الدراسة.

دلت القياسات الخاصة بمحصول القمح ومكوناته زيادة ملحوظة فى وزن السنبلة ، وزن ١٠٠٠ حبة علاوة على محصول الحبوب والقش الناتج.

ولقد تم تحليل النبات الناتج كيماويا والتى بينت نتائجه زيادة فى النسبة المئوية لكل من النيتروجين والفسفور والبوتاسيوم لكل من الحبوب والقش وذلك علاوة على زيادة محصول القمح وصلاحية العناصر الغذائية بالتربة. وقد دلت نتائج هذا البحث على أهمية تعظيم إستخدام الأسمدة الحيوية والتى أدت إلى تقليل نسبة إستخدام السماد الكيماوى مما يقلل من تلوث التربة الزراعية علاوة على حل مشكلة الفوسفات المترسبة فى الأراضى المصرية نتيجة إضافة الأسمدة الفوسفاتية سنويا نظرا لطبيعة الأراضى المصرية لكونها أراضى تميل للقلوية ومحتوية على كربونات كالسيوم.