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POSITIVE INFLUENCE OF INOCULATION WITH PHOSPHATE DISSOLVING BACTERIA AND APPLICATION OF PHOSPHORUS ON WHEAT GROWN ON A CALCAREOUS SOIL

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ABSTRACT

A field experiment was conducted at Nubaria Agricultural Research Station Farm on a calcareous soil of 26% $CaCO_3$ to study the positive effect of inoculation with phosphate dissolving bacteria (PDB) and phosphorus application on wheat. There were two factors under study (1) phosphorous fertilization (5 rates, 0-26 kg P/fed.) and (2) bacterial inoculation using phosphate dissolving bacteria (PDB), under a trade name of Phosphorine, (two treatments: with, B_1, and without, B_0). Wheat (*Triticum aestivum* L., var. Sakha 8) was sown in the two winter seasons 1998/99 and 1999/2000. Grain yield of wheat (Sakha 8) increased by 30.4 % (almost one third, 0.67 Mg/fed equals 670 kg/fed) with using A_2B_1 treatment (13 kg P/fed + inoculation). Inoculation with PDB increased grain yield of wheat by 10 % when combined with 13 kg P/fed (A_2B_1 treatment) . This treatment might be recommended as a good P fertilization treatment for the tested wheat variety grown under the same condition of soil. Dry weight of individual plant was increased by 8.3 and 11.7 % in the case of absence P addition and in the presence of inoculation (A_0B_1 treatment) in the first and second seasons, respectively. Weight of plant increased more with P up to A_3 rate (13 kgP/fed) with inoculation. Inoculation with PDB had a little effect on P concentration in plant after 30 days of sowing, but a significant positive effect was noticed after 60 days of sowing. The action of inoculation and activity of phosphorus dissolving bacteria needed time to affect soil reaction, as the beneficial effect of inoculation with PDB on decreasing soil pH increased with time. Available P decreased with time indicating P fixation by soil components and/or utilization of available P by the growing wheat plants. The highest activities of PDB were observed after 60 days of sowing, since the populations of PDB were higher than those after 30 days of sowing and after harvest.

Keywords: Phosphate dissolving bacteria, wheat inoculation, phosphorus application.

INTRODUCTION

A calcareous soil is a soil that has free calcium carbonate ($CaCO_3$) in the profile (Loeppert and Suarez, 1996). It can also be defined as a soil of extractable Ca and Mg levels exceeding the cation exchange capacity (Hagen and Tucker, 1982). The carbonates buffer the pH of most calcareous soils within a range of 7.5 to 8.5 and affect the chemistry and availability of N, P, K, Mg, Mn, Zn, Cu, and Fe (Marschner,1995 and Obreza *et al.*,1993). Calcareous soils cover more than 30% of the earth's surface (Marschner, 1995). In Egypt, the newly reclaimed soils in Nubaria region represent about

900,000 feddans of which 290,000 are calcareous and the rest are sandy soils. Phosphorus is considered the second important fertilizer nutrient and the amount of available P for plant uptake is low even after fertilization with phosphate fertilizer (Olsen, 1973). Chemical water-soluble phosphate fertilizers are routinely applied to crops, and their P rapidly reacts with soil and becomes progressively less available for plant uptake (Kucey, 1989). Calcareous soils have high fixation capacity for applied phosphorus which is reflected in poor crop responses to applied P fertilizer (Bauder *et al*., 1997). Phosphorus fertilizer added to a calcareous soil is subject to a series of chemical reactions with soil components that decrease its solubility and availability to plants (Talibudeen, 1981). When water soluble phosphate fertilizers were added to soil of pH 8.0, reactions forming less soluble compounds occur and P availability decreases (Cope, 1981 and Eghball *et al*, 1990, and Yang and Jacobsen, 1990).

Biological fertilization becomes an important factor to increase the availability of some nutrients such as phosphorus correcting their deficiency in soils. With regard to soil phosphorus, microrhizal fungi and phosphate dissolving bacteria (PDB) also called "phosphorus solubilizing bacteria; PSB" are considered as biological agents that have an important role in increasing the solubility of soil P and enhancing its absorption by plants (Tinker and Sanders, 1975; El-Attar *et al*., 1979; Fawaz *et al*., 1980; FAO, 1995 and Koreish *et al*., 1998). Inoculants of (PDB) are used as bio-fertilizers for increasing the supply of phosphorus to plants in soil. Although many of P-solubilizer organisms have been studied, some of these have been developed into commercial phosphate inoculates: Bacillus megatherium (var. phosphaticum), polymyxa, Pseudomonas striata and Penicillium billaii have been reported as efficient phosphate solubilizing bacteria and were successfully tested on different crops (FAO, 1995). Solubilization of inorganic phosphates in soils is well established and stressed the importance of certain soil microorganisms in supplying the growing plants with available P (Abd El-Azeem ,1998). The role of PDB is important through the release of neutral and alkaline phosphate enzymes that is capable of solubiizing less soluble phosphorus (Chhonkar and Taraeder, 1984). Inoculation of germinating seeds or plant roots by PDB organisms can also modify the amounts and nature of root exudates leading to increased P availability and root growth (Barber and Lynch, 1977; Prikryl and Vancura, 1980 and Kraffczyk *et al*., 1984). Increased yields of wheat and chickpea due to seed inoculation with rhizobium and PDB was attributed to enhancement of plant growth by providing it with more available forms of N and P, respectively (Kundu and Gaur, 1980 and Alagwadi and Gaur, 1988). Inoculation of wheat seeds with Pseudomonas striata or Bacillus polymyxa culture increased the availability of soil P as well as grain yield (Shinde and Patil, 1985). Hashem (1996) inoculated wheat seeds with a mixture of N-fixation bacteria and PDB and obtained considerable increase in grain yield.

The objective of this study was to: investigate the response of wheat plants grown on a calcareous soil to seed inoculation with PDB and phosphorus application.

MATERIALS AND METHODS

Field experiment was conducted at Nubaria Agricultural Research Station Farm. The farm is located at 30° 54' latitude and 29° 30' longitude. Its attitude is 22 meter above sea level. Soil samples from the experimental site were collected for physical and chemical analysis before planting (initial state). Results of the analysis are presented in Table (1). The soil is a calcareous one having 26% $CaCO_3$. A factorial randomized complete block (RCB) design with four replicates was used. The plot area was 28-m^2 (5 X 5.6 m), each plot consisted of 26 rows, 20 cm apart. There were two factors under study (1) mineral phosphate fertilization and (2) bacterial inoculation using PDB inoculate. The following five rates of mineral phosphorus (ordinary calcium super-phosphate 6.5%P) were used : A_0, zero P fertilizer; A_1, 6.5 kg P/ fed (15 kg P_2O_5/fed); A_2, 13 kg P/fed (30 kg P_2O_5/fed); A_3, 19.5 kg P/fed (45 kg P_2O_5/fed); and A_4, 26 kg P/fed (60 kg P_2O_5/fed). Two treatments of PDB inoculation (using P-dissolving bacteria with a trade name of PHOSPHORINE) were used as: B_0, without inoculation, and B_1, inoculation with PHOSPHORINE (*Bacillus megatherium* var. phosphaticum adsorbed on peat powder as a carrier). Treatments were carried out in 4 replicates. Thus the overall number of experimental plots equals 40 (i.e., 5 P-rates x 2 inoculation treatments x 4 replicates).

Table (1): Some physical and chemical properties and fertility status of the studied site.

A. Physical properties of the studied locations

Season	Depth cm	Bulk density gm/cm^3	Sand %	Silt %	Clay %	Texture Class
1998-1999 (location 1)	0-20	1.32	49.43	22.11	28.46	Sandy clay loam
	20-40	1.36	47.42	23.35	28.93	
1999-2000 (location 2)	0-20	1.33	48.26	19.90	31.84	Sandy clay loam
	20-40	1.35	47.65	21.76	30.59	

B. Chemical properties of the studied sites.

Season	Depth cm	$CaCO_3$ %	pH 1:2.5	E.C. dSm^{-1}	Soluble anions me/L				Soluble cations me/L			
					CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}	Ca^{++}	Mg^{++}	Na^+	K^+
1998-1999	0-20	26.33	8.34	2.9	---	8.20	17.3	4.6	7.5	5.2	15.1	2.3
	20-40	27.41	8.48	2.7	---	7.40	16.0	3.8	6.4	4.9	13.8	2.1
1999-2000	0-20	25.88	8.41	3.2	---	10.80	18.2	5.5	9.2	6.6	15.3	2.6
	20-40	26.16	8.41	2.9	---	8.50	16.6	4.1	7.7	5.2	13.9	2.4

C. Soil fertility of the studied sites.

Season	Depth cm	O.M %	Total N %	P mg kg^{-1}	K mg kg^{-1}	DTPA extractable mg kg^{-1}			
						Zn	Fe	Mn	Cu
1998-1999	0-20	0.53	0.031	6.84	126	0.46	5.02	1.63	0.28
	20-40	0.41	0.022	5.12	114	0.43	4.91	1.50	0.26
1999-2000	0-20	0.51	0.33	6.75	119	0.52	4.36	1.74	0.31
	20-40	0.43	0.25	5.55	108	0.47	3.87	1.62	0.27

Cultural Practices:

'Wheat (*Triticum aestivum*, var. Sakha 8) was sown in the two winter seasons 1998/99 and 1999/2000 (in another location within the same site of

1998/99 season) at a rate of 70 kg/fed. During land preparation, mineral P (for treatments receiving P) was added. At sowing time, seeds of wheat (for the inoculated treatments) were washed and inoculated through mixing with PDB inoculant for 30 minutes using arabic gum as an adhesive agent, immediately before sowing. The experiment field was put under flood irrigation.

Nitrogen fertilizer in the form of ammonium nitrate (33.5% N) was added to all plots of the experiment at 100kgN/fed in two equal doses, four and eight weeks from planting. Potassium fertilizer was also added to all plots in the form of potassium sulfate (40% K) at the rate of 25 kg K/fed during land preparation. All other cultivation practices used in the region by wheat farmers were followed.

Soil sampling:

Soil samples were taken from the surface layer (0-25 cm). Three samplings were taken during the growth period after 30, 60 days from sowing and at harvest. Soil samples were air-dried, ground, sieved through a 2-mm sieve and stored for the analysis of pH and available P.

Soil analysis:

Particle size distribution was determined by the hydrometer method, Bulk density was determined using the core technique (Black *et al.,* 1965). Total calcium carbonate was measured by the calcimeter (Black *et al.*, 1965). Soil reaction (pH) was determined in 1:2.5 soil water suspension by a pH meter (Jackson, 1973). Electrical conductivity (EC) and soluble ions were measured in the saturated paste extract (Richards, 1954, and Black *et al* ., 1965). Soil organic matter was determined using the Walkley-Black method (Black *et al.,* 1965). Total nitrogen in soil was determined by the Kjeldahl method (Page *et al.*, 1982). Available phosphorous in soil was extracted with 0.5 M sodium bicarbonate solution of pH 8.5 (Olsen *et al.*, 1954) and measured colorimetrically by the ascorbic acid method of Murphy and Riley (1962) modified by Watanabe and Olsen (1965) and cited by Page *et al.* (1982). Available potassium was extracted by 1.0 M ammonium acetate pH 7.0 and measured using flame-photometer (Jackson, 1973). Available Zn, Fe and Mn was extracted by DTPA (Lindsay and Norvell, 1978) and measured using atomic absorption spectrophotometer.

Plant Analysis:

Plant samples (each sample = whole plant) were taken concurrently with soil samplings, washed firstly with tap water, then with distilled water before oven drying at 70 °C; then stored for analysis. Wet digestion with the mixture of sulfuric acid and hydrogen peroxide was carried out and P was determined by the vanadate-molybdate yellow method (Chapman and Pratt, 1961) using Millton Roy spectrophotometer 21 D.

Bacterial Counts:

Bacterial counting of phosphate dissolving bacteria (PDB) in the rhizosphere of wheat was done after 30, 60 days and after harvest by dilution plate technique according to Taha, *et al.* (1969).

RESULTS AND DISCUSSION

Grain and straw yields of wheat:

The effect of the tested variables (phosphorus rates with and without inoculation with PHOSPHORINE) on wheat grain and straw yields (biological yield) is presented in Table (2). Yield increased with application of fertilizer as well as with inoculation with PDB. The positive effect of PDB is attributable to the release of neutral and alkaline phosphate enzymes capable of solubilizing less soluble phosphorus (Chhonkar and Taraeder, 1984). The maximum increase of 30.4% in grain yield for the average of the two seasons was obtained by A_2B_1 i.e. inoculation with PDB combined with 13 kg P/fed. The similar P treatment with no PDB (A_2B_0) increased grain yield by 20.4 %. Thus PDB gave grain yield gain of 10%. An increase of 21.2 % was obtained from the 19.5 P/fed without inoculation (A_3B_0). Grain yield was increased by 10.2 % with the application of 6.5 kg P/fed without PDB (A_1B_o). Thus increasing P rate over 13 kg /fed had no effect in such a soil of about 7 mg available P/kg.

Inoculation with PDB increased grain yield by 14.7% in plots not receiving P in the first season and 7.7% in the second season. P application minimized the positive effect of PDB. Increasing wheat grain yield in response to increasing phosphorus rates and inoculation with PDB was reported by several researchers (Gaur *et al.*, 1980; Kundu and Gaur, 1980; Gaur, 1985 and Hashem, 1996).

Regarding wheat straw, results show that increasing phosphorus rates and inoculation with (PDB) increased wheat straw yield. Average of the two seasons shows a maximum 35.6% increase in straw yield occurred by application of 19.5 kgP/fed + inoculation with PDB (A_3B_1). There was 26.9 % increase from the addition of 19.5 kgP/fed alone (A_3B_o). Inoculation with PDB without P application gave 8.7 % increase . The magnitude of such effect was lower with increased P rate.

It may be stated that applying 13 kgP/fed combined with PDB inoculation is recommended for Sakha 8 wheat grown on calcareous soils. Prasad *et al.* (1985) reported a maximum wheat yield in response to 26.2kg P/ha (equivalent to 11 kg P/fed). Campbell *et al.* (1995) and Peterson *et al.* (1981) reported highest wheat yields at 20 kg P/ha in a brown silt loam soil.

Phosphorus concentrations in wheat grains and straw:

Phosphorus concentration in wheat gains and straw was positively affected by application of P or inoculation with PDB (Table 3). The highest P concentration in grains (0.688%) was with A_2B_1 treatment, and the highest in straw (0.374%) was with A_3B_1.

Table (2): Wheat grain and straw yields (Mg/fed) as affected by application of phosphorus fertilizer and inoculation with phosphorus dissolving bacteria (PDB) in 1998 and 1999 seasons.

Phosphorus Fertilizer (A) (kg P/fed)	PDB treatment (B)					
	Without PDB (B_0)	With PDB (B_1)	Mean	Without PDB (B_0)	With PDB (B_1)	Mean
	Grain yield (Mg/fed)					
	1998			1999		
A_0	2.160	2.478	2.335	2.224	2.395	2.310
A_1 (6.5kg/fed)	2.465	2.616	2.540	2.363	2.558	2.460
A_2 (13kg/fed)	2.613	2.848	2.730	2.665	2.866	2.765
A_3 (19.5kg/fed)	2.660	2.726	2.593	2.653	2.803	2.728
A_4 (26kg/fed)	2.625	2.664	2.630	2.627	2.759	2.693
Mean	2.510	2.662		2.506	2.676	
LSD $_{0.05}$	A 0.22 B 0.14 A*B ns			A 0.179 B 0.113 A*B ns		
	Straw yield (Mg/fed)					
	1998			1999		
A_0	5.418	6.343	5.880	5.142	5.817	5.479
A_1 (6.5kg/fed)	5.961	5.647	6.004	5.824	6.608	6.216
A_2 (13kg/fed)	6.373	6.786	6.560	6.296	6.829	6.563
A_3 (19.5kg/fed)	6.735	7.490	6.963	6.658	6.832	6.745
A_4 (26kg/fed)	6.612	6.716	6.664	6.566	6.621	6.594
Mean	6.220	6.796		6.097	6.459	
LSD $_{0.05}$	A 0.615 B 0.528 A*B ns			A 0.771 B 0.512 A*B ns		

Mg = 10^6 g (metric ton).

Table (3): Phosphorus concentration in wheat grains and straw as affected by application of phosphorus fertilizer and inoculation with phosphorus dissolving bacteria (PDB) in 1998 and 1999 seasons.

Phosphorus Fertilizer (A) (kg P/fed)	PDB treatment (B)					
	Without PDB (B_0)	With PDB (B_1)	Mean	Without PDB (B_0)	With PDB (B_1)	Mean
	P % in grains					
	1998			1999		
A_0	0.318	0.405	0.362	0.320	0.419	0.370
A_1 (6.5kg/fed)	0.426	0.543	0.485	0.456	0.561	0.509
A_2 (13kg/fed)	0.581	0.672	0.627	0.569	0.663	0.616
A_3 (19.5kg/fed)	0.612	0.615	0.614	0.637	0.642	0.640
A_4 (26kg/fed)	0.554	0.582	0.568	0.580	0.574	0.577
Mean	0.498	0.563		0.512	0.572	
LSD $_{0.05}$	A 0.061 B 0.053 A*B ns			A 0.054 B 0.048 A*B ns		
	P % in straw					
	1998			1999		
A_0	0.165	0.214	0.190	0.173	0.222	0.198
A_1 (6.5kg/fed)	0.229	0.286	0.258	0.251	0.304	0.278
A_2 (13kg/fed)	0.335	0.348	0.342	0.366	0.369	0.373
A_3 (19.5kg/fed)	0.357	0.365	0.360	0.370	0.383	0.377
A_4 (26kg/fed)	0.316	0.327	0.322	0.348	0.317	0.333
Mean	0.280	0.307		0.302	0.321	
LSD $_{0.05}$	A 0.112 B ns A*B ns			A 0.101 B ns A*B ns		

Dry weight of plant (after 30 and 60 days of sowing):

Results of dry weight after 30 and 60 days from sowing as affected by phosphate application and PDB inoculation are presented in Table (4). Thirty days after sowing, P application significantly increased plant weight, but inoculation with PDB showed a slight insignificant increase. Average values of the two seasons for the main effect of P addition were 0.626, 0.660, 0.719, 0.786 and 0.749 g/plant for the A_0 , A_1, A_2, A_3 and A_4, treatments, respectively. Increases over 13 kgP/fed (i.e. > A_2) were not significant. Thus increasing P level to 13 kgP/fed or more gave no significant increase in wheat growth at this stage. Increasing the rate from 19.5 to 26 kgP/fed suppressed plant growth slightly, this occurred in both seasons. High levels of P were reported to have a negative effect on formation of hair in plant roots (Romer *et al.*, 1988; Gahoonia and Nielsen, 1997 and Gahoonia *et al.*, 1999). Average values for the main effect of PDB were 0.693 and 0.723 for B_0 and B_1, respectively indicating an insignificant 4 % increase. This slight increase was probably due to a low number and poor activity of PDB in the soil during the first 30 days following inoculation

Sixty days after sowing there was an increase in plant weight due to P application as well as to PDB inoculation. Average weights for the main effect of P in the two seasons were 2.656, 2.895, 3.042, 3.345 and 3.193 g/plant for the A_0, A_1, A_2, A_3 and A_4 treatments, respectively. The average weights of the seasons for the inoculation treatments were 3.036 and 3.338 g/plant for B_0 and B_1, respectively. At this stage the effect was significant (10 %) . The markedly positive effect of inoculation after 60 days as compared with 30 days indicates that this period was sufficient to show the activity of the bacteria. Growth promoting substances such as auxine, gibberellins and cytokinins were reported to have been produced by the PDB (Brown, 1972 and Barea and Brown, 1974). Kundu and Gaur, (1980) reported that such bacteria improve plant growth, due to solubilizing insoluble phosphate as well as to producing growth promotion substances. The no significant effect of PDB after 30 days of sowing, and a significant effect after 60 days reflect a necessity for a time period for PDB to exert their full effect. Dry weight after 60 days increased by 8.3 and 11.7 % under conditions of no P application in season 1 and 2, respectively. Dry weight increased more under P application. Significant differences between with and without PDB after 60 days were observed in the two growing seasons. Such significant differences occurred with the P rates up to 13 kg P/fed. Using the high P rates of 19.5 and 26.0 kg P/fed minimized the positive effect of the inoculation on dry weight of plant.

Laheurte and Bertheline (1988) reported that the effect of bacterial inoculation on plant growth depended on the amount of P available to plant, since plant growth was promoted by inoculation when P was 5 to 15mg/L in the solution of soluble P in the medium, around plant roots. They also added that, the bacteria might increase plant growth by producing plant growth hormone-like substances.

Table (4): Dry weight of wheat (g/plant) after 30 and 60 days of sowing as affected by application of phosphorus fertilizer and inoculation with phosphorus dissolving bacteria (PDB) in 1998 and 1999 seasons.

Phosphorus Fertilizer (A)	PDB treatment (B)					
(kg P/fed)	Without PDB (B_0)	With PDB (B_1)	Mean	Without PDB (B_0)	With PDB (B_1)	Mean
	Dry weight of wheat (g/plant) after 30 days of sowing					
	1998			1999		
A_0	0.600	0.644	0.622	0.569	0.688	0.629
A_1 (6.5kg/fed)	0.611	0.652	0.631	0.677	0.702	0.689
A_2 (13kg/fed)	0.696	0.709	0.702	0.727	0.743	0.735
A_3 (19.5kg/fed)	0.773	0.788	0.781	0.789	0.792	0.791
A_4 (26kg/fed)	0.725	0.757	0.741	0.759	0.753	0.756
Mean	0.681	0.710		0.704	0.736	
LSD 0.05	A 0.087	B ns	A*B ns	A 0.037	B ns	A*B ns
	Dry weight of wheat (g/plant) after 60 days of sowing					
	1998			1999		
A_0	2.830	3.066	2.948	2.481	2.772	2.626
A_1 (6.5kg/fed)	3.033	3.341	3.187	2.757	3.149	2.953
A_2 (13kg/fed)	3.18	3.847	3.514	2.903	3.450	3.177
A_3 (19.5kg/fed)	3.556	3.816	3.685	3.133	3.179	3.026
A_4 (26kg/fed)	3.461	3.639	3.550	2.925	3.119	3.002
Mean	3.212	3.542		2.860	3.134	
LSD 0.05	A 0.493	B 0.311	A*B ns	A 0.247	B 0.156	A*B ns

Phosphorus concentration and uptake:

The effect of P and PDB on the percentage of P in wheat plant are presented in Table (5). Phosphorus concentration in plants increased progressively with P application up to the rate of 19.5 kgP/fed, and decreased at 26.0 kg P/fed. There was an increase due to inoculation by PDB. In the first sample (30 days after sowing), P percentage ranged between 0.240 (A_oB_o) and 0.286 (A_3B_1). In the second sample (60 days after sowing), P percentage ranged from 0.133 (A_oB_o) to 0.176 (A_3B_1). Therefore, phosphorus concentrations increased by application of fertilizer P as well as by inoculation with PDB. However, the very high rate of 26.0 kgP/fed proved less efficient than a lower rate of 19.5 kg P/fed.

Inoculation with PDB had no significant effect on P concentration in plant after 30 days of sowing over the two seasons. However, after 60 days of sowing, there was a significant and positive effect of PDB inoculation on P concentration in plant. Phosphorus concentration in plant was positively affected by inoculation and increased in season 1 by 19.1 and 6.7 % with the no P and the high P rate, respectively. In the second season, in presence of PDB, P concentration increased by 11.9 and 4.9% with no P and with the highest dose of P, respectively.

After 60 days of growth P concentrations were positively affected by inoculation particularly under conditions of no P or low P (6.5 kg P/fed). Also, P concentrations after 60 days were lower than those after 30 days of growth. The decrease in P concentration with time may be attributed to the dilution effect since the uptake of P by plants after 60 days was higher than that after 30 days.

Phosphorus uptake depends on P concentration in plant and also on the amount of plant growth and both were positively affected by P-application. Table (6) shows that P uptake after 30 days of sowing increased progressively with increased P up to 13 kgP/fed in season 1 and 2. However, P uptake showed a slight but not significant increase due to PDB inoculation in season 1 and 2. After 60 days of sowing, P uptake was significantly influenced by either PDB inoculation or P application. Thirty days after sowing the average uptake of P (for 2 seasons) ranged between 1.40 mg/plant (A_0B_0) to 2.27 mg/plant (A_3B_1). Sixty days after sowing comparable values were 3.54 and 5.88 mgP/plant for the same two treatments, respectively. The effect of P occurred in presence as well as in absence of PDB. Mean values for PDB treatments were 1.83 and 2.81 mg/plant for B_0 and B_1, respectively, in season 1. Comparable values in season 2 were 4.72 and 5.45 mg/plant, respectively.

Organisms that act on solubilizing inorganic P and making it available to plants are reported to have little effect on P uptake if the level of available P in the soil is high (Banik and Dey, 1982). Kundu and Gaur (1980) reported that the combined positive effect of growth factors and increased nutrient availability caused by the development of bacteria in rhizosphere may account for the positive effect of the P-dissolving bacteria on crop growth and nutrients uptake. Morel and Fardean (1990) showed that an input of fresh P fertilizer increased P uptake in soils poor or medium in available P, but decreased it in soils high in available P.

Soil pH

The effect of P rates and inoculation with PDB on soil pH measured after 30 and 60 days from sowing as well as after harvest is presented in Table (7). Results show that application of P resulted in a significant decrease in soil pH, for both timing dates. This occurred in presence or absence of PDB inoculation. An average pH of 8.15 (over the two seasons and after 30 days of sowing) was shown in the no P treatments compared with an average pH of 7.90 in the P treatments. A trend of a decreased pH was noticed with increased P. Baravalle *et al.* (1995) showed that pH decreased with P addition up to 50mg P/kg.

Inoculation with PDB had a significant effect on soil pH values particularly after 60 days from sowing as well as at harvest. Therefore, the action of inoculation and activity of phosphorus dissolving bacteria needed time to affect soil reaction. During the first 30 days after sowing, there was a low count of phosphate dissolving bacteria (Table 9), thus their activity seemed not enough to affect pH. Phosphate dissolving bacteria have the ability to solubilize insoluble P in soil by producing organic acids that lower the pH values and bring about the dissolution of bound forms of phosphate (Ralston and McBride, 1976 and Boutros *et al.*, 1987).

At harvest, P application decreased pH, and pH values were higher than the corresponding ones after 30 or 60 days of growth. Inoculation with PDB significantly decreased soil pH. Such a decrease may be attributed to the decomposition of soil organic matter via PDB and the subsequent release of organic acids. Also, the inoculation with bacteria can modify amounts and nature of root exudates (Barber and Lynch, 1977; Prikryl and Vancura, 1980 and Laheurte and Bertheline, 1988).

Table (5): Phosphorus concentration in wheat plant after 30 and 60 days of sowing as affected by application of phosphorus fertilizer and inoculation with phosphorus dissolving bacteria (PDB) in 1998 and 1999 seasons.

Phosphorus Fertilizer (A) (kg P/fed)	PDB treatment (B)					
	Without PDB (B_0)	With PDB (B_1)	Mean	Without PDB (B_0)	With PDB (B_1)	Mean
	P % in plant after 30 days of sowing					
	1998			1999		
A_0	0.242	0.261	0.252	0.238	0.250	0.244
A_1 (6.5kg/fed)	0.268	0.267	0.268	0.250	0.264	0.261
A_2 (13kg/fed)	0.273	0.276	0.274	0.268	0.274	0.276
A_3 (19.5kg/fed)	0.278	0.285	0.281	0.274	0.288	0.281
A_4 (26kg/fed)	0.271	0.277	0.274	0.269	0.273	0.271
Mean	0.266	0.270		0.260	0.270	
$LSD_{0.05}$	A 0.014 B ns A*B ns			A 0.012 B ns A*B ns		
	P % in plant after 60 days of sowing					
	1998			1999		
A_0	0.131	0.156	0.144	0.135	0.151	0.143
A_1 (6.5kg/fed)	0.151	0.165	0.158	0.146	0.162	0.154
A_2 (13kg/fed)	0.163	0.171	0.167	0.160	0.168	0.164
A_3 (19.5kg/fed)	0.168	0.175	0.172	0.166	0.177	0.172
A_4 (26kg/fed)	0.165	0.176	0.171	0.163	0.171	1.67
Mean	0.156	0.169		0.154	0.166	
$LSD_{0.05}$	A 0.018 B 0.011 A*B ns			A 0.015 B 0.009 A*B ns		

The measurements of available P values in soil samples collected after 30, 60 days from sowing and after harvest are presented in Table (8). Results show that the contents of available P increased by P application as well as by PDB inoculation. Increased application rates gave increased available P. Thirty days from sowing, the average of the two seasons for the A_0B_0 treatment was 9.3mgP/kg increased to 16.3mgP/kg for the A_4B_0 treatment and 18.2mgP/kg for A_4B_1 treatment. Sixty days after sowing, the averages of available P (over the two seasons) were 7.5, 13.4 and 15.3 mg/kg for A_0B_0, A_4B_0, and A_4B_1, respectively. At harvest, comparable average values were 6.6, 10.7 and 12.1 mg/kg for the same treatments, respectively. This shows that available P decreased with time. Such decrease could be attributed to the P fixation by soil components, i.e., $CaCO_3$ and clay beside the utilization of P by plants. El-Gamal (1996), El-Sayed (1998), Koreish *et al.* (1998) and Mehana and Farag (2000) showed that the PDB increased available phosphorus in soil and decreased soil capacity for P fixation. Shinde and Patil (1985) reported that inoculation of wheat seeds with PDB increased the availability of soil P and resulted in grain yields similar to those obtained with 21 kg P/ha applied as calcium super-phosphate. Availability of added phosphorus was reported to decrease with increasing time after application (Afif *et al.*, 1993 and Castro and Torrent, 1995). It was concluded by Rubeiz *et al.* (1992) that, P availability decreased with time after applying soluble phosphate fertilizers in a calcareous soil; and that available P showed a gradual decrease (from 500mg/kg to 200mg/kg) after each week of inoculation.

Table (6): Phosphorus uptake by wheat plant after 30 and 60 days of sowing as affected by application of phosphorus fertilizer and inoculation with phosphorus dissolving bacteria (PDB) in 1998 and 1999 seasons.

Phosphorus Fertilizer (A) (kg P/fed)	PDB treatment (B)					
	Without PDB (B_0)	With PDB (B_1)	Mean	Without PDB (B_0)	With PDB (B_1)	Mean
	P uptake (mg/plant) after 30 days of sowing					
	1998			1999		
A_0	1.45	1.68	1.56	1.35	1.72	1.54
A_1 (6.5kg/fed)	1.58	1.74	1.66	1.69	1.86	1.77
A_2 (13kg/fed)	1.90	1.96	1.92	1.95	2.04	1.99
A_3 (19.5kg/fed)	2.11	2.25	2.18	2.11	2.28	2.20
A_4 (26kg/fed)	2.02	2.10	2.06	2.08	2.06	2.07
Mean	1.81	1.95		1.84	1.97	
LSD $_{0.05}$	A 0.26 B ns A*B ns			A 0.24 B ns A*B ns		
	P uptake (mg/plant) after 60 days of sowing					
	1998			1999		
A_0	3.72	4.78	4.25	3.36	4.18	3.77
A_1 (6.5kg/fed)	4.57	5.51	5.04	4.04	4.98	4.51
A_2 (13kg/fed)	5.15	6.53	5.84	4.66	4.94	4.80
A_3 (19.5kg/fed)	5.95	6.73	6.34	4.93	5.03	4.98
A_4 (26kg/fed)	5.74	6.40	6.07	5.02	5.34	5.18
Mean	5.03	5.99		4.40	4.90	
LSD $_{0.05}$	A 0.47 B 0.41 A*B ns			A 0.53 B 0.33 A*B ns		

Available phosphorus in soil:

Available soil P was affected to a large extent by PDB inoculation, particularly in the absence of P addition . After 30 days of sowing, available soil P increased by 49.5 and 13.1% with the A_0 and A_4 rates in the first season, while in the second season, comparable values were 44.2 and 10.2%, respectively. A trend similar to that of 30 days of sowing was obtained after 60 days of sowing as well as after harvest. Cavazza and Patruno (1997) reported that fertilizer application rapidly increased available P in soil, and was gradually decreased to approximately one half in a period of about 5 months

Table (7): Soil pH after 30 and 60 days of sowing and after harvest as affected by application of phosphorus fertilizer and inoculation with phosphorus dissolving bacteria (PDB) in 1998 and 1999 seasons.

Phosphorus Fertilizer (A) (kg P/fed)	PDB treatment (B)					
	Without PDB (B_0)	With PDB (B_1)	Mean	Without PDB (B_0)	With PDB (B_1)	Mean
	Soil pH after 30 days					
	1998			1999		
A_0	8.16	8.05	8.11	8.21	8.14	8.18
A_1 (6.5kg/fed)	8.01	7.96	7.98	8.04	7.94	7.99
A_2 (13kg/fed)	7.93	7.85	7.88	7.97	7.91	7.94
A_3 (19.5kg/fed)	7.86	7.85	7.85	7.85	7.80	7.88
A_4 (26kg/fed)	7.87	7.86	7.86	7.82	7.80	7.81
Mean	7.96	7.91		7.98	7.92	
LSD $_{0.05}$	A 0.15	B ns	A*B ns	A 0.13	B ns	A*B ns
	Soil pH after 60 days of sowing					
	1998			1999		
A_0	8.11	7.96	8.04	8.22	8.05	8.13
A_1 (6.5kg/fed)	8.09	7.95	8.02	8.06	7.88	7.97
A_2 (13kg/fed)	7.99	7.91	7.95	8.02	7.78	7.90
A_3 (19.5kg/fed)	7.95	7.78	7.87	7.88	7.89	7.88
A_4 (26kg/fed)	7.85	7.84	7.85	7.92	7.90	7.91
Mean	8.00	7.89		8.02	7.91	
LSD $_{0.05}$	A 0.13	B 0.08	A*B ns	A 0.17	B 0.11	A*B ns
	Soil pH after harvest					
	1998			1999		
A_0	8.21	7.98	8.09	8.19	8.08	8.13
A_1 (6.5kg/fed)	8.13	7.98	8.05	8.14	8.07	8.11
A_2 (13kg/fed)	8.06	8.00	8.03	8.01	7.93	7.97
A_3 (19.5kg/fed)	8.01	7.98	7.99	8.05	7.87	7.96
A_4 (26kg/fed)	8.02	7.89	7.95	8.00	7.89	7.95
Mean	8.08	7.96		8.08	7.97	
LSD $_{0.05}$	A ns	B 0.11	A*B ns	A 0.13	B 0.08	A*B ns

Population counts of PDB in soil:

Population counts of phosphate dissolving bacteria (PDB) after 30 and 60 days of sowing and after harvest are shown in Table (9). Counts of phosphate dissolving bacteria were increased by 3.22 times with inoculation in the absence of P addition after 30 days of inoculation. With A_1, A_2, A_3 and A_4 levels of P, the counts of PDB increased by 3.22, 2.52, 2.59, and 2.70 times, respectively. After 60 days of sowing, the PDB counts increased by 3.63, 3.57, 3.22, 3.31, and 3.82 times with A_0, A_1, A_2, A_3, and A_4 treatments, respectively. At harvest, the PDB counts increased by 4.08, 2.99, 2.57, 2.60, and 2.63 times with A_0, A_1, A_2, A_3, and A_4 treatments as compared to uninoculated treatments, respectively. Also, PDB inoculation showed a considerable increase in PDB population reaching 2.81, 3.63, and 2.85 times (average for all P treatments) at 30 and 60 days after sowing, and at harvest, respectively. It could be noted that the highest activities of PDB were observed after 60 days of sowing as their multiplication was higher than that after 30 days of sowing and after harvest. Ralston and McBride (1976) reported that, inoculation of calcium phosphate dissolving bacteria (PDB); on day 35 after inoculation, increased the number of these microbes in the treated soil. Ocampo *et al*, (1975) and Kundu and Gaur, (1980) reported that bacterial counts were remarkably increased during the 5[th] - 7[th] week following inoculation.

Table (8): Available P in soil after 30 and 60 days of sowing and after harvest as affected by application of phosphorus fertilizer and inoculation with phosphorus dissolving bacteria (PDB) in 1998 and 1999 seasons.

Phosphorus Fertilizer (A) (kg P/fed)	PDB treatment (B)					
	Without PDB (B_0)	With PDB (B_1)	Mean	Without PDB (B_0)	With PDB (B_1)	Mean
	Available P in soil (after 30 days of sowing)					
	1998			1999		
A_0	9.1	13.6	11.3	9.5	13.7	11.6
A_1 (6.5kg/fed)	14.4	16.4	15.4	13.5	15.4	14.4
A_2 (13kg/fed)	16.0	16.7	16.4	16.1	17.7	16.9
A_3 (19.5kg/fed)	15.8	18.1	16.9	15.7	17.6	16.7
A_4 (26kg/fed)	16.0	18.1	17.0	16.6	18.3	17.5
Mean	14.3	16.6		14.3	16.6	
LSD 0.05	A 2.8	B 1.8	A*B ns	A 2.8	B 1.18	A*B ns
	Available P in soil (after 60 days of sowing)					
	1998			1999		
A_0	7.5	12.4	9.9	7.6	11.8	9.7
A_1 (6.5kg/fed)	10.3	13.3	11.8	11.9	13.6	12.8
A_2 (13kg/fed)	11.6	13.7	12.6	12.8	14.4	13.6
A_3 (19.5kg/fed)	13.9	13.8	13.8	13.0	15.0	14.0
A_4 (26kg/fed)	14.0	14.9	14.4	12.8	15.6	14.2
Mean	11.4	13.6		11.6	14.1	
LSD 0.05	A 2.6	B 1.6	A*B ns	A 2.7	B 1.7	A*B ns
	Available P in soil after harvest					
	1998			1999		
A_0	7.1	10.4	8.8	6.0	11.6	8.8
A_1 (6.5kg/fed)	9.8	11.5	10.6	9.9	11.9	10.9
A_2 (13kg/fed)	10.0	11.6	10.8	10.7	12.5	11.6
A_3 (19.5kg/fed)	9.9	11.8	11.0	11.1	11.5	11.7
A_4 (26kg/fed)	10.4	12.1	11.2	11.0	12.0	11.0
Mean	9.5	11.5		9.8	11.9	
LSD 0.05	A 2.0	B 1.2	A*B ns	A 2.2	B 1.4	A*B ns

Table (9): Population counts of phosphate dissolving bacteria (PDB) after 30 and 60 days of sowing and after harvest (average of two seasons, 1998 and 1999).

P treatment (kg P/fed)	PDB population (x 10^3 cells g^{-1} dry soil)						
	30 days		60 days		harvest		
	B_0	B_1	B_0	B_1	B_0	B_1	Mean
A_0	1.76	5.67	2.27	8.25	1.27	5.18	4.07
A_1 (6.5kg/fed)	2.04	6.57	2.83	10.10	2.12	6.35	5.00
A_2 (13kg/fed)	2.72	6.86	3.11	10.04	2.46	6.33	5.59
A_3 (19.5kg/fed)	2.84	7.38	3.82	12.63	2.51	6.52	5.65
A_4 (26kg/fed)	2.61	7.06	2.79	10.67	2.32	6.11	5.26
Mean	2.39	6.71	2.96	10.74	2.14	6.10	5.17

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التأثير الإيجابي للتلقيح بالبكتريا المذيبة للفوسفات وإضافة الفوسفور على القمح المنمى فى الأرض الجيرية

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أجريت تجربة حقلية فى محطة بحوث النوبارية على أرض جيرية بها ٢٦% كربونات كالسيوم لدراسة التأثير الإيجابى للتلقيح بالبكتريا المذيبة للفوسفات وإضافة الفوسفور على القمح . كان هناك عاملين تحت الدراسة الأول هو التسميد الفوسفاتي (A) (٥ معدلات من صفر – ٢٦ كجم فو / فدان) والثانى هو التلقيح البكتيري (B) بإستخدام البكتريا المذيبة للفوسفات " فوسفورين " (معاملتين هما عدم التلقيح B_0 و التلقيح B_1) . تم زراعة القمح صنف سخا ٨ في موسمين هما ١٩٩٨/١٩٩٩ و ١٩٩٩/٢٠٠٠. تزايد محصول الحبوب لصنف سخا ٨ بنسبة ٣٠,٤% (تقريبا الثلث الذى يبلغ ٦٧٠ كجم / فدان) بإستخدام المعاملة A_2B_1 (١٣كجم فو / فدان + التلقيح البكتيرى). أدي التلقيح مع نفس معاملة الفوسفور السابقة إلى زيادة محصول الحبوب بمقدار ١٠%. يجب أن يوصى بهذه المعاملة كأفضل معاملة تسميد فوسفاتي للصنف المختبر عندما ينمي فى نفس الأرض تحت نفس الظروف . تزايد الوزن الجاف للنبات الواحد بنسبة ٨,٣ و ١١,٧% فى حالة غياب الفوسفور المضاف ووجود التلقيح (A_0B_1) فى الموسم الأول والثانى على التوالى بالإضافة إلى ذلك فإن الوزن الجاف تزايد أكثر بإضافة الفوسفور حتي المعدل الثالث (A_3) فى وجود التلقيح . وجد تأثير قليل للتلقيح علي تركيز الفوسفور فى النبات بعد ٣٠ يوم من الزراعة بينما وجد تأثير معنوى موجب للتلقيح على تركيز الفوسفور بعد ٦٠ يوم من الزراعة . يحتاج كل من التلقيح ونشاط البكتريا المذيبة للفوسفات إلى الوقت حتى يؤثران على تفاعل التربة (رقم الـ pH) حيث لوحظ أن التأثير الايجابي للتلقيح على خفض رقم الـ pH يتزايد مع الوقت . تناقص الفوسفور الميسر فى التربة مع الزمن مما يؤكد تثبيت الفوسفور بواسطة مكونات التربة وإستخدام الفوسفور الميسر بواسطة نبات القمح النامى. لوحظت الزيادة العالية فى نشاط البكتريا المذيبة للفوسفات بعد ٦٠ يوم من الزراعة حيث أن أعدادها كانت أعلى من تلك الأعداد المقدرة بعد ٣٠ يوم من الزراعة و بعد الحصاد.

الأهمية :-

لهذا البحث أهمية تطبيقية حيث يمكن أن يوصى بإضافة فوسفات أحادي الكالسيوم بمعدل ١٣ كجم فو/ فدان (٢٠٠كجم سوبر فوسفات) بالإضافة إلى تلقيح حبوب القمح بلقاح البكتريا المذيبة للفوسفات (الفوسفورين) بالمعدل الموصى به وذلك للحصول على زيادة قدرها ٦٧٠ كجم حبوب/ فدان مع ملاحظة إستخدام نفس الصنف و نفس ظروف التربة.