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Effect of Bacterial Inoculation and Spraying with K_2HPO_4 on Egyptian Clover in A Calcareous Soil

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TWO FIELD experiments were conducted in a normal calcareous soil at Noubaria to assess the effects of inoculation with N2-fixing bacteria and /or two doses of K_2HPO_4 on Egyptian clover (*Trifolium alexandrinum*) growth.

In both rials, seeds were inoculated with *Rhizobium leguminosarum* biovar trifolii on sowing day. Then, plants were sprayed three times with some N2-fixing bacteria, *Bacillus polymyxa, Azospirillum brasilense* and *Azotobacter chroococcum*. Plants were sprayed three times with two rates of K_2HPO_4, the first 0.8 kg P and 2 kg K/fed as recommended rate and the second was twice the recommended one. Three cuts were taken after 11, 20 and 30 weeks of planting in the first season and two cuts were taken after 11, 30 weeks of planting the second one.

The obtained data showed that inoculation with N fixing micro - organisms to increase fresh and dry yield of clover significantly over the non inoculated plant. Uptake of N, P and K in all the cuts increased significantly with that treatment which also increased nitrogenase activity in root nodules and dehydrogenase in rhizosphere soil over the significancy limits.

Apart from the applied foliar fertilizer rate, the foliar application of K_2HPO_4 resulted in same trend noticed in yield and N, P and K uptake achieved by bacterial inoculation in most tested treatments. Thus low rate of P and K applications (0.8 kg P / fed nd 2 kg K / fed) were enough to correct both P and K deficiency in such tested calcareous soil.

The combination of both inoculation and K_2HPO_4 foliar application resulted in some interaction effects on those studied parameters and in the other cases, However, either of bacterial inoculation or K_2HPO_4 foliar application played its role individually.

Finally, seed clover inoculation and / or plant foliation with 4.5 kg K_2HPO_4 applied in 3 equal doses may be recommended to obtain the best quantity and quality of clover under such soil condition. Di potassium phosphate did not increase significantly nitrogenase and dehydrogenase activities.

Keywords: Calcareous soil, Egyptian clover (*Trifolium alexandrinum*), *Rhizobium leguminosarum* biovar trifolii, *Bacillus polymyxa, Azospirillum brasilense, Azotobacter chroococcum*, K_2HPO_4.

Egyptian clover is the most important forage winter crop in Egypt. Its cultivated area almost amounts to 1.25 million feddans of the total area around 7.5 million feddans. The new reclaimed area including the sandy and calcareous soils should be associated in producing more forages for animal wealth. Therefore increasing productivity of quality and quantity of clover in such new reclaimed area should be given more attention.

Phosphorus and potassium are important elements for increasing clover productivity. Phosphorus has a role in plant life throw energy storage and transfer. It acts as a linkage unit, has a vital metabolic role and is considered an important structural component of wide varieties of biochemical materials including nucleic acids, coenzymes, phosphoproteins, phospholipid and sugar phosphates (Daniel *et al.,* 1998). Potassium is a mobile element in the plant tissues and it plays an important role in photosynthesis through carbohydrate metabolism, osmotic regulation, nitrogen uptake and translocation of assimilates. Also, it has a role in physiological processes in the plant such as respiration, transpiration, translocation of sugars and carbohydrates and enzyme transformation (Kelling *et al.,*1998). Mengel & Kirkby (1979) suggested for that purpose potassium meta phosphate and potassium phosphate as a complete soluble fertilizer to correct the deficiency of both P and K in soils suffered phosphate fixation or low availability, such as in calcareous soils, which was investigated later to foliate peanut (Sparks, 1986), citrus (Hassaballa *et al.,* 1991) and maize (Hamdia *et al.,* 2000). They reported that potassium phosphate corrected directly P and K deficiencies and indirectly by the nutrition balance in plants and helps plants to tolerate more salt stress.

One of increasing clover productivity factors is inoculation with N_2-fixing bacteria not only for clover productivity but also to enrich soil with more available N (Gray & Williams, 1979 and Mengel & Kirkby, 1979). Inoculation of legume with rhizobia and *Azospirillum brasilense* generally increased root nodules and yields in field experiments (Tilak *et al.,* 1981). The ability of rhizosphere bacteria·to solubilize phosphorus may be important in Egypt, where soil available phosphorus is low. Several workers reported the efficiency of phosphoric fertilizers through solubilizing the fixed forms by acids produced from bacteria (Badr El-Din, 1986). *Bacillus* species have mechanisms other than N_2- fixation such as increasing phosphate availability (Rai *et al.,* 1981), antibiotics which may suppress pathogenic organism in the rhizosphere (Brown, 1974) or plant growth substances (Burns *et al.,* 1981). Holl *et al.* (1988) found that the effect of inoculation of white clover with *Bacillus polymyxa* was highly positive on root and shoot dry weights . When leguminous plants were inoculated with mixed cultures of *Azospirillum and rhizobia* in equal cell ratio, nodules number and seed yields increased (Rai, 1983), as well as a substantial increase in N_2-fixation and shoot dry matter (Iruthayathas *et al.,* 1983 and Plazinski & Rolfe, 1985). El-Sayed (1999) found that combined inoculation of lentil with *Rhizobium leguminosarum* and Pseudomonas striata or *Bacillus polymyxa* increased nodulation and nitrogenase activity, dry matter of plants and their uptake of N and P.

Thus, the purpose of this study was to evaluate the role of Egyptian clover inoculation with some N_2-fixing bacteria and foliation it with different rates of K_2HPO_4 on yields, N, P and K taken up by plants, in addition to some enzyme activities like nitrogenase in the root nodules and dehydrogenase in the soil rhizosphere.

Material and Methods

Two field experiments were carried out at Noubaria Agric. Res. Station Farm where the soil is normaly calcareous one having sandy clay loam as Table 1 shows according to the analytical methods described by Black *et al.* (1965) in the two winter agricultural seasons 2002 / 2003 and 2003 / 2004.

Clover (*Trifolium alexandrinum*) was chosen for this study. In the first trial, seeds were inoculated with Rhizobium leguminosarum biovar trifolii which was carried on peat (1g contains 10^8 cells of bacteria) on the sowing day 15^{th} of October 2002 or not inoculated as main treatments. Treated plots were leaf sprayed with some associative N_2-fixing bacteria, *i.e.*, Azospirillum brasilense, *Azotobacter chroococcum* and *Bacillus polymyxa* which carried on vinasse as a liquid carrier (1ml contains 109 cells of bacteria) three times on the 15^{th} of November, 2003, 8^{th} of January and 12^{th} of March 2003. Three submain treatments were 0, 2 and 4 kg k / fed corresponding to 0, 0.8 and 1.6 kg P / fed respectively as K_2HPO_4 (mono hydrogen di potassium phosphate) sprayed three times on 15^{th} of November, 2003, 8^{th} of January and 12^{th} of March 2003. Three cuts were taken on the 5^{th} January, 9^{th} March and 24^{th} April 2003.

In the second season and in another neigbouring site, clover seeds, which were inoculated with *Rhizobium leguminosarum* biovar trifolii or not, planted on the 19^{th} October 2003 and treated with the same associative N_2- fixing bacteria, *i.e. Azospirillum brasilense, Azotobacter chroococcum* and *Bacillus polymyxa,* which were carried on vinasse as a liquid carrier (1ml contains 10^9 cells of bacteria) for foliar application. The triple times of spray treatments were more near one another than in the previous season on 16^{th} of November, the 22^{nd} of December 2003 and the 4^{th} of February 2004. The same sub treatments of H_2KPO_4 foliar application on the16^{th} of November, the 22^{nd} of December 2003 and the 4^{th} of February 2004. Two cuts were taken on the12^{th} of January and 2^{nd}. of March 2004. Samples of each cut of that season were dried, wet digested according to Sommers & Nelson (1972) and their contents of nitrogen, while phosphorus and potassium were determined according to Chapman & Pratt (1961).

TABLE 1. Physical and chemical analyses of the surface and subsurface soil layers for the two experiments .

Soil properties		Season 2002		Season 2003	
		0-20 cm	20-40 cm	0-20 cm	20-40 cm
Coarse sand	% without $CaCO_3$ removal	15.31	13.95	9.71	8.49
Fine sand		37.46	37.52	23.22	24.14
Silt		20.13	23.80	44.76	45.45
Clay		27.10	24.73	22.21	21.92
Textural class		Sandy clay loam			
$CaCO_3$		23.11	25.21	24.15	23.72
Water saturation %		48.00	48.50	33.25	33.33
Total soluble salts %		0.08	0.08	0.13	0.13
PH (in 1-2.5 water suspension)		8.00	8.08	8.20	8.11
Cation exchange capacity (meq/100 g soil)		14.33	13.26	11.75	11.75
Organic matter %		0.62	0.57	1.08	0.99
Total N %		0.042	0.037	0.018	0.018
C / N ratio		8.56	8.93	34.80	31.90
Available P (mg / kg soil)		8.32	7.70	4.15	3.84
Available K (mg / kg soil)		500.0	447.0	311.0	292.0

Rhizosphere samples, after each cut, were collected to determined dehydrogenase enzyme activity by the method described by Thalmann (1967). The activity of nitrogenase enzyme in clover root nodules was assayed by the acetylene reduction assay (Hardy *et al.*, 1973). Data were statistically analyzed according to Snedecore & Cochran (1971).

Results and Discussion

a . Fresh yield

Table 2 represents fresh yield values of the 1^{st} and 2^{nd} seasons, respectively, in tons / fed. Data indicted that clover inoculation with N_2 – fixing bacteria had significantly increased the fresh yields of the 1^{st} and 2^{nd} cut in each season over the uninoculated plants. For the 3^{rd} cut in the first season, clover had not significantly increased by inoculation. The total increases of cuts in fresh yields, as a result of inoculation with rhizobia were about 18.52%. This result is in line with that obtained by Hemandez & Hill (1983) and Emskin *et al.* (1993).

TABLE 2. Effect of K_2HPO_4 application and inoculation with N_2-fixing bacteria on fresh yield weight of clover (Ton/fed).

Cut	Inoculation	K_2HPO_4 foliation			Mean	Increase %	L.S.D at 0.05 level
		0	I	II			
in the first season							
First	Without	2.90	4.90	5.20	4.33	---	Inoc: 0.69 PK: 0.85 Inoc.PK: n.s.
	With	5.00	5.50	6.30	5.60	25.28	
	Mean	3.95	5.20	5.75	---	---	
	Increase %	---	18.18	25.00			
Second	Without	2.50	3.00	3.90	3.13	---	Inoc: 0.88 PK: 1.23 Inoc.PK: n.s.
	With	2.90	4.60	4.76	4.09	30.67	
	Mean	2.70	3.80	4.33	---	---	
	Increase %	---	40.74	60.37			
Third	Without	3.90	4.60	5.15	4.55	---	Inoc: n.s. PK: 0.91 Inoc.PK: n.s.
	With	3.95	5.00	5.20	4.72	3.74	
	Mean	3.93	4.80	5.18	---	---	
	Increase %	---	22.14	31.81			
Total	Without	9.30	12.50	14.25	12.02	---	Inoc: 1.23 PK: 0.80 Inoc.PK: n.s.
	With	11.85	15.10	16.26	14.40	18.52	
	Mean	10.58	13.80	15.26	---	---	
	Increase %	---	25.11	36.08			
in the second season							
First	Without	5.69	9.07	8.50	7.57	--	Inoc: 1.29 PK: 1.64 Inoc.PK: n.s
	With	6.76	8.94	10.88	8.86	14.32	
	Mean	6.23	9.01	9.69	---	---	
	Increase %	---	44.62	55.54			
Second	Without	3.63	4.16	5.88	4.46	---	Inoc: 0.96 PK: 0.96 Inoc.PK: n.s.
	With	4.49	4.89	7.08	5.49	20.39	
	Mean	4.06	4.53	6.48	---	---	
	Increase %	---	11.58	59.61			
Total	Without	9.32	13.23	14.38	12.31	---	Inoc: 1.88 PK: 2.20 Inoc.PK: n.s.
	With	11.25	13.83	17.96	14.35	16.57	
	Mean	10.29	13.53	16.17	---	---	
	Increase %	---	31.49	57.14			

I: K_2HPO_4 dose contains 2 kg of K and 0.8 kg of P/fed.
II: K_2HPO_4 dose contains 4 kg of K and 1.6 kg of P/fed.

Also, the increases of cuts, as a result of inoculation with rhizobia and some N2-fixing bacteria were observed. These results are in harmony with those obtained by Dashit *et al.*, (1997) and El-Sayed (1999) who stated that the inoculated bacteria like *Azospirillum, Azotobacter* and *Bacillus* may produce growth promoting substances such as auxins, gibberellins and cytokinins. Such substances may improve plant growth and stimulate the microbial development.

Foliar application of K_2HPO_4 increased fresh yield proportionally with doubling the rate of application in both seasons. That increases, which reached to about 60 % over the control in the 2nd cut in both seasons, were significant in the 2nd, 3rd cuts of the first season and 1st and 2nd cut of the second season causing significant increases in the total fresh yields.

There was no interaction effect of these two factors on the fresh yield in all cases except the 1st cut in the second season.

b. Dry yield

Data in Table 3 show the dry yield of the first season in kg/ fed and 2nd season in ton / fed, respectively. Results indicated that inoculation with N_2-fixing bacteria caused significant increases of the yields in all the 3 cuts of the 1st season and the 1st one only in the 2nd season, These increases ranged between 10 and 37 % over uninoculated treatments. Referring to the yield weight in both seasons, which was more than 4 or 5 folds in the 2nd season than that in the 1st one, it could be concluded that inoculation with N_2-fixing bacteria were more effective in soil with low productivity. However inoculation with N_2-fixing bacteria increased the total dry matter significantly in the both seasons (Gray & Williams, 1979 and El-Sayed, 1999).

Spraying with K_2HPO_4 resulted in significant increases in dry yield for, all the 2nd season cuts when the doubled rate was applied, while the low rate had insignificant effect on the 2nd cut of the 2nd season dry yield. Soil application of K_2HPO_4 increased dry yield of each 2nd season cut down to significance on 0.05 level but total yield was higher than that level. The combination of N_2-fixing bacteria and K_2HPO_4 application did not gave significant interaction effect on dry yield in all cases.

c. Nitrogen uptake

Table 4 shows nitrogen uptake in kg / fed clover plant of the both of first and second seasons. Inoculation with N_2-fixing bacteria and K_2HPO_4 foliar application individually increased significantly N uptake over the control in all cuts and their summation in each season. El-Sayed (1999) confirmed that data and declared the increases in N uptake may due to inoculation with the special strains of N_2-fixing bacteria. Regarding K_2HPO_4 foliation effect on increasing N uptake by plant, Sparks (1986) and Hassaballa *et al.* (1991) attributed that effect to correction of the nutritional balance in plant.

TABLE 3. Effect of K_2HPO_4 application and inoculation with N_2-fixing bacteria on dry yield of clover .

Cut	Inoculation	K_2HPO_4 foliation #			Mean	Increase %	L.S.D at 0.05 level
		0	I	II			
Dry yield in the first season (Kg/fed.)							
First	Without	400.7	470.3	494.7	455.2	---	Inoc: 18.9 KP: 28.1 Inoc. PK: n.s.
	With	395.1	499.8	608.7	501.2	10.11	
	Mean	397.9	485.1	551.7	---	---	
	Increase %	---	21.92	38.65			
Second	Without	439.9	552.9	671.8	554.9	---	Inoc : 59.0 KP: 102.5 Inoc. PK: n.s.
	With	640.3	745.4	804.1	729.9	31.54	
	Mean	540.1	649.1	738.0	---	---	
	Increase %	---	20.18	36.64			
Third	Without	311.4	361.7	352.6	341.9	---	Inoc: 92.7 KP: 53.2 Inoc. PK: n.s.
	With	349.9	549.3	513.3	470.8	37.70	
	Mean	330.7	455.5	433.0	---	---	
	Increase %	---	37.74	30.93			
Total	Without	1172.0	1390.8	1519.1	1360.6	---	Inoc: 125.5 KP: 120.9 Inoc. PK: n.s.
	With	1380.2	1774.6	1926.1	1693.6	24.47	
	Mean	1276.1	1582.7	1722.6	---	---	
	Increase %	---	24.03	34.99			
Dry yield in the second season (Ton/fed.)							
First	Without	1.252	2.086	1.530	1.623	---	Inoc: 0.399 PK: 0.537 Inoc.PK: n.s..
	With	1.555	2.056	2.510	2.040	25.69	
	Mean	1.404	2.071	2.020	---	---	
	Increase %	---	47.51	43.87			
Second	Without	0.937	1.331	1.881	1.383	---	Inoc: 0.381 PK: 0.386 InocPK: n.s..
	With	1.555	1.415	2.271	1.747	26.32	
	Mean	1.246	1.373	2.076	---	---	
	Increase %	---	11.47	59.66			
Total	Without	2.414	3.418	3.412	3.001	---	Inoc: 0.461 KP: 0.631 Inoc PK: n.s..
	With	2.992	3.369	4.677	4.121	36.99	
	Mean	2.703	3.394	4.045	---	---	
	Increase %	---	25.56	49.63			

I: K_2HPO_4 dose contains 2 kg of K and 0.8 kg of P/fed.
II: K_2HPO_4 dose contains 4 kg of K and 1.6 kg of P/fed.

TABLE 4. Effect of K_2HPO_4 application and inoculation with N_2-fixing bacteria on N uptake by plants (kg/fed).

Cut	Inoculation	K_2HPO_4 foliation # 0	I	II	Mean	Increase %	L.S.D at 0.05 level
in the first season							
First	Without	8.05	12.56	9.94	10.18	=	Inoc. : 0.40 PK : 0.67 Inoc. PK: 0.70
	With	10.55	13.35	22.40	15.43	51.52	
	Mean	9.30	12.95	16.17			
	Increase %	=	39.24	73.87			
Second	Without	11.17	14.63	22.43	16.08	=	Inoc. : 2.06 PK : 3.15 Inoc. PK: 3.57
	With	21.22	25.06	21.47	22.59	40.47	
	Mean	16.19	19.85	21.95			
	Increase %	=	13.75	25.83			
Third	Without	7.29	7.27	12.98	9.18	=	Inoc. : 2.85 PK : 2.81 Inoc. PK: n.s.
	With	8.88	14.66	18.89	14.15	54.15	
	Mean	8.08	10.97	15.93			
	Increase %	=	35.69	97.13			
Total	Without	26.50	34.45	45.56	35.44	=	Inoc. : 4.09 PK : 3.03 Inoc. PK: n.s.
	With	42.15	53.07	62.76	52.66	48.60	
	Mean	34.33	43.76	54.16			
	Increase %	=	27.48	57.78			
in the second season							
First	Without	8.76	24.53	12.90	15.40	---	Inoc: 5.55 PK: 6.78 Inoc. PK: n.s..
	With	18.68	23.63	27.43	23.25	50.97	
	Mean	13.72	24.08	20.17	---	---	
	Increase %	---	75.51	47.01			
Second	Without	9.30	11.66	15.24	12.07	---	Inoc: 3.22 PK: 5.18 Inoc. PK: n.s..
	With	13.29	15.27	21.64	16.73	38.61	
	Mean	11.30	13.47	18.44	---	---	
	Increase %	---	19.20	63.19			
Total	Without	18.06	36.19	28.14	27.46	---	Inoc: 7.58 PK: 6.16 Inoc.P K: n.s.
	With	31.97	38.90	49.07	39.98	45.59	
	Mean	25.02	37.55	38.61	---	--	
	Increase %	---	50.08	54.32			

I: K_2HPO_4 dose contains 2 kg of K and 0.8 kg of P/fed.
II : K_2HPO_4 dose contains 4 kg of K and 1.6 kg of P/fed.

The interaction effect of the two studied factors on nitrogen uptake by plants of the early two cuts in the first season was significant, Where in the first cut, N uptake was increased by K_2HPO_4 spraying rate II and bacteria inoculation more than twice of that without inoculation. Also, in the 2^{nd} cut of that first season, inoculation with N fixing bacteria raised N uptake by plants without PK spraying to be statistically as the same as those sprayed with the doubled K_2HPO_4 rate . In the 3^{rd} cut, total 3 cuts of the 1^{st} season and 2^{nd} cut of the 2^{nd} season, there was no significance withen the interaction effects.

d. Phosphorus uptake

Table 5 represents the quantities of P taken up by plants in kg/fed. Inoculation with N_2-faxing bacteria was significantly converted where inoculation depressed P uptake in the 1^{st} cut of the 1^{st} season only and consequently reflected on total P, while the increases in the 2^{nd} and 3^{rd} ones were significant as the same as the 2^{nd} season cuts by inoculation. These increases may due to the effect of inoculation with N_2-fixing bacteria on plant growth and dry matter production, which, reflected on more P uptake (El-sayed, 1999). Also, *Bacillus* species have mechanisms other than N_2-fixation such as increasing phosphate availability (Rai *et al.*, 1981).

Concerning potassium phosphate application, same trend of inoculation was observed here also for foliar application only apart from the K P rate foliation. However, the highest increase percent for P uptake in the 2^{nd} cut, which reached about 58 and 57 % in K_2HPO_4 I and II foliar application over the control, respectively. The increases in P uptake by using K_2HPO_4 are in agreement with Hassablla *et al.* (1991) and Hamdia *et al.* (2000).

The interaction effect of these two factors was found to be insignificant in the 3^{rd} cut of the 1^{st} season and both cuts of the second season. It means that each factor played its role individually. The interaction effect was significantly more pronounced in the early two cuts and total uptake of P in the 1^{st} season where in the 1^{st} cut the inoculation confirmed significantly the effect of the higher K_2HPO_4 spraying rate and in the 2^{nd} cut the difference between PK rates was significantly negligible without N fixing bacteria inoculation.

e) Potassium uptake

Table 6 shows K uptake by clover plants in kg / fed. Data cleared that there was a positive significant effect by inoculation with N_2-fixing bacteria in all cuts of the 1^{st} season . In the 2^{nd} season, that effect was insignificant but it caused 18.07,29.60 and 22.78 % increase in K taken up compared with that taken up by noninoculated plots for 1^{st}, 2^{nd} cuts and total K uptake, respectively. The explanation previously mentioned in case of P uptake as positively affected by plant inoculation with N_2- fixing bacteria, which can be true also in case of K uptake.

TABLE 5. Effect of K_2HPO_4 application and inoculation with N_2-fixing bacteria on P uptake by plants (kg/fed) .

Cut	Inoculation	K_2HPO_4 foliation # 0	I	II	Mean	Increase %	L.S.D at 0.05 level
in the first season							
First	Without	6.49	9.00	8.36	7.95	=	Inoc 0.48
	With	2.21	3.15	4.63	3.33	- 58.11	PK: 0.39
	Mean	4.35	6.07	6.49			Inoc.PK: 0.84
	Increase %	=	39.57	49.22			
Second	Without	3.74	4.60	4.64	4.33	=	Inoc: 0.56
	With	3.24	5.70	8.12	5.69	31.51	PK: 0.75
	Mean	3.49	5.15	6.38			Inoc.KP: 0.97
	Increase %	=	47.61	82.80			
Third	Without	1.18	1.70	2.08	1.66	=	Inoc: 2.69
	With	1.78	2.69	2.93	2.47	49.06	PK: 4.79
	Mean	1.48	2.20	2.50			Inoc.P K: n.s.
	Increase %	=	47.97	68.73			
Total	Without	11.41	15.05	15.08	13.85	=	Inoc: 0.74
	With	7.24	11.54	15.67	11.49	17.05	PK: 0.49
	Mean	9.33	13.30	15.38			Inoc.PK: n.s.
	Increase %	=	42.60	64.88			
in the second season							
First	Without	6.64	12.36	8.15	9.05	---	
	With	7.74	14.03	17.56	13.11	44.86	
	Mean	7.19	13.20	12.86	---	---	Inoc: 2.60 KP: 2.57
	Increase %	---	83.59	78.86			Inoc.P K 4.50
Second	Without	2.24	4.00	3.15	3.13	---	
	With	4.03	5.16	6.15	5.11	63.26	Inoc: 1.68
	Mean	3.14	4.58	4.68	---	---	KP: 1.27
	Increase %	---					Inoc KP: n.s.
Total	Without	9.48	16.62	11.30	12.18	---	
	With	11.77	19.19	23.76	18.22	49.59	Inoc: 3.79
	Mean	10.62	17.90	17.53	---	---	KP: 3.25
	Increase %	---					Inoc. KP: n s..

I: K_2HPO_4 dose contains 2 kg of K and 0.8 kg of P/fed.
II: K_2HPO_4 dose contains 4 kg of K and 1.6 kg of P/fed.

Spray application of K_2HPO_4 resulted in significant effect on increasing K uptake over the control in all cases . In some cases the higher spray rate of K_2HPO_4 was significantly effective more than the lower one in the early two cuts and total K uptake of the 1st season and the 2nd cut of the second season .

Benefits of K_2HPO_4 in increasing K uptake was noticed by Sparks (1986) on peanut, Hassaballa *et al.* (1991) on citrus trees and fruits, and Hamdia *et al.* (2000) on maize.

TABLE 6. Effect of K_2HPO_4 application and inoculation with N_2-fixing bacteria on K uptake by plants (kg/fed).

Cut	Inoculation	K_2HPO_4 foliation #			Mean	Increase %	L.S.D at 0.05 level
		0	I	II			
in the first season							
First	Without	2.00	2.56	2.70	2.42	=	Inoc: 0.10 PK: 0.15 Inoc.PK: 0.17
	With	1.90	2.69	3.51	2.70	11.57	
	Mean	1.95	2.63	3.10			
	Increase %	=	34.82	59.18			
Second	Without	1.77	2.29	3.03	2.36	=	Inoc: 0.24 PK: 0.39 Inoc.PK: n.s.
	With	2.50	2.99	3.38	2.96	25.25	
	Mean	2.14	2.64	3.20			
	Increase %	=	23.47	50.01			
Third	Without	2.79	3.48	3.51	3.26	=	Inoc: 0.88 PK: 0.53 Inoc.PK: n.s.
	With	3.06	5.15	5.12	4.44	36.19	
	Mean	2.93	4.31	4.31			
	Increase %	=	47.40	47.47			
Total	Without	6.56	8.33	9.24	8.04	=	Inoc: 0.94 PK: 0.53 Inoc.PK: n.s.
	With	7.46	11.32	12.01	10.26	24.85	
	Mean	7.01	9.82	10.62			
	Increase %	=	35.52	51.54			
in the second season							
First	Without	10.24	18.08	13.03	13.78	---	Inoc: n.s. PK: 4.03 Inoc .PK: n.s
	With	11.44	17.35	20.02	16.27	18.07	
	Mean	10.84	17.72	16.53	---	---	
	Increase %	---	63.47	52.49			
Second	Without	7.95	9.28	11.45	9.56	---	Inoc: n.s. PK: 3.47 Inoc PK: n.s
	With	9.87	11.27	16.02	12.39	29.60	
	Mean	8.91	10.28	13.73	---	---	
	Increase %	---	15.38	54.10			
Total	Without	18.19	27.36	24.48	23.34	---	Inoc: n.s. PK: 4.74 Inoc PK: n.s
	With	21.31	28.62	36.04	28.66	22.78	
	Mean	19.75	27.99	30.26	---	---	
	Increase %	---	41.72	53.22			

I: K_2HPO_4 dose contains 2 kg of K and 0.8 kg of P/fed.
II: K_2HPO_4 dose contains 4 kg of K and 1.6 kg of P/fed.

Each factor of inoculation and K_2HPO_4 application was individually affecting K uptake without interaction effect of them. However, the 1^{st} cut of the 1^{st} season, the interaction effect was significantly succeeded in raising amount of K in plants when sprayed with the lower rate of K_2HPO_4 without N_2-fixing bacteria inoculation. These results were statistically as the same as those inoculated ones.

f. Enzyme activities

Each of nitrogenase enzyme in clover root nodules in μ mole C_2H_4 /g dry nodules / h and dehydrogenase enzyme in rhizosphere zone of roots in μg TPF/g dry soil /day are presented on Table 7 . With respect to nitrogenase, it could be found that inoculation increased significantly its activity in both cuts. In the 1^{st} cut that activity was about 94 % over the control, while it was about 35% only over the control in the 2^{nd} cut. Similar results were obtained by Rai (1983) on chick pea.

Soil or foliar application of K_2HPO_4 did not increase N_2-ase activity over the control as the same trend as interaction effect of both studied factors.

Regarding dehydrogenase enzyme, its activity in soil was significantly increased by plant inoculation with N_2-fixing bacteria in the both cuts. These results were in harmony with those reported by Mpepereki *et al.* (1997) who considered that the increase in dehydrogenase activity as a good indicator of biological activity in soil, also Chander *et al.* (1998) who concluded that activity of dehydrogenase related closely with the size of microbial biomass and microbiological redox system.

Dipotassium phosphate application had insignificant effect on that enzyme activity except for K_2HPO_4 foliation with the low rate, which was significantly higher than the control in the 1^{st} cut only. Nevertheless, higher rate of K_2HPO_4 foliar application reduced that activity with significant differences down of all the treatments in that cut. The reduction of the enzyme activity by that treatment in the 2^{nd} cut was insignificant. There was insignificant interaction effect on dehydrogenase activity in any cut.

TABLE 7. Effect of K_2HPO_4 application and inoculation with N_2-fixing bacteria on some enzymes activities in nodules and root zone soil of clover in 2nd season.

Enzyme Activity	Cut	Inoculation	K_2HPO_4 foliation #			Mean	Increase%	L.S.D at 0.05 level
			Without	I	II			
Nitrogenase (u mole C_2H_4/g dry nodules/h)	First	Without	304.23	344.27	420.91	372.6	---	Inoc: 78.97 PK: n.s. Inoc PK: n.s
		With	621.28	773.86	757.25	722.37	93.86	
		Mean	462.72	559.07	589.08	---	---	
		Increase %	---	20.82	27.31			
	Second	Without	411.21	543.42	590.42	515.23	---	Inoc: 110.77 PK: n.s. Inoc PK: n.s
		With	662.45	695.63	743.28	697.43	35.36	
		Mean	536.83	619.53	666.85	---	---	
		Increase %	---	15.41	24.22			
Dehydrogenase (ug TPF / g dry soil / day)	First	Without	41.79	48.97	29.26	41.51	---	Inoc: 2.79 PK: 3.40 Inoc PK: n.s
		With	49.74	55.58	34.68	48.08	15.83	
		Mean	45.77	52.28	31.97	---	---	
		Increase %	---	14.23	-30.15			
	Second	Without	50.40	58.10	43.50	50.88	---	Inoc: 6.27 PK: 7.53 Inoc PK: n.s.
		With	70.21	67.60	52.60	63.43	24.68	
		Mean	60.31	62.85	48.05	---	---	
		Increase %	---	4.22	-20.33			

I: K_2HPO_4 dose contains 2 kg of K and 0.8 kg of P/fed.
II: K_2HPO_4 dose contains 4 kg of K and 1.6 kg of P/fed.

References

Badr El-Din, S. M. S.; Khalafallah, M. A. and Moawad, H. (1986) Response of soybean to inoculation with Rhizobium japonicum. *Z. Pflanz. Bod.*, 149.

Black, C.A.; Evans, D.O.; Ensmingers, L.E.; White, J.L.; Clark, F.E. and Dionouer, R.C. (1965) *"Methods of Soil Analysis. II- Chemical and Microbiological Properties"*, pp. 1149-1176, Amer. Soci. Agronomy, Inc. Madison, Wisconsin, USA.

Brown, M. E. (1974) Seed and root bacterization. *Annual Review of Phytopathology* **12**, 181.

Burns, T.A. Jr.; Bishop, P.E. and Israel, D.W. (1981) Enhanced nodulation of leguminous plant roots by mixed cultures of *Azotobacter vinelandii* and *Rhizobium*. *Plant & Soil* **62**, 399.

Chander, K.; Goyal, S.; Nandal, D.P. and Kapoor, K.K. (1998) Soil organic matter, microbial biomass and enzyme activities in tropical agroforestry system. *Biol. Fert. Soils* **27**, 168.

Chapman, H.D. and Pratt, P.F. (1961) *"Methods of Analysis for Soils, Plants and Waters,"* Univ. of Calif., Division of Agricultural Sciences.

Daniel, T. C.; Sharpley, A. N. and Lemunyon, J. L. (1998) Agricultural phosphorus and eutrophication: A symposium overview. *J. Environ. Qual.* **27**, 251.

Dashi, N.; Zhang, F.; Hynes, R. and Smith, D.L. (1997) Application of plant growth – promoting rhizobacteria to soybean (Glycine max. (L) Merr.) increases protein and dry matter yield under short-season conditions. *Plant & Soil* **188**, 33.

El-Sayed, S.A.M. (1999) Influence of Rhizobium and phosphate solubilizing bacteria on nutrient uptake and yield of Lentil in the New Valley. *Egypt J. Soil Sci.* **39** (2), 175.

Emskine, W.; Saxina, N. P.; and Saxena, M. C. (1993) Iron deficiency in lentil: Yield loss and geographic distribution in a germplasm collection. *Plant & Soil.* **151**, 249.

Gray, T.R.G. and Williams, S.T. (1979) "*Soil Micro-Organisms*", Longman Inc., New York.

Hamdia, M.A.; El-Komy, H.M.A. and Barakate, N. (2000) The rate of foliar phosphorus and potassium fertilization and / or *Azospirillum lipoferum* or *Bacillus polymyxa* inoculation in nitrogen fixation (N15) and mineral nutrition of maize grown under salt stress. Xth *International Colloquium for the Optimization of Plant Nutrition,* Cairo, Egypt April 8-13. 2000 Session (13).

Hardy, R.W.F.; Burns, R.C. and Holsten, R.D. (1973) Application of the acetylene-ethylene reduction assay for measurement of nitrogen fixation. *Soil Biol.* Biochem. **5**, 47.

Hassaballa, I.A.; Sharaf, M.M.; Komh, R.N. and El-Deeb, M.D. (1991) Effect of foliar K and P fertilization on growth, yield and mineral content of citrus grown in different localities. *Desert Inst. Bull, A. R. E.* **41** (2), 323.

Hemandez, L. G. and Hill, G. S. (1983) Effect of population and inoculation on yield and yield component of chickpea (Cicer arietinum). *Proc. Agron. Soc.NZ.*, 43 .

Holl, F.B.; Chanway, C.P.; Turkington, R. and Radley, R.A. (1988) Response of crested wheatgrass (*Agropyron cristatum L.*), Perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens L.*) to inoculation with *Bacillus polymyxa. Soil Biol. Biochem.* **20**, 19.

Iruthayathas, E.E.; Gunasekaran, S. and Vlassak, K. (1983) Effect of combined inoculation of *Azospirillum* and *Rhizobium* on nodulation and N_2-fixation of winged bean and soybean. *Scientia Hortic.* **20**, 231.

Kelling, K.A.; Bundy, L.G.; Combs, S.M. and Peters, J.B. (1998) Soil test recommendation for field, vegetable and fruit crops. UWEX Bull. A 2809, 54p.

Mengel, K. and Kirkly, E.A. (1979) "*Principles of Plant Nutrition*", International Potash Inst.,Bern, Switzerland.

Mpepereki, S.; Makonese, F. and Wollum, A. G. (1997) Physiological characterization of indigenous rhizobia nodulating *Vigna unguiculata* in Zimbabwean soils. *Symbiosis* 22.

Plazinski, J. and Rolfe, B. G. (1985) *Azospirillum-Rhizobium* interaction leading to a plant growth stimulation without nodule formation. *Can. J. Microbiol.* **31**, 1026.

Rai, J.; Bagyaraj, D.J. and Manjunath, A. (1981) Influence of soil inoculation with vesicular arbuscular mycorrhizae and a phosphate-dissolving bacterium on plant growth and P32-uptake. *Soil Biochemistry* **13**, 105.

Rai, R. (1983) Efficacy of associative N_2-fixation by streptomycin- resistant mutants of Azospirillum brasilense with genotypes of chick pea Rhizobium strains. *J. Agric. Sci. Camb.* **100**, 75.

Snedecore, G. W. and Cochran, W. G. (1971) *"Statistical Methods"*, 7^{th} ed., Iowa State Univ., Press Amer, Iowa, USA.

Sommers, L.E. and Nelson, D.W. (1972) Determination of total Phosphorus in soil. A rapid perchloric acid digestion procedure. *Soil Sci. Soc. Amer. Proc.* **36**, 902.

Sparks, D. (1986) Growth and nutrition of peanut seedlings from potassium phosphate foliar sprays. *Hortscience* 21 (31.7) ((*c.f. Soils and Fert.* **50** (2738)).

Thalmann, A. (1967) Uber die microbiello aktiviatat undihr benziehung zu frucht- bartkeits merkmalen einiger acherboden unter besonderer beruksi chtigung der Dehydrogenase aktiviatat (TTC.Reduktion). Biss, Gieben. *pH. D. Thesis*, W. Germany.

Tilak, K. V. B. R.; Singh, C. S. and Rana, J. P. S. (1981) Effects of combined inoculation of Azospirillum brasilense with Rhizobium trifolii, Rhizobium meliloti and Rhizobium sp. (Cowpea miscellancy)on nodulation and yield of clover (Trifolium repens), Lucerne (Medicago sativa) and Chickpea (Civer arietinum). *Zbl. Bakt. II. Abt.* **136**, 117.

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تأثير التلقيح البكتيرى والرش بالفوسفات ثنائية البوتاسيوم علي البرسيم المصري في أرض جيرية

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أقيمت تجربتان في منطقة النوبارية (تربة جيرية) لدراسة تأثير التلقيح البكتيرى ببعض البكتيريا المثبتة للأزوت الجوي و/ أو معدلات مختلفة من الفوسفات ثنائية البوتاسيوم علي نمو البرسيم المصري (*Trifolium alexandrinum*).

تـــم تلقيـــح بـــذور البرسيم في التجـــربتين ا ببكتيـــريا الريزوبيا (*Rhizobium leguminosarum* biovar trifolii) يوم الزراعة ثم رشت النباتات ببعض البكتيريا المثبتة للأزوت اللاتكافلية وهي *Bacillus polymyxa*, *Azospirillum brasilense*، *Azotobacter chroococcum* ثلاث مرات كما تم رش التجربة بمعدلين من ال الفوسفات ثنائية البوتاسيوم (٠,٨ كجم فو و ٢ كجم بو كمعدل موصي به و الآخر ضعف الأول) و أخذ ثلاث حشات بعد ١١ ، ٢٠ ، ٣٠ أسبوعا من الزراعة فى الموسم الأول و فى الموسم الثانى حشتان بعد ١١ ، ٢٠ أسبوعا من الزراعة. وتم وزن المحصول الرطب و الجاف في كل من الموسمين و كذلك قدرت النسبة المئوية للنيتروجين و الفوسفور والبوتاسيوم وحسب الممتص من كل منها كما تم تقدير نشاط إنزيمى النيتروجينيز في العقد الجذرية و الديهيدروجينيز في ريزوسفير التربة للبرسيم / حشة.

أوضحت النتائج في التجربة الأولى أن التلقيح بالريزوبيا أدى إلي الزيادة المعنوية للمحصول الطازج والجاف للبرسيم مقارنة بمعاملة الكنترول غير الملقحة . أثبتت النتائج أن التلقيح البكتيرى ادى إلي الزيادة المعنوية لإمتصاص عناصرالنيتروجين و الفوسفور و البوتاسيوم كما زاد نشاط انزيمى النيتروجينيز والديهيدروجينيز في التجربة الثانية .

إستخدام الرش بالفوسفات ثنائية البوتاسيوم أدي إلي نفس نتائج التلقيح البكتيري بالنسبة للمحصول وكان المعدل الموصى به (٠,٨ كجم فو و ٢ كجم بو/فدان) يغنى عن المعدل المضاعف.أما بالنسبه لنشاط إنزيمى النيتروجينيز والديهيدروجينيز في التجربة الثانية فلم يؤد إستخدام الرش بالفوسفات ثنائية البوتاسيوم إلي زيادة في النشاط الإنزيمى.

هذا وقد نوقشت حالات التأثير المتبادل بين العاملين المدروسين وهى حالة قليلة عامة لكن فى أغلب الأحوال كان كل منهما يؤثر منفردا عن الآخر .

وفى النهاية يمكن من خلال هذه التجربة أن نوصي باستخدام التلقيح البكتيرى بالبكتيريا المثبتة للأزوت الجوي والرش بمحاليل تحضيرات السلالات التكافلية المذكورة مع ٤,٥ كجم فوسفات ثنائية البوتاسيوم/ فدان بالتساوي علي ثلاث فترات أدي إلي أفضل نوعية و كمية للمحصول.