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COMPARATIVE STUDY BETWEEN BIOFERTILIZTAION AND SULPHUR ON AVAILABILITY OF ADDED PHOSPHORUS TO FABA BEAN PLANTS UNDER HIGH PH SOIL CONDITIONS

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ABSTRACT

Faba bean is one of the most important crops as a protein source for human and livestock. It's consumed as green pods in the vegetable varieties or dry seeds in the others. The agricultural practices clearly affect green pods and dry seeds quality. Phosphorus is one of the most vital elements for living organisms and plant production. High pH soils affect phosphorus availability. The objective of the present study is to investigate the effect of biofertilization and sulphur on faba bean seed and straw yields, hundred seeds, weight, phosphorus uptake and protein content. Two field experiments were carried out at Sakha Agric. Res. Sta. Farm during two successive winter seasons of 2001/2002 and 2002/2003 using faba bean (*Vicia faba*) var. Giza 461. split plot design was used. The main plots were allotted for two phosphorus levels of 15 and 30 kg P_2O_5 fed.$^{-1}$. The sub plots were randomly assigned to four treatments: 1- without application (check treatment), 2- Inoculating faba bean seeds with phosphate solubilizing bacteria (*Bacillus megatherium* var. *Phosphaticum*), called (Phosphorin), 3- Inoculating faba bean seeds with mixture of non-symbiotic fixing bacteria (Azospirillum, Azotobacter and Bacillus) called (microbin). and 4-Application of 120 kg fed.$^{-1}$ sulphur to the soil before sowing. The other recommended agricultural practices were followed. The results can be summarized as:

The highest faba bean seed and straw yields were obtained with 15 kg P_2O_5 fed.$^{-1}$. Inoculating faba bean seeds by microbin as a biofertilization gave the highest seed and straw yields with the two studied phosphorus levels. Inoculating faba bean seeds with phosphorin as a biofertilization achieved the highest hundred seeds, weight and phosphorus uptake by the seeds and straw. Microbin had the highest protein content in the seeds, as well as phosphorus use efficiency.

INTRODUCTION

Phosphorus is particularly important for leguminous plants (Mengel and Kirkby, 1987). Legumes usually require large amounts of phosphorus and their ability to utilize soil phosphate is often less pronounced than that of cereals and grasses (Gunawardena *et al.*, 1992). Shortage of phosphorus element can reduce legumes nodulation, N_2-fixation and suppress seed yield (Abdel-Reheem *et al.*, 1992). Most Egyptian soils contain considerably high total phosphorus, yet, the amount available for plant uptake is low even after fertilization with superphosphate. In such case the two possible ways to increase plant available phosphorus are the use of phosphate solubilizing microorganisms or by decreasing soil pH. A great attention has been paid to the use of phosphate dissolving microorganisms (Saber *et al.*, 1983; Ibrahim *et al.*, 1995; Abdul Wahid and Mehana 1999; Hamissa *et al.*, 2000). Oxidation of the reduced sulphur compounds by soil microorganisms under aerobic

conditions, results in the production of sulphuric acid and/or sulphate which can affect plants grown on alkaline soils (Gendy *et al.*, 1995 and Knany *et al.*, 2000). The objective of the present work is to study the effect of phosphate solubilizing bacteria. Some non symbiotic N_2-fixing bacteria and sulphur on faba bean yield, phosphorus uptake and phosphorus use efficiency under different levels of phosphate fertilization at high pH soils.

MATERIALS AND METHODS

Two field experiments were carried out at Sakha Agriculture Research Station Farm using faba bean (*Vicia faba*) var. 461 during the two successive winter seasons of 2001-2002 and 2002-2003. The main properties of the experimental soils were 3.4 and 3.2 dS/m in EC, 7.94 and 7.82 in pH, 1.84 and 1.80% in soil organic matter, 26 and 33 ppm in available nitrogen, 5.4 and 5.9 ppm in available phosphorus and the soil texture was clayey in the first and second season, respectively.

Recommended seed rate was planted on 7 and 5 November in the first and second season.

Split plot design was used. The main plots were allotted for two phosphorus levels of 15 and 30 kg P_2O_5/fed. in the form of super phosphate 15% P_2O_5. The sub-plots were randomly assigned to four treatments: 1- Check treatment without any application, 2- Inoculating the faba bean seeds with the phosphate solubilizing biofertilizer consisted of an efficient strain of *Bacillus megatherium* var. *Phosphaticum* adsorbed on peatmoss powder as carrier and registered under a local name of phosphorin, 3- Inoculating the faba bean seeds by a mixture of non symbiotic N_2-fixer (i.e., Azotobacter, Azospirillum and Bacillus) adsorbed on peatmoss powder as carrier and registered under a local name of microbin, and 4-Application of 120 kg/fed. sulphur. The treatments were replicated four times. The other agricultural practices were done. At the harvest time seed and straw yields were measured and calculated as kg/fed. Representative seed samples were collected, dried at 70°C, crashed and wet-digested using sulphuric and percloric acids. Total nitrogen was measured by Kjeldahl method and phosphorus was colorimetrically measured according to Jackson (1958). Protein percent was calculated by multiplying the total nitrogen by 6.25. Soluble nitrogen was determined according to Piper (1947) and phosphorus uptake (PU) by the following equation:

$$PU = \frac{P\% \times \text{yield kg}}{100}$$

Phosphorus use efficiency (PUE) was calculated by the following equation:

$$PUE = \frac{\text{Seed yield kg/fed.}}{\text{Applied P kg/fed.}}$$

The main characteristics of the studied soil were analysed according to Black *et al.* (1965). The data were statistically analysed according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The data presented in Tables 1, 2 and 3 show the effect of phosphorus levels, biofertilization, sulphur and their interactions on faba bean seeds, straw yields and hundred seeds, weight in both seasons.

1.Seed yield:

Table 1 shows that the phosphorus level of 15 kg P_2O_5 fed^{-1} highly significantly increased faba bean seed yield (2024.8 and 2005.3 kg $fed.^{-1}$ in the first and second season) as compared with 30 kg P_2O_5 $fed.^{-1}$ level (1788.6 and 1818.5 kg $fed.^{-1}$) in the first and second season, respectively. This may be due to that the experimental soils had additional amount of available phosphorus (5.4 and 5.9 ppm) beside the phosphorus fertilizer and the other reason is the effect of the inoculation by the microorganisms on increasing the availability of added and native phosphorus to faba bean plants.

There are high significant differences in faba bean seed yield between check, phosphorin, microbin and sulphur treatments (Table 2). The highest seed yield was obtained with the microbin treatment of (2118 and 2132 kg $fed.^{-1}$) followed by phosphorin treatment of (1960 and 1984 kg $fed.^{-1}$) followed by sulphur treatment of (1914 and 1898 kg $fed.^{-1}$) and the lowest values of (1635 and 1634 kg $fed.^{-1}$) were recorded with the check treatment. The interaction between phosphorus levels and biofertilizers (Table 3) shows that microbin treatment gave the highest seed yield under the phosphorus level of 15 kg P_2O_5 $fed.^{-1}$. This may be due to two reasons, the first is the promoting effect of non-symbiotic N_2-fixing bacteria to plants which ascribed to their ability to fix free molecular atmospheric nitrogen. The second one is the role of these bacteria in improving the availability of soil nutrients through chelator substances which are important for solubilizing sparingly soluble inorganic compounds and make easy forms available for plants uptake. Similar results were obtained by Hassanein 1995, Abdul Wahid and Mehana, 1999 and Hamissa *et al.*, 2000.

Table (1):Effect of phosphorus levels on faba bean seed and straw yields kg/fed. and 100 seed weight (g). in the first and second seasons.

Treatments	Seed yield		Straw yield		100 seed weight	
	1st	nd	1st	2nd	1st	2nd
15 kg P_2O_5/fed.	2024.8	2005.3	1889.8	1894.6	64.3	64.5
30 kg P_2O_5/fed.	1788.6	1818.5	1630.8	1673.8	64.7	64.8
F-test	**	**	**	**	**	N.S

2.Straw yield:

It is clear from the data in Table 1 that the phosphorus level of 15 kg P_2O_5 $fed.^{-1}$ had the higher faba bean straw yield of 1889.8 and 1894.6 kg $fed.^{-1}$ in the two growing seasons, respectively. The difference between the two phosphorus levels was highly significant. Microbin treatment highly

significantly increased straw yield followed by check treatment (Table 2). On the other hand, the lowest straw yield was recorded at sulphur treatment in both seasons. The interaction between the two phosphorus levels and biofertilization (Table 3) indicates that the microbin under 15 kg P_2O_5 fed.$^{-1}$ treatment had the highest straw yield of 2331 and 2229 kg fed.$^{-1}$ in the first and second seasons, respectively. This may be due to the balance between nitrogen and phosphorus fertilization, where increasing the available phosphorus led to increasing fruit growth rather than vegetative growth. The yield of straw at sulphur treatment clearly illustrates a competitive effect between the nitrogen and sulphur which resulted in a low straw low straw yield (from the review). Similar results were obtained by Shams El-Din *et al.*, 1995 and Knany *et al.*, 2000.

Table (2):Effect of microbin, phosphorin and sulphur on faba bean seed and straw yields kg/fed. and 100 seed weight (g) in the first and second seasons.

Treatments	Seed yield		Straw yield		100 seed weight	
	1st	2nd	1st	2nd	1st	2nd
Check	1635	1634	1764	1815	64.9	64.8
Phosphorin	1960	1984	1662	1707	65.4	65.6
Microbin	2118	2132	2006	1981	64.5	64.8
Sulphur	1914	1898	1609	1635	63.3	63.5
F-Test	**	**	**	**	**	**
L.S.D. 0.05	3.21	12.59	2.21	44.27	0.23	0.33

Table (3):Effect of the interaction between phosphorus levels and bio-fertilizers on faba bean seed and straw yields kg/fed. and 100 seed weight (g) in both seasons.

Treatments		Seed yield		Straw yield		100 seed weight	
Main	Sub-	1st	2nd	1st	2nd	1st	2nd
15 kg P_2O_5/ fed.	Check	1589	1551	1918	2008	64.3	64.1
	Phosphorin	2100	2076	1680	1717	65.2	65.6
	Microbin	2310	2300	2331	2229	65.0	65.1
	Sulphur	2100	2098	1630	1624	62.7	63.2
30 kg P_2O_5/ fed.	Check	1690	1717	1610	1621	65.5	65.4
	Phosphorin	1820	1892	1645	1697	65.5	65.5
	Microbin	1925	1964	1680	1732	64.0	64.4
	Sulphur	1729	1700	1588	1645	63.8	63.7
F-Test		**	**	**	**	**	**
L.S.D. 0.05		4.54	17.8	3.12	62.6	0.32	0.46

3.Hundred seed weight:

The data of Table 1 show a highly significant effect of phosphorus levels on faba bean hundred seed-weight in the first season. No significant effect on the hundred seed weight was detected in the second season. The highest values of 65.4 and 65.6 were obtained with phosphorin treatment. Whereas, the lowest values of 63.3 and 63.5 were recorded with sulphur treatment in the first and second seasons, respectively (Table 2). Similar

results were obtained by Knany *et al.* (2000) on the soya bean. The effect of the interaction between phosphorus levels and biofertilization on hundred seed weight was highly significant. The highest values were obtained by phosphorin treatment under the higher level of phosphorus (30 kg P_2O_5/fed.). This may indicate the beneficial effect of phosphorus for good filling in faba bean seeds. Similar results were obtained by Hassanein (1995).

4.Phosphorus uptake of the seed yield:

Phosphorus levels significantly affected phosphorus uptake of faba bean seed yield in the first season, while no significant effect was detected in the second season as shown in Table 4. Inoculating faba bean seeds with biofertilizers, phosphorin highly significant increased phosphorus uptake of faba bean seeds in both seasons (Table 5). It followed by microbin treatment in both seasons. The lowest phosphorus uptake was recorded with check treatments. The interaction between phosphorus levels and biofertilization (Table 6) show that phosphorin treatment under the lower phosphorus level of 15 kg P_2O_5 fed.$^{-1}$ had the highest phosphorus uptake of faba bean seed yield in the first season. In the second season the highest value of 7.38 kg fed^{-1}. was obtained with phosphorin and 30 kg P_2O_5 fed^{-1}. The differences between the treatments were highly significant in the first season, but non significant in the second season. The increase of phosphorus uptake due to phosphorin treatment may be due to increasing phosphorus availability along the growth period. In the presence of the high level of phosphorus fertilizer may be microorganisms become not effect and the fertilizer become fixed. Similar results were reported by Abdul Wahid and Mehana, 1999 and Hamissa *et al.*, 2000.

Table (4):Effect of phosphorus levels on phosphorus uptake of faba bean seeds, straw kg/fed. and phosphorus use efficiency in the first and second seasons.

Treatments	P-uptake in seed yield		P-uptake in straw yield		P-use efficiency	
	1st	2nd	1st	2nd	1st	2nd
15 kg P_2O_5/fed.	5.56	5.14	2.28	2.16	134.98	133.68
30 kg P_2O_5/fed.	5.11	5.28	2.03	2.14	59.63	60.60
F-test	*	N.S	*	N.S	**	**

Table (5):Effect of biofertilization on phosphorus uptake of seeds, straw yields and phosphorus use efficiency in the first and second seasons.

Treatments	P-uptake in seed yield		P-uptake in straw yield		P-use efficiency	
	1st	2nd	1st	2nd	1st	2nd
Check	4.12	4.12	2.18	2.24	80.98	80.30
Phosphorin	7.45	7.22	2.50	2.50	100.33	100.72
Microbin	5.39	5.26	2.53	2.42	109.08	109.38
Sulphur	4.39	4.24	1.42	1.45	98.62	98.17
F-Test	**	**	**	**	**	**
L.S.D. 0.05	0.34	0.32	0.21	0.18	0.20	0.60

5.Phosphorus uptake by straw yield:

The data in Table 4 show that 15 kg P_2O_5 fed.$^{-1}$ significantly increased phosphorus uptake by straw yield in the 1st season, while in the 2nd season phosphorus uptake was not significant changed as a result of applying any of the studied two P levels. Inoculating faba bean seeds by biofertilizers of phosphorin and microbin highly significantly increased phosphorus uptake of faba bean straw (Table 5). This due to that biofertilization led to clear increase of straw yield. Results of Table 6 show that microbin under 15 kg P_2O_5 fed.$^{-1}$ treatment had the highest phosphorus uptake of the faba straw in the first season. While in the second season the highest value was obtained with phosphorin under 30 kg P_2O_5 fed^{-1}.

Table (6):Effect of the interaction between phosphorus levels and bio-fertilizers on phosphorus uptake of seeds, straw yield kg/fed. and phosphorus use efficiency in the first and second seasons.

Treatments		P-uptake in seed yield		P-uptake in straw yield		P-use efficiency	
P-level	bio-fertilizer	1st	2nd	1st	2nd	1st	2nd
15 kg P_2O_5/ fed.	Check	3.86	3.87	2.26	2.47	105.93	103.37
	Phosphorin	7.98	7.06	2.52	2.41	140.0	138.40
	Microbin	5.78	5.21	2.88	2.30	154.0	153.30
	Sulphur	4.62	4.40	1.47	1.46	140.0	139.67
30 kg P_2O_5/ fed.	Check	4.37	4.36	2.09	2.00	56.03	57.23
	Phosphorin	6.92	7.38	2.47	2.60	60.67	63.03
	Microbin	5.01	5.30	2.18	2.54	64.17	65.46
	Sulphur	4.15	4.08	1.38	1.43	57.63	56.67
F-Test		**	N.	*	**	**	**
L.S.D. 0.05		0.47	-	0.30	0.25	0.29	0.85

6.Phosphorus use efficiency (PUE):

Data in Tables 4, 5 and 6 show that phosphorus use efficiency was highly significantly increased with the phosphorus level of 15 kg P_2O_5 fed.$^{-1}$ in the both seasons. Inoculating faba bean seeds by biofertilizer of microbin led to a higher significant increase of phosphorus use efficiency. The highest (PUE) value was obtained with microbin treatment, followed by phosphorin and sulphur treatments. The lowest value was recorded with the check treatment. The interaction between phosphorus levels and biofertilization highly significantly affected (PUE) in both seasons. The highest (PUE), value was obtained with microbin treatment under 15 kg P_2O_5/fed.$^{-1}$ level in both seasons.

This may indicate that the nutrients status became more efficient with biofertilization. These results are agree with the obtained by Monib *et al.*, 1990; Saber and Gomaa, 1993 and Barakat and Gabr, 1998.

7.Total N(%), soluble-N (Mg kg^{-1}) and protein (%) of the seeds:

The data in Table 7 show that total nitrogen percentage and soluble nitrogen in the faba bean seeds were higher with 15 kg P_2O_5 fed^{-1} level. The

protein percent in the seeds also highly significantly increased with the same phosphorus level. Inoculating faba bean seeds with microbin as a biofertilizer increased total nitrogen %, soluble N as well as protein %. The increase of the protein was highly significant (Table 8). The interaction between phosphorus levels and biofertilization (Table 9) show that microbin under the first phosphorus level (15 kg P_2O_5/fed.$^{-1}$) had the highest values of total N%, soluble N and protein %.

Table (7):Effect of phosphorus levels on total nitrogen %, soluble nitrogen mg kg^{-1} and protein % in the faba bean seeds in the first and second seasons.

Treatments	Total N%		Soluble-N		Protein % in seeds	
	1st	2nd	1st	2nd	1st	2nd
15 kg P_2O_5/fed.	3.49	3.50	0.54	0.53	21.87	21.86
30 kg P_2O_5/fed.	3.40	3.42	0.41	0.43	21.23	21.39
F-test	-	-	-	-	**	**

Table (8):Effect of bio-fertilization on total nitrogen %, soluble nitrogen mg kg^{-1} and protein % in faba bean seeds in the first and second seasons.

Treatments	Total N%		Soluble-N		Protein % in seeds	
	1st	2nd	1st	2nd	1st	2nd
Check	3.40	3.39	0.46	0.49	21.23	21.20
Phosphorin	3.43	3.44	0.47	0.46	21.45	21.48
Microbin	3.75	3.74	0.56	0.55	23.40	23.33
Sulphur	3.21	3.28	0.40	0.42	20.12	20.48
F-Test	-	-	-	-	**	**
L.S.D. 0.05	-	-	-	-	0.17	0.17

Table (9):Effect of the interaction between P-levels and bio-fertilization on total-N%, soluble-N mg/kg and protein % of the seeds in the first and second season.

Treatments		Total N%		Soluble-N		Protein % in seeds	
P levels	Bio-fertilizer	1st	2nd	1st	2nd	1st	2nd
15 kg P_2O_5/ fed.	Check	3.36	3.37	0.54	0.54	21.03	21.07
	Phosphorin	3.43	3.43	0.52	0.49	21.43	21.43
	Microbin	3.98	3.93	0.69	0.67	24.87	24.53
	Sulphur	3.20	3.26	0.39	0.41	20.13	20.40
30 kg P_2O_5/ fed.	Check	3.43	3.41	0.38	0.44	21.43	21.33
	Phosphorin	3.43	3.45	0.42	0.43	21.47	21.53
	Microbin	3.51	3.54	0.42	0.42	21.93	22.13
	Sulphur	3.22	3.29	0.41	0.42	20.10	20.57
F-Test		-	-	-	-	**	**
L.S.D. 0.05		-	-	-	-	0.25	0.25

These may be due to non symbiotic N-fixing process and the hormonal exudates such as indole acetic acid, gibberillin and cytokinins of these microorganisms which can modify root growth resulting in more efficient absorption of available nutrients from the soil. Similar results were reported by Jagnow *et al.*, 1991; Noel *et al.*, 1996 and Hamissa *et al.*, 2000.

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دراسة مقارنة بين التسميد الحيوى والكبريت على صلاحية الفوسفور المضاف لنباتات الفول البلدى تحت ظروف الأراضي عالية الرقم الهيدروجينى

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يعتبر الفول البلدى واحد من أهم المحاصيل كمصدر للبروتين للإنسان وحيواناته. ويستخدم الفول البلدى كقرون خضراء فى أصناف فول الخضر ، كما يستخدم كمحصول بذور جافة فى الأصناف الأخرى. والعمليات الزراعية تؤثر بوضوح فى جودة القرون والبذور الجافة. ويعتبر الفوسفور من أهم العناصر الأساسية فى حياة الكائنات الدقيقة وإنتاجية النبات.

ارتفاع الرقم الهيدروجينى للأرض يؤثر على صلاحية الفوسفور بها. ويتلخص الهدف من البحث فى دراسة أثر التسميد الحيوى مقارنة بالكبريت على يسر الفوسفور المضاف للفول البلدى وأثره على محصول البذور والقش ونسبة البروتين والفوسفور الممتص. ولتحقيق هدف الدراسة أجريت تجربتان حقليتان خلال الموسمين الشتويين ٢٠٠١-٢٠٠٢ ، ٢٠٠٢-٢٠٠٣ بمزرعة محطة البحوث الزراعية بسخا على صنف الفول البلدى جيزه ٤٦١ ، واستخدم التصميم الإحصائى القطع المنشقة فى أربعة مكررات. شغلت القطع الرئيسية بمستويين من الفوسفور (١٥ ، ٣٠كجم فو٢أ٥ للفدان) كما شغلت القطع الشقية بأربع معاملات هى:

١-معاملة المقارنة.

٢-التلقيح بالبكتريا المذيبة للفوسفات.

٣-التلقيح بخليط من البكتريا المثبتة للأزوت لا تكافليا.

٤-إضافة ١٢٠ كجم كبريت للفدان.

وتتلخص النتائج المتحصل عليها فى الآتى:

- أعطى المستوى الأول من الفوسفور (١٥ وحدة للفدان) أعلى محصول بذور وأعلى محصول تبن.
- التلقيح البكتيرى بالبكتريا المثبتة للأزوت لا تكافليا (الميكروبين) أعطى أعلى محصول من البذور والتبن مع كل من مستويي الفوسفور. أما التلقيح بالبكتيريا المذيبة للفوسفات أدى الى أعلى امتصاص من الفوسفور المضاف وأعلى امتلاء للبذور وأعلى نسبة بروتين فى البذور. كما أعطى التلقيح بالبكتريا المثبتة للأزوت لا تكافليا أعلى القيم فى كفاءة استخدام وحدة السماد الفوسفاتى.