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## Associative Effect of Phosphate Dissolving Fungi, *Rhizobium* and Phosphate Fertilizer on Some Soil Properties, Yield Components and the Phosphorus and Nitrogen Concentration and Uptake by *Vicia faba* L. Under Field Conditions

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**Abstract:** The effect of interactions between three phosphate-dissolving fungi (PDF) (*Aspergillus niger*, *A. fumigatus* and *Penicillium pinophilum*) and *Rhizobium leguminosarum* biovar *viciae* (RH) was studied on some soil chemical properties, phosphorus and nitrogen uptake and yield of faba beans (*Vicia faba* L.). Analysis of the soil after crop harvest indicated that the inoculation with PDF and RH significantly increased the levels of soil available P, mineral N ( $\text{NH}_4^+$ -N+  $\text{NO}_3^-$ -N) as compared to the untreated soil. The highest level of available P was recorded under the treatment  $\text{RH}_1 + 31 \text{ Kg P}_2\text{O}_5 \text{ fed}^{-1} + P. \text{ pinophilum}$ . The highest value of soil total N was observed with the treatment  $\text{RH}_1 + 15.5 \text{ kg P}_2\text{O}_5 \text{ fed}^{-1} + A. \text{ niger}$ . Values of soil pH and total N were not significantly affected by the soil inoculation with PDF. The inoculation with RH and PDF significantly increased seeds and straw yields of faba beans as compared to the untreated plants. The maximum seeds and straw yields of 1272 and 1871  $\text{Kg fed}^{-1}$  respectively, were achieved in the treatment  $\text{RH}_1 + 31 \text{ Kg P}_2\text{O}_5 \text{ fed}^{-1} + P. \text{ pinophilum}$ . The highest P uptake by plants was also achieved under the same treatment. The highest N uptake was recorded with the combination  $\text{RH}_1 + 31 \text{ Kg P}_2\text{O}_5 \text{ fed}^{-1} + A. \text{ fumigatus}$ . It was clearly evident that the ordinary used quantity of SP fertilizer could be reduced by 50%, in the presence of PDF, without any significant effect on the yield of *Vicia faba* (L.).

**Key words:** Phosphate-dissolving fungi, *Rhizobium leguminosarum*, superphosphate, faba bean, P and N uptake

### Introduction

Most of the reclaimed areas, which planned to be cultivated in Egypt, are sandy soils with alkaline pH. Under such conditions, considerable amounts of the available forms of phosphorus are usually subjected to rapid transformation to less available or unavailable forms. Therefore, the heavy application of phosphatic fertilizers is a routine followed to supply the plant with the required amount of phosphorus.

Several published studies showed the importance of certain soil microorganisms increasing the availability of phosphorus in soils (Saber *et al.*, 1983; Mohod *et al.*, 1989; Salih *et al.*, 1989; Manjunatha and Devi, 1990; Abdel Azeem, 1998). In previous pot and column experiments, the inoculation of soil with three fungal isolates, namely, *Aspergillus niger*, *A. fumigatus* and *Penicillium pinophilum*, significantly increased the yield and P uptake by wheat and faba bean plants. This encouraged us to evaluate the potentiality of these fungi under field conditions. The biofertilizer *Rhizobium leguminosarum* is routinely used in faba bean cultivation, especially in light texture soil. It gives more than 23  $\text{Kg N fed}^{-1}$  (Mahmoud *et al.*, 1988; Abdul Alla *et al.*, 1993). In addition, about 25-50  $\text{Kg (NH}_4)_2\text{SO}_4 \text{ fed}^{-1}$  or any other equivalent nitrogen fertilizer is added to the soil (Abdul Alla *et al.*, 1993). The present work aimed to study the effect of interaction between these fungal isolates, the bacterium *Rhizobium leguminosarum* biovar *viciae* (RH) and superphosphate (as ordinary phosphate fertilizer) on the yield of faba beans and uptake of P and N.

### Materials and Methods

**Inoculum preparation:** The PDF were grown on a mixture of Martin's medium (100 ml) (Allen, 1959), 10%  $\text{CaCl}_2$  (10 ml) and 10%  $\text{K}_2\text{HPO}_4$  (5 ml) and incubated at 30°C in the dark. After one week, the fungal spores were harvested and resuspended in sterile distilled water to give a final concentration of about  $1-2 \times 10^9$  colony forming units per ml. The fungal preparation was added to the soil with the irrigation after 15 and 60 days of seed sowing @100 ml per hill, at this stage the irrigation was carried out manually.

**Field experiment:** A field experiment was conducted in a sandy soil at the Agricultural Experimental Farm of Suez Canal University, Ismailia, Egypt. The required physical and chemical properties

Table 1: Some physical and chemical properties of the investigated soil

Particle size distribution (%):	
Sand	95.25
Silt	2.25
Clay	2.50
Texture class	Sandy
pH *	8.02
$\text{CaCO}_3$ (g $\text{Kg}^{-1}$ soil)	35.0
E C (d $\text{Sm}^{-1}$ )**	1.20
Soluble cations (c mol $\text{Kg}^{-1}$ soil) **:	
Na <sup>+</sup>	0.08
K <sup>+</sup>	0.01
Ca <sup>2+</sup>	0.13
Mg <sup>2+</sup>	0.03
Soluble anions (c mol $\text{Kg}^{-1}$ soil) **:	
$\text{CO}_3^{2-}$	0.00
$\text{HCO}_3^-$	0.03
Cl <sup>-</sup>	0.08
$\text{SO}_4^{2-}$	0.14
Organic C (g $\text{Kg}^{-1}$ soil)	1.40
Total N (g $\text{Kg}^{-1}$ soil)	0.15
$\text{NaHCO}_3$ - soluble P (mg $\text{Kg}^{-1}$ soil)	4.01
Soluble N (mg $\text{Kg}^{-1}$ soil):	
$\text{NH}_4^+$ - N	5.50
$\text{NO}_2^-$ - N	0.25
$\text{NO}_3^-$ - N	2.25

\* In soil -water suspension 1:2.5. \*\* In soil saturation extract.

(Table 1) of the surface soil (0-30 cm depth) used were determined according to Page *et al.* (1982).

The experimental design was split-split plot with four replicates. It involved two main plots assigned for two *Rhizobium leguminosarum* biovar *viciae* (RH) treatments, i.e. without (RH<sub>0</sub>) and with (RH<sub>1</sub>) *Rhizobium* inoculation. Plus three levels of superphosphate (SP, 15.5%  $\text{P}_2\text{O}_5$ ), i.e. 0, 15.5 and 31  $\text{Kg P}_2\text{O}_5 \text{ fed}^{-1}$  as subplots. In addition to four phosphate-dissolving fungi (PDF), i.e. none, *Aspergillus niger* (Asp. n), *A. fumigatus* (Asp.f) and *Penicillium pinophilum* (Pen) randomly arranged in sub-subplots. These fungi have been isolated before from the rhizosphere of faba beans (*Vicia faba* L.), kidney bean (*Phaseolus vulgaris* L.), peas (*Pisum sativum* L.) and wheat (*Triticum aestivum*

L.). They were tested for their ability to dissolve both rock phosphate and tricalcium phosphate (Wahid and Mehana, 2000). The experimental unit consisted of 6 rows, 60 cm apart and 4 m long with 4 replicates. Potassium sulphate (50% K<sub>2</sub>O) @ 5 Kg K<sub>2</sub>O fed<sup>-1</sup> was added to all plots before cultivation, while ammonium sulphate (20.6% N) @ 30 Kg N fed<sup>-1</sup> was added after 50 days. Faba bean seeds (cv. Giza 2) were sown in hills 20 cm apart @ 2 seeds per hill. The seeds were previously soaked in water for 12 h and either thoroughly mixed or not with cell suspension of an effective strain of RH (6x10<sup>8</sup> cell ml<sup>-1</sup>).

The crop was harvested after 140 days and yields of straw and seeds were estimated. The concentration of P and total N in plant samples were determined according to Jackson (1958) and Chapman and Pratt (1961) respectively. In addition, some soil features were analyzed after crop harvest. Soil pH was measured in soil-water suspension (1:2.5), while sodium bicarbonate soluble P was determined by method of Olsen *et al.* (1954). Nitrate was determined by the ultraviolet spectrophotometric method (Anonymous, 1992), ammonium by the indophenol blue method (Page *et al.*, 1982) and the total nitrogen by the Kjeldahl method (Chapman and Pratt, 1961).

**Statistical analysis:** Data were statistically analyzed according to Snedecor and Cochran (1969). ANOVA and LSD at 0.05 were applied to determine the significance of the obtained results.

## Results and Discussion

**Soil chemical properties:** This part of the experiment is focussed on studying the effect of the additives PDF, RH and SP on P availability and mineral N in soil as well as the changes in the soil pH. These factors were chosen because of their crucial effect on plant growth. The effect of a single additive or their interaction (s) was considered in the following sections.

**Soil pH:** Soil pH values did not change significantly with the addition of PDF or other additives i.e. RH or SP (Tables 2, 3). This indicated that the solubilization of phosphate was not due to the change in soil pH. Salih *et al.* (1989) suggested that the nature of the secreted organic acids is more important in releasing P from its insoluble materials than the pH changes. In this context, Luo *et al.* (1993) stated that the amount of the secreted organic acids, succinic, oxalic, acetic and malonic, accounted for the amount of P released from AlPO<sub>4</sub>, FePO<sub>4</sub> and Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>. On the other hand, phosphate-solubilizing ability of PDF could be attributed to their acid and neutral phosphatase production (Casida, 1959, Chhonkar and Tarafdar, 1984). In a previous pot-experiment study with the same fungi, there was a significant negative correlation between changes in the values of soil pH and the amount of available P (Wahid and Mehana, 2000).

**Available P:** The seed inoculation with RH significantly increased the level of available P compared to the untreated ones. This result is in agreement with that of Halder *et al.* (1992), who found that the strain of *R. leguminosarum* biovar *viciae* was able to solubilize the insoluble phosphate "hydroxyapatite". Similar effect was obtained when the soil was treated with PDF. This indicated the ability of the used fungi to dissolve the insoluble phosphate in soil. These fungi can be arranged in decreasing order according to their ability to solubilize the insoluble form of phosphate as follow: *P. pinophilum* > *A. fumigatus* > *A. niger*. This result can be supported by previous work done on the same fungi and showed high ability to dissolve insoluble P (Wahid and Mehana, 2000). Meantime, raising the application rate of SP significantly increased the level of available P.

The level of available P significantly increased as a result of the interaction between RH, PDF and SP (Table 3). The highest level was recorded with the treatment RH<sub>1</sub> + 31 Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup> + *P. pinophilum*. It is clear that in the presence of RH, the addition of SP @ 15.5 Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup> in combination with PDF increased the available P in soil as much as did the addition of 31 Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>

without PDF. It is evident that the combined application of PDF, RH and SP significantly improves the efficiency of SP through increasing the level of available P. Consequently, the traditional heavy application of SP could be reduced.

**Soil mineral and total N:** Seed inoculation with RH significantly increased the levels of NO<sub>3</sub><sup>-</sup>-N, mineral N (NH<sub>4</sub><sup>+</sup> -N+ NO<sub>3</sub><sup>-</sup>-N) and total N (Table 2). This result could be ascribed to the relatively higher N<sub>2</sub>-fixation in the presence of RH. Field beans (*Vicia faba*), like all other well nodulating legumes, obtained most of their nitrogen requirements from the atmosphere through symbiotic relationship with *Rhizobium* (Richards and Soper, 1979).

On the other hand, addition of PDF to the soil increased levels of NH<sub>4</sub><sup>+</sup> -N, NO<sub>3</sub><sup>-</sup>-N and total N (Table 2). However, the increases in soil total N were not significant. The increase in mineral N may be attributed to the increase in rate of ammonification and heterotrophic nitrification processes carried out by PDF. Balasubramanya and Patel (1980) demonstrated that some species of *Aspergillus* and *Penicillium* were able to produce NO<sub>3</sub><sup>-</sup> during degradation of the pesticides carboxin and oxycarboxin. They also found that these fungi were able to oxidize several nitrogenous compounds such as NH<sub>4</sub><sup>+</sup>, pepton, urea, cyanide and azide to NO<sub>2</sub><sup>-</sup>. Similarly, the addition of SP @ 15.5 and 31 Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup> significantly increased levels of NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N comparing to untreated soil. The application of SP did not significantly increase the soil total N. Hammad *et al.* (1990) noticed that increasing rate of P fertilizers decreased total N of sandy soil cultivated with broad bean plants. The increasing effect of SP on N levels can be explained on the basis that, increasing the level of P significantly enhanced the symbiotic N<sub>2</sub>-fixation which in turn increases the N uptake by plants (Table 6).

The interaction (s) among the three additives, i.e. PDF, RH and SP, significantly affected the levels of NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N and total N (Table 3). The highest level of soil total N was recorded with the treatment RH + 15.5 Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup> + *A. niger* followed by the treatment RH + 15.5 Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup> + *P. pinophilum*. No significant difference was observed between these two treatments. This indicated that the combine inoculation of RH and *A. niger* or *P. pinophilum* with simultaneous application of SP @ 15.5 Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup> enhanced the symbiotic N<sub>2</sub> fixation.

**Yield components:** Concerning the main effect of the inoculation with RH, on the yield components. The seed and straw yields of faba beans were significantly increased comparing to RH-uninoculated plants (Table 4). This could be explained on the basis that the values of available P and N as well as that of total N, in the soil, were greatest in the presence of RH (Table 2).

With respect to the main effect of the PDF, Table 4 indicates that PDF significantly increased both the straw and the seed yields of faba beans compared to untreated plants. The highest yield was recorded with the fungus *P. pinophilum*. This fungus was proved to be the most efficient among the tested fungal isolates. This results could be partially attributed to the relatively higher ability of that fungus to increase the available P in the soil (Table 2). The result obtained coincides with previous work conducted on wheat and faba bean plants (Wahid and Mehana, 2000), where straw, seed and biological yield increased significantly with the application of the same PDF.

Regarding the main effect of the P level, Table 4 indicates that seed and straw yields significantly increased with increasing P levels up to 31 Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>. This stimulatory effect of P may be due to its role in enhancing metabolic activities of faba bean. Such activities may include photosynthesis, starch synthesis, glycolysis and synthesis of fats and protein (Thomson and Troech, 1979; Shalaby and Ahmed, 1993). On the other hand, the addition of P significantly increased root surface area that is important in supplying the plant with the nutrients needed (Hallmark and Barber, 1984) which in turn results in increasing P and N contents of plants.

**Mehana and Wahid:** Phosphate-dissolving fungi, *Rhizobium leguminosarum*, superphosphate, faba bean, P and N uptake

Table 2: The main effects of *Rhizobium leguminosarum* (RH), phosphate-dissolving fungi (PDF) and superphosphate (Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>) on some soil chemical properties after faba bean harvest

Treatments	pH 1:2.5	Available P (mg Kg <sup>-1</sup> )	Mineral N* (mg Kg <sup>-1</sup> )			Total N mg Kg <sup>-1</sup>
			NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	Total	
RH <sub>0</sub>	7.85	13.4	4.30	5.72	10.02	154
RH <sub>1</sub>	7.82	16.6	4.09	6.97	11.06	178
LSD 0.05	NS	1.69	NS	0.51	1.02	18
PDF:						
None	7.88	10.8	3.20	4.63	7.83	158
Asp. n.	7.84	14.9	4.90	7.46	12.36	169
Asp. f.	7.82	16.6	4.72	7.07	11.79	162
Pen.	7.82	17.6	3.96	6.21	10.17	173
LSD 0.05	NS	2.87	1.07	0.69	1.42	NS
P <sub>2</sub> O <sub>5</sub> levels:						
0	7.86	5.3	3.57	5.37	8.94	162
15.5	7.83	18.0	4.58	6.48	11.06	173
31	7.82	21.6	4.44	7.18	11.62	162
LSD 0.05	NS	3.22	0.70	0.42	0.82	NS

Table 3: Effect of the interaction between *Rhizobium leguminosarum* (RH), phosphate-dissolving fungi (PDF) and superphosphate (Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>) on some soil chemical properties after faba bean harvest

RH	P <sub>2</sub> O <sub>5</sub>	PDF	pH (1:2.5)	Available P (mg Kg <sup>-1</sup> soil)	Mineral N* (mg Kg <sup>-1</sup> soil)			Total N (mg Kg <sup>-1</sup> soil)	
					NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	Total		
RH <sub>0</sub>	0	None	7.91	3.3	1.78	2.80	4.58	140	
		Asp. n.	7.88	5.1	4.48	6.74	11.22	149	
		Asp. f.	7.81	4.7	3.18	6.32	9.50	176	
		Pen.	7.90	5.5	4.90	3.66	8.56	176	
		15.5	None	7.80	6.3	3.18	3.92	7.10	142
			Asp. n.	7.83	16.5	4.62	8.04	12.66	144
	Asp. f.		7.90	16.8	6.38	7.01	13.39	148	
	Pen.		7.90	19.4	4.76	4.19	8.95	180	
	31		None	7.86	17.9	3.26	4.77	8.03	140
			Asp. n.	7.86	19.0	5.56	9.10	14.66	140
		Asp. f.	7.81	22.9	4.72	7.14	11.86	147	
		Pen.	7.75	23.0	4.82	4.94	9.76	162	
RH <sub>1</sub>		0	None	7.91	4.7	2.36	4.60	6.96	176
			Asp. n.	7.88	6.2	4.60	5.76	10.36	176
	Asp. f.		7.77	6.3	4.28	7.02	11.30	151	
	Pen.		7.82	6.6	3.00	6.05	9.05	150	
	15.5		None	7.87	13.5	4.18	5.45	9.63	176
			Asp. n.	7.77	21.5	4.94	7.25	12.19	219
Asp. f.		7.80	24.7	5.38	7.04	12.42	180		
Pen.		7.80	25.2	3.16	8.94	12.10	196		
31		None	7.91	18.8	4.46	6.21	10.67	176	
		Asp. n.	7.79	21.1	5.18	7.87	13.05	186	
	Asp. f.	7.81	24.3	4.40	7.91	12.31	168		
	Pen.	7.75	26.0	3.12	9.48	12.60	176		
	LSD	0.05	NS	4.98	1.05	1.15	1.36	26	

Asp. n.: *Aspergillus niger*; Asp. f.: *A. fumigatus*; Pen.: *Penicillium pinophilum*. RH<sub>0</sub>: no *Rhizobium*; RH<sub>1</sub>: with *Rhizobium*. \* NO<sub>2</sub><sup>-</sup> was not detected. NS: non significant.

Table 4: The main effects of *Rhizobium leguminosarum* (RH), phosphate-dissolving fungi (PDF) and superphosphate (Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>) on the yield components (Kg fed<sup>-1</sup>) of faba bean plants grown on a sandy soil

Treatments	Seed yield	Straw yield	Biological yield
RH			
RH <sub>0</sub>	867	1293	2160
RH <sub>1</sub>	1027	1520	2547
LSD 0.05	60	99	153
PDF			
None	833	1232	2065
Asp.n.	947	1429	2376
Asp.f.	948	1417	2365
Pen.	1062	1548	2610
LSD 0.05	100	130	210
P <sub>2</sub> O <sub>5</sub> level			
0	819	1216	2035
15.5	939	1391	2330
31	1084	1613	2697
LSD 0.05	91	139	220

Asp. n.: *Aspergillus niger*; Asp. f.: *A. fumigatus*; Pen.: *Penicillium pinophilum*. RH<sub>0</sub>: no *Rhizobium*; RH<sub>1</sub>: with *Rhizobium*.

Table 5: Effect of the interaction between *Rhizobium leguminosarum* (RH), phosphate-dissolving fungi (PDF) and superphosphate (Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>) on the yield components (Kg fed<sup>-1</sup>) of faba bean plants grown on a sandy soil

RH	P <sub>2</sub> O <sub>5</sub>	PDF	Seed yield	Straw yield	Biological yield
RH <sub>0</sub>	0	None	642	823	1465
		Asp. n.	739	1197	1936
		Asp. f.	743	1111	1854
		Pen.	848	1290	2138
	15.5	None	700	1029	1729
		Asp. n.	926	1323	2249
		Asp. f.	709	1125	1834
		Pen.	960	1352	2312
	31	None	948	1458	2406
		Asp. n.	1014	1513	2527
		Asp. f.	1020	1618	2638
		Pen.	1159	1678	2837
RH <sub>1</sub>	0	None	832	1156	1988
		Asp. n.	883	1402	2285
		Asp. f.	982	1466	2448
		Pen.	886	1284	2170
	15.5	None	892	1416	2308
		Asp. n.	1102	1621	2723
		Asp. f.	976	1448	2424
		Pen.	1245	1813	3058
	31	None	983	1512	2495
		Asp. n.	1018	1519	2537
		Asp. f.	1257	1736	2993
		Pen.	1272	1871	3143
LSD	0.05		203	269	464

Table 6: The main effects of *Rhizobium leguminosarum* (RH), phosphate-dissolving fungi (PDF) and superphosphate (Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>) on the concentration (%) and uptake (mg plant<sup>-1</sup>) of N and P by faba bean plants grown on a sandy soil

Treatments	N					P				
	Seed		Straw		Total uptake	Seed		Straw		Total uptake
	Conc.	Uptake	Conc.	Uptake		Conc.	Uptake	Conc.	Uptake	
RH										
RH <sub>0</sub>	3.67	636	1.58	409	1045	0.35	60.7	0.16	41.4	102.1
RH <sub>1</sub>	3.80	781	1.92	584	1365	0.37	76.0	0.20	60.8	136.8
LSD 0.05	0.07	46	0.17	54	114	0.01	5.15	0.02	8.3	12.4
PDF										
None	3.53	588	1.58	389	977	0.34	56.6	0.14	37.0	93.6
Aps.n.	3.86	731	1.76	503	1234	0.37	70.1	0.19	57.2	127.3
Asp.f.	3.86	732	1.81	513	1245	0.36	68.3	0.19	56.7	125.0
Pen.	3.71	788	1.87	579	1367	0.36	76.5	0.20	65.0	141.5
LSD 0.05	NS	130	NS	98	227	0.02	10.2	0.02	9.9	17.0
P <sub>2</sub> O <sub>5</sub>										
0	3.65	598	1.66	406	1004	0.34	55.7	0.15	36.5	92.2
15.5	3.77	708	1.76	490	1198	0.35	65.7	0.18	50.1	115.8
31	3.79	822	1.84	594	1416	0.38	82.4	0.21	67.7	150.1
LSD 0.05	NS	117	NS	86	202	0.02	6.79	0.02	9.0	15.4

Asp. n.: *Aspergillus niger*; Asp. f.: *A. fumigatus*; Pen.: *Penicillium pinophilum*. RH<sub>0</sub>: no *Rhizobium*; RH<sub>1</sub>: with *Rhizobium*. NS: non significant.

In the light of the interactions between RH, PDF and P, levels (Table 5), it was evident that the application of RH in combination with PDF significantly increased the yield components comparing to the untreated soil or to the single application of either RH or PDF, regardless of the P level. This could be attributed to the cumulative effects, such as the increased supply of N and P as well as the growth promoting substances produced by the added organisms (Alagawadi and Gaur, 1988). Meanwhile, combined application of RH and different P levels increased the yield components in comparison with the single application. This could be due to the establishment of an effective plant-*Rhizobium*-symbiosis system and to the direct influence of P on N fixation (Subba Rao, 1976). Likewise, application of PDF with different levels of P enhanced the yield components, particularly in the presence of RH. However, the differences were not always significant.

The present results reflected the important role played by these fungal isolates in increasing P availability in soil (Table 3). The maximum seed and straw yields (1272 and 1871 Kg fed<sup>-1</sup>, respectively) were recorded in the application of *R. leguminosarum* in combination with *P. pinophilum* plus 31 Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>. However, no significant difference, in the yield component,

was observed between the two applied levels of SP in the presence of RH + *P. pinophilum* (Table 5). It was clear that the application of RH with *P. pinophilum* could reduce the amount of phosphatic fertilizer applied to the soil. As the application of RH with high level of P (31 Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>) only produced less yield than the application of RH with low level of P (15.5 Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>) in presence of *P. pinophilum*.

**Nitrogen and phosphorus content in plant:** The concentration of P and N, in seeds and straw of faba beans, responded differently to the treatment with RH, PDF or different levels of P (15.5 or 31 Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>). While the concentration of P significantly increased, that of N was insignificant (Table 6). However, no significant difference was observed among the used PDF. The used PDF can be arranged, in a descending order, according to their effect on the total uptake of N as follow: *P. pinophilum* > *A. fumigatus* > *A. niger*. But for the total uptake of P, the arrangement will be: *P. pinophilum* > *A. niger* > *A. fumigatus*. The highest enhancement effect of *P. pinophilum* on P uptake was reported in wheat and faba bean plants fertilized with either superphosphate or rock phosphate (Wahid and Mehana, 2000).

Table 7: Effect of the interaction between *Rhizobium leguminosarum* (RH), phosphate-dissolving fungi (PDF) and superphosphate (Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>) on the concentration (%) and uptake (mg plant<sup>-1</sup>) of N and P by faba bean plants grown on a sandy soil

RH	P <sub>2</sub> O <sub>5</sub>	PDF	N				P					
			Seeds		Straw		Total uptake	Seeds		Straw		Total uptake
			Conc.	Uptake	Conc.	Uptake		Conc.	Uptake	Conc.	Uptake	
RH <sub>0</sub>	0	None	3.43	440	1.38	227	667	0.32	41.1	0.11	18.1	59
		Asp.n.	3.50	517	1.51	361	878	0.34	50.3	0.15	35.9	86
		Asp.f.	3.68	547	1.55	344	891	0.34	50.5	0.13	28.9	79
		Pen.	3.80	644	1.74	449	1093	0.33	56.0	0.15	38.7	94
	15.5	None	3.50	490	1.40	288	778	0.33	46.2	0.13	26.8	73
		Asp.n.	4.25	787	1.76	466	1253	0.35	64.8	0.18	47.6	112
		Asp.f.	3.50	496	1.45	326	822	0.35	49.6	0.15	33.8	83
		Pen.	3.55	682	1.96	530	1212	0.33	63.4	0.15	40.6	104
	31	None	3.50	664	1.46	426	1090	0.35	66.4	0.14	40.8	107
		Asp.n.	3.58	726	1.61	487	1213	0.38	77.1	0.19	57.5	134
		Asp.f.	4.25	867	1.59	515	1382	0.35	71.4	0.19	61.5	132
		Pen.	3.50	811	1.58	530	1341	0.40	92.7	0.22	73.8	166
RH <sub>1</sub>	0	None	3.50	582	1.55	358	940	0.33	54.9	0.12	27.7	82
		Asp.n.	3.63	641	1.64	460	1101	0.35	61.8	0.17	47.7	109
		Asp.f.	3.75	737	1.82	534	1271	0.35	68.7	0.15	44.0	112
		Pen.	3.88	688	2.11	542	1230	0.35	62.0	0.18	46.2	108
	15.5	None	3.63	648	1.79	507	1155	0.35	62.4	0.17	48.1	110
		Asp.n.	4.50	992	1.80	584	1576	0.36	79.3	0.20	64.8	144
		Asp.f.	3.65	712	1.82	527	1239	0.38	74.2	0.24	69.5	143
		Pen.	3.58	891	2.10	761	1652	0.35	87.2	0.25	90.7	177
	31	None	3.60	708	1.87	565	1273	0.38	74.7	0.18	54.4	129
		Asp.n.	3.68	749	2.23	677	1426	0.43	87.5	0.27	82.0	169
		Asp.f.	4.30	1081	2.63	913	1994	0.38	95.5	0.25	86.8	182
		Pen.	3.93	1000	1.72	644	1644	0.40	101.8	0.25	93.6	195
LSD	0.05		0.60	120	NS	169	291	0.03	15.6	0.04	17.4	22

Asp. n.: *Aspergillus niger*; Asp. f.: *A. fumigatus*; Pen.: *Penicillium pinophilum*. RH<sub>0</sub>: no *Rhizobium*; RH<sub>1</sub>: with *Rhizobium*. NS: non significant.

Hallmark and Barber (1984) found that P may increase the physiological activity of roots, consequently increases P concentration and uptake by plants. On the other hand, the increasing effect of phosphatic fertilizers on N content of plant may be attributed to their influence on number and weight of nodules. This influence in turn increases the rate of N fixation (Balasubramanian *et al.*, 1980; Hammad *et al.*, 1990).

The interactions between RH, PDF and P levels showed interesting results (Table 7). The highest total N uptake was achieved with the application of RH and *A. fumigatus* in the presence of 31 Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>. But for the total P uptake the highest value was recorded with RH plus *P. pinophilum* in presence of 31 Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>. However, there was no significant difference between levels of P (15.5 or 31 Kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>), particularly on P uptake, with RH plus *P. pinophilum*. This finding proposes the possibility of reducing the amount of superphosphate by about 50% of the ordinary used quantity, without any significant decrease in P uptake or in the yield of faba bean plants. However, this investigation needs to be re-evaluated under different soils and environmental conditions before being generalized.

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