Response of Faba Bean (*Vicia faba* L.) to Rhizobium, VA-Mycorrhizal and Phosphorine Inoculation under Starter Doses from Nitrogen Fertilization

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ABSTRACT

To study the effect of three nitrogen starter doses (0, 10 and 20 kg N/fed.) and inoculation with Rhizobium, (Rh), VA-mycorrhize (VA- M) and Phosphorine (Ph) and their interaction on seed yield and its components of faba bean, two field experiments were conducted at the Experimental Farm, Fac. of Agric. At Kafr El-Sheikh, Tanta Univ., Egypt during the two successive winter seasons 1999/2000 and 2000/2001. A split plot design with four replicates was used in this study. The main results could be summarized as follows:

1- Increasing nitrogen starter doses up to 20 kg N/fed. markedly increased plant height at harvest, number of branches/plant, number of pods/plant, number of seeds/pod, number of seeds/plant, 100-seed weight, seed yield/plant, seed yield/fed. and seed protein content. Each increament of applied nitrogen as starter doses exhibited a significant increase in all estimated traits.

2- Significant increases in all traits under study were recorded by the inoculation treatments over the control. In general, inoculation with Rhizobium alone or combined with other biefertilizer was more effective in increasing seed yield and its attributes as well as seed protein content. The highest value of all studied characters was observed for inoculation treatment with mixture of Rhizobium, VA- mycorrhize and Phosphorine together.

3- It can be stated that inoculation of faba bean seed before planting with (Rhizobium + VA-mycorrhizae + Phosphorine) as well as (Rhizobium + VA-mycorrhizae) or (Rhizobium + Phosphorine) and fertilization with 20 kg N/fed. as starter dose was the recommended for raising faba bean productivity and reducing the environmental pollution under the conditions of the present study.

INTRODUCTION

Faba bean (*Vicia faba*, L.) is one of the most important food legume crops in Egypt as a source of vegetable protein and carbohydrates for human consumption. Thus it is important to increase its production either by increasing the cultivated area or raising per unit area yield by applying the most suitable cultural practices.

Application of nitrogen fertilization to faba bean as a starter dose might be useful, Attia *et al.* (1987) pointed out that maximum seed yield/fed. was produced by nitrogen application at the rate of 20 kg N/fed. More, Soliman (1992) stated that a gradual significant increase in seed yield/fed. was detected as the nitrogen level increased up to 48 kg N/fed. Metwally (1997) found that increasing nitrogen rate up to 45 kg N/fed. markedly increased faba bean plant

height, number of branches, pods and seeds/plant, 100-seed weight as well as seed yield per plant and per feddan. Said (1998) came to the same results with raising nitrogen starter doses up to 20 kg N/fed. Furthermore, a significant stimulation influence of nitrogen application on seed yield/fed. of faba bean, was detected, particularly at the rate of 20 kg N/fed. (Hanna, 1999) as well as at the rate of 30 kg N/fed. in calcareous soil (Kerlous, 1999).

Biofertilization is very important for legumes as well as for faba bean because it supplies plants with a part of the reqirements from essential nutrients, saves a great amount of mineral fertilizers and reduces environmental pollution and costs of crop production. Moreover, biofertilizers are not expensive compared to mineral ones. Faba bean seed or soil treatment with microbial inoculants such as specific *Rhizobium* strains could supply the plants with a part of nitrogen required during different growth stages and increase seed yield and its components as well as seed protein content (Monib *et al.*, 1994). In addition, inoculation of faba bean seed with VA- mycorrhizae could supply the plants with a part of phosphorus required and increase seed yield, its attributes and seed quality (Radwan and Sheta, 1993 and Radwan, 1997). The dual inoculation of faba bean seed with both VA- mycorrhizae and Rhizobium was found to be capable of fulfilling requirements of P and N in plants and induce pronounced increases in plant growth and biological yield of faba bean and other legume crops (Hayman, 1983; Isach *et al.*, 1994; Armanios *et al.*, 1996; Hanna, 1999; Hauka, 2000; Bayoumy, 2001 and Selim and El-Saei, 2001). Furthermore, Sherif *et al.*, 1997 observed that Rhizobium inoculation and/or phosphorine increased number of pods/plant, seed index and lentil seed yield, but the increases, which resulted from Phosphorine inoculation were less than those obtained by Rhizobium inoculation. However, the highest values for all studied characters were obtained when Rhizobium inoculation and Phosphorine were used under the application of 30 kg P_2O_5/fed.

The objectives of this investigation were to study the effect of starter doses from nitrogen fertilization and inoculation with Rhizobium, Phosphorine and VA-mycorrhize on faba bean yield and its components as well as saving a great amount of mineral fertilizers and hence reducing environmental pollution and costs of crop production.

MATERIALS AND METHODS

Two field experiments were carried out at the Experimental Farm, Fac. of Agric. at Kafr El-Sheikh, Tanta Univ., Egypt during the two successive winter seasons 1999/2000 and 2000/2001. In both seasons, the experimental design was a split plot design with four replicates. The main plots were conducted for the three nitrogen starter doses, i.e., 0, 10 and 20 kg N/fed. The sub-plots were occupied by the following eight inoculation treatments, i.e., control (without inoculation), Rhizobium (Rh.), VA-mycorrhizae (VA-M), Phosphorine (Ph.), Rh.

+ VA-M, Rh. + ph., VA-M + ph and Rh. +VA-M + ph. Nitrogen fertilizer in the form of urea (46% N) was applied at the above mentioned levels after sowing and just before the sowing irrigation. Inoculation of faba bean seed was done just before sowing. (Rhizobium leguminosarum biovar, Vicea) culture prepared in the Microbiology Division, Sakha Agric. Res. Station. Phosphorine as a biofertilizer contain a highly active dissolving bacteria, which converse the insoluble tricalcium phosphate to the soluble mono-calcium phosphate and supplying the plants with its needs during different growth stages. Phosphorine produced by General Organization for Agric. Equalization Fund, Ministry of Agriculture. Seed treatment with mycorrhiza spores was done just before sowing. VA-M inoculum was prepared and added as described by Armanios et al., (1996) and Radwan (1997). Local strain of Glomus macrocarpum was kindly obtained from plant Production Dept., Fac. of Agric. (Saba Basha), Alexandria Univ., Alexandria, Egypt.

The experimental soil was clay in texture boor in organic matter (1.83%) with pH of 8.1. Available phosphorus was (14.62 ppm) and available nitrogen was (18.64 ppm) (average of the two seasons for the upper 30 cm of the soil surface).

The preceding crop was cotton and corn in the first and second seasons, respectively. Each sub plot included five ridges, each of 3.5 m long and 60 cm in width. Sowing of faba bean seed (Giza 461 variety) took place at Nov. 18th and 20th in the two seasons, respectively on two sides of ridge in hills 20 cm apart. Before the first irrigation the plants were thinned to two plants/hill. Calcium super phosphate (15.5% P_2O_5) was added during seedbed preparation at the rate of 100 kg/fed. Other cultural practices for growing faba bean were conducted as recommended.

At harvest, ten guarded plants were randomly taken from each sub plot and the following data were recorded: plant height (cm), number of branches and pods/plant, number of seeds/pod, number of seeds/plant, 100-seed weight and seed yield/plant. Seed yield/fed. was estimated from the three inner ridges of each experimental unit. Seed protein content was determined using the improved Kjeldahl method according to A.O.A.C. (1980).

All the collected data were subjected to statistical analysis as described by Snedecor and Cochran (1980). The treatment means were compared according to Duncan's multiple range test (Duncan, 1955). All statistical analysis was performed using analysis of variance technique by means of "IRRISTAT" computer software package.

RESULTS AND DISCUSSION

1. Effect of nitrogen fertilization:

The obtained results, given in Tables (1, 2 and 3) clearly show that nitrogen fertilizer level exhibited a significant effect on all estimated traits in both seasons. A gradual and significant in crease in plant height at harvest, number of branches, pods and seeds/plant, number of seeds/pod, 100-seed weight, seed yield plant, seed yield/fed. and seed protein content accompanied each increament of applied nitrogen as starter doses. The maximum values of all characters under this study were achieved when the nitrogen fertilization was applied at the rate of 20 kg N/fed. as starter dose. This trend was similar in the two seasons of experimentation. Application of 10 and 20 kg N/fed. as starter doses significantly increased seed yield/fed. by 17.1 and 36.5% as well as by 16.4 and 34.6% in the first and second seasons, respectively compared with the unfertilized treatment. The positive response of faba bean crop to nitrogen fertilizer indicated that the nitrogen demand of the crop is not being completely met by N2- fixation and therefore, symbiotic nitrogen fixation could be limiting. The low rate of inorganic nitrogen (10 and 20 kg N/fed.) stimulated both faba bean plant growth and biological nitrogen fixation process. This may be attributed to the fact that faba bean plants could obtain its nitrogen requirements from the native nitrogen in the soil and the atmospheric N_2-fixation. The previous results agreed with the findings of Attia et al., 1987; Soliman, 1992; Metwally, 1997; Said, 1998; Hanna, 1999 and Kerlous, 1999.

2. Effect of inoculation:

Data in Tables (1, 2 and 3) reveal that inoculation treatments alone or combined together exhibited significant differences in all traits under study in both seasons. The highest values of all studied characters were achieved when VA-mycerhize, Phosphorine and Rhizobium inoculation were used together. Inoculation with Rhizobium, VA-mycorhizae, Phosphorine, Rh + VA-M, Rh + Ph, VAM + Ph and Rh + VA-M + Ph increased seed yield/fed. by 8.1, 2.9, 4.7, 15.6, 17.7, 8.8 and 23.0% as well as by 7.7, 3.0, 4.4, 14.8, 16.7, 8.3 and 21.8 over the control treatment in the first and second seasons, respectively. In general, the inoculation with highly effective strain of Rhizobium alone or combined with other biefertilizer was more effective in increasing seed yield and its attributes as well as seed protein content. This could be attributed to the increase in nitrogen supply, fixed by root nodules, which is required for growth and productivity of faba bean plants. It is clear also that the combined inoculation with Rhizobium, VA-mycorrhizae and Phosphorine ensured adequate amounts of nitrogen and phosphorus, which enabled faba bean plants to produce the highest seed yield and its components. These results are in good agreement with those obtained by Radwan and Sheta (1993), Monib et al. (1994); Isach et

al. (1994); Armanios et al. (1996); Sherif et al. (1997); Hanna (1999); Hauka (2000); Bayoumy (2001) and Selim and El-Saei (2001).

3. Effect of interaction:

The interaction between nitrogen fertilizer level and inoculation treatment caused a marked effect on number of pods/plant and seed yield per plant as well as per feddan during the two growing seasons (Tables 1, 2 and 3). Data in Tables (4, 5 and 6) show clearly that the highest values from number of pods/plant (22.15 and 25.18), seed yield/plant (46.37 and 47.52 g/plant) and seed yield/fed. (22.34 and 22.97 ardab/fed.) were obtained by the application of biofertilization (Rhizobium + VA-mycorrhizae + Phosphorine) under the level of 20 kg N/fed. as starter dose in both seasons, respectively. Whereas the lowest one of these traits were given by the control treatment (without inoculation and without nitrogen fertilization) in the two seasons. From the above mentioned results, it can be stated that inoculation of faba bean seed before planting with (Rhizobium + VA-mycorrhizae + Phosphorine) as well as with (Rhizobium + VA-mycorrhizae) or (Rhizobium + Phosphorine) and fertilization with 20 kg N/fed. as starter dose could be recommended for raising faba bean productivity and decreasing pollution through decreasing nitrogen application under the environmental conditions of the present study.

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Table 1. Averages of plant height, number of branches and pods/plant and number of seeds/pod as influenced by nitrogen level and inoculation treatment in 1999/2000 and 2000/2001 growing seasons.

Factor	Plant height (cm)		No. of branches/plant		No. of pods/plant		No. of seeds/pod	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
	Season		Season		Season		Season	
N-level (kg N/fed.) (A):								
0	97.26c	98.48c	3.49c	3.89c	15.54c	17.51c	2.95c	2.98c
10	103.29b	104.39b	3.74b	4.25b	16.64b	19.30b	3.09b	3.14b
20	107.68a	109.16a	4.12a	4.69a	18.52a	22.58a	3.23a	3.26a
F- test	**	**	**	**	**	**	**	**
Inoculation treatment (B):								
Control	99.71d	100.98d	3.60d	4.05d	15.84f	18.15h	3.01c	3.05c
Rh.	102.68abc	102.19cd	3.76bcd	4.23bc	16.75cde	19.58e	3.08bc	3.10bc
VA-M.	101.40cd	103.06bcd	3.63cd	4.18bc	16.33e	18.88g	3.04bc	3.07bc
Ph.	101.80bcd	103.60bcd	3.69cd	4.15c	16.61de	19.22f	3.06bc	3.10bc
Rh+ VA-M.	103.52abc	104.54abc	3.83bc	4.40a	17.08bc	20.38c	3.09bc	3.14bc
Rh.+ Ph.	104.40ab	105.59ab	3.92ab	4.46a	17.45b	20.78b	3.12b	3.17b
VA-M. + Ph.	103.04abc	104.94abc	3.82bc	4.28a	16.82cd	20.04d	3.08bc	3.12bc
Rh.+ VA-M. + Ph.	105.38a	107.19a	4.06a	4.47a	18.34a	21.36a	3.25a	3.28a
F- test	**	**	**	**	**	**	**	**
Interaction (AXB):	NS	NS	NS	NS	**	**	NS	NS

Rh., VA- M. and Ph means inoculation with Rhizobium, VA- mycorrhize and Phosphorine, respectively.
** and NS indicate P<0.01 and not significant, respectively. Means of each factor designated by the same latter are not significantly different at 5% level, using Duncan's multiple range test.

Table 2. Averages of number of seeds/plant, 100- seed weight, seed yield/plant and seed yield/fed. as influenced by nitrogen level and inoculation treatment in 1999/2000 and 2000/2001 growing seasons.

Factor	No. of seeds/plant		100- seed weight (g)		Seed yield (g/plant)		Seed yield (ardab/fed.)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
	Season		Season		Season		Season	
N-level (kg N/fed.) (A):								
0	44.53c	51.34c	69.66c	69.86c	33.96c	35.11c	13.15c	14.21c
10	59.70b	59.24b	74.53b	74.64b	37.30b	38.46b	15.82b	16.54b
20	58.92a	72.41a	78.72a	78.52a	40.96a	42.10a	18.44a	19.12a
F- test	**	**	**	**	**	**	**	**
Inoculation treatment (B):								
Control	46.46f	54.41f	71.81e	71.92e	35.35e	36.51e	14.46e	15.17e
Rh.	50.65cde	59.81d	74.08bcd	74.13bcd	37.00c	38.14c	15.63c	16.34c
VA-M.	48.71ef	56.78e	72.32de	72.61de	35.98de	37.14de	14.88de	15.62de
Ph.	49.96de	58.64e	73.38cde	73.41cde	36.32cd	37.44cd	15.14cd	15.83cd
Rh+ VA-M..	51.93bc	62.77c	75.40ab	75.56ab	38.54b	39.68b	16.72b	17.41b
Rh.+Ph.	53.65b	64.65b	75.82ab	76.04a	38.95b	40.10b	17.02b	17.71b
VA-M. + Ph.	50.87bcd	61.62c	74.70bc	74.82abc	37.14c	38.30c	15.73c	16.43c
Rh.+ VA-M. + Ph.	58.83a	69.34a	76.92a	76.21a	40.00a	41.16a	17.79a	18.48a
F- test	**	**	**	**	**	**	**	**
Interaction (AXB):	NS	NS	NS	NS	**	**	**	**

Rh., VA- M. and Ph means inoculation with Rhizobium, VA- mycorrhize and Phosphorine, respectively.
** and NS indicate P<0.01 and not significant, respectively. Means of each factor designated by the same latter are not significantly different at 5% level, using Duncan's multiple range test.

Table 3. Averages of seed protein content as influenced by nitrogen level and inoculation treatment in 1999/2000 and 2000/2001 growing seasons.

Season	N-level (kg N/fed.) (A)				Inoculation treatment (B)									Interaction (AXB)
	F- test	0	10	20	F- test	Control	Rh.	VA-M.	Ph.	Rh+ VA-M..	Rh.+Ph.	VA-M. + Ph.	Rh.+ VA-M. + Ph.	
1999/2000	*	19.3c	19.8b	20.4a	*	19.1d	19.4cd	19.5c	19.7b	19.8b	20.2a	20.3a	20.4a	NS
2000/2001	*	19.5c	19.9b	20.5a	*	19.2d	19.6c	19.8bc	20.1ab	20.0ab	20.2a	20.3a	20.4a	NS

Rh., VA- M. and Ph means inoculation with Rhizobium, VA- mycorrhize and Phosphorine, respectively.
* and NS indicate P<0.01 and not significant, respectively. Means of each factor designated by the same latter are not significantly different at 5% level, using Duncan's multiple range test.

Table 4. Effect of interaction between N-level and different inoculation treatments on No. of pods/plant in the two growing seasons.

Inoculation treatment	1999/2000 season			2000/2001 season		
	N-level (kg N/fed.)			N-level (kg N/fed.)		
	0	10	20	0	10	20
Control	14.30l	16.23g-j	17.00efg	15.75m	17.95j	20.75e
Rh.	15.65jk	16.65f- i	17.95d	17.40kl	19.08hi	22.25d
VA-M.	15.30k	16.58f- i	17.10ef	16.95l	18.83l	20.88e
Ph.	15.63jk	16.60f- i	17.60de	17.55jk	18.98hi	21.13e
Rh+ VA-M.	15.78jk	16.75fgh	18.73c	18.03j	19.80fg	23.30c
Rh.+ Ph.	15.90ijk	16.78fgh	19.68b	18.05j	20.08f	24.20b
VA-M. + Ph.	15.75jk	16.73fgh	17.98d	17.73jk	19.43gh	22.98c
Rh.+ VA-M. + Ph.	16.03h-k	16.85e-h	22.15a	18.65i	20.25f	25.18a

Rh., VA- M. and Ph means inoculation with Rhizobium, VA- mycorrhize and Phosphorine, respectively.
Means designated by the same latter are not significantly different at 5% level, using Duncan's multiple range test

Table 5. Effect of interaction between N-level and different inoculation treatments on seed yield (g/plant) in the two growing seasons.

Inoculation treatment	1999/2000 season			2000/2001 season		
	N-level (kg N/fed.)			N-level (kg N/fed.)		
	0	10	20	0	10	20
Control	31.48m	36.21g- i	38.35cde	32.67l	37.31ghi	39.56cde
Rh.	34.35kl	37.45d-g	39.20cd	35.54jk	38.54d-g	40.36c
VA-M.	33.12l	36.35f- i	38.47cde	34.34k	37.51fgh	39.56cde
Ph.	33.52l	36.79fgh	38.65cd	34.67k	37.95e-h	39.72cd
Rh+ VA-M.	34.63jkl	37.75c-g	43.24b	35.76ijk	38.89c-g	44.38b
Rh.+ Ph.	34.74i-l	38.00c-f	44.10b	35.86ijk	39.18c-f	45.28b
VA-M. + Ph.	34.44kl	37.66c-g	39.33c	35.56jk	38.87c-g	40.46c
Rh.+ VA-M. + Ph.	35.4h-k	38.22cde	46.37a	36.52hij	39.43cde	47.52a

Rh., VA- M. and Ph means inoculation with Rhizobium, VA- mycorrhize and Phosphorine, respectively.
Means designated by the same latter are not significantly different at 5% level, using Duncan's multiple range test.

Table 6. Effect of interaction between N-level and different inoculation treatments on seed yield (ardab/fed.) in the two growing seasons.

Inoculation treatment	1999/2000 season			2000/2001 season		
	N-level (kg N/fed.)			N-level (kg N/fed.)		
	0	10	20	0	10	20
Control	11.76k	15.06fgh	16.57cde	12.48l	15.72ghi	17.32cde
Rh.	13.78ij	15.93def	17.17cd	14.53jk	16.59d-g	17.89c
VA-M.	12.92j	15.07fgh	16.66cde	13.69k	15.87fgh	17.32cde
Ph.	13.18j	15.46efg	16.79cd	13.88k	16.18e-h	17.43cd
Rh+ VA-M.	13.99hij	16.14c-f	20.04b	14.65ijk	16.85c-g	20.74b
Rh.+ Ph.	14.07hij	16.32cde	20.65b	14.72ijk	17.05c-f	21.38b
VA-M. + Ph.	13.86ij	16.08c-f	17.27c	14.51jk	16.83c-g	17.96c
Rh.+ VA-M. + Ph.	14.55ghi	16.48cde	22.34a	15.21hij	17.23cde	22.97a

Rh., VA- M. and Ph means inoculation with Rhizobium, VA- mycorrhize and Phosphorine, respectively.
Means designated by the same latter are not significantly different at 5% level, using Duncan's multiple range test.

الملخص العربي

استجابة الفول البلدي للتلقيح ببكتريا العقد الجذرية وفطر الميكروهيزا والفسفورين تحت الجرعات المنشطة من التسميد الأزوتى

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قسم المحاصيل- كلية الزراعة بكفرالشيخ- جامعة طنطا

لدراسة تأثير ثلاث مستويات من التسميد الأزوتي كجرعات منشطة و هي (صفر ،١٠، ٢٠ كجم أزوت /فدان) و التلقيح ببكتريا العقد الجذرية (الريزوبيم) فطر الميكروهيزا و الفسفورين و التفاعل بينهم علي محصول الفول البلدي و مكوناته، أجريت تجربتين حقليتين بالمزرعة البحثية لكلية الزراعة بكفر الشيخ – جامعة طنطا – مصر خلال موسمي ١٩٩٩/٢٠٠٠، ٢٠٠٠/٢٠٠١م . وقد استخدم في تنفيذ التجارب تصميم القطع المنشقة في أربع مكررات. و يمكن تلخيص أهم النتائج المتحصل عليها فيما يلى:

أدت زيادة مستويات التسميد الأزوتى كجرعات منشطة حتى ٢٠ كجم أزوت للفدان الى زيادة واضحة فى كل من صفات ارتفاع النبات عند الحصاد، عدد الفروع والقرون لكل نبات، عدد البذور فى القرن، وعدد البذور لكل نبات، وزن الـ١٠٠ بذرة، محصول البذرة لكل نبات، محصول البذور للفدان وكذلك محتوى البذور من البروتين. وقد أدت كل زيادة فى مستويات الأزوت المضافة كجرعات منشطة الى زيادة معنوية فى كل الصفات المدروسة. وقد سجلت معاملات التلقيح زيادة معنوية فى كل الصفات المدروسة. و بصفة عامة فان التلقيح ببكتريا العقد الجذرية بمفردها أو مع أى من فطر الميكروهيزا أو الفسفورين كان له التأثير الفعال الواضح على زيادة محصول البذور ومكوناته وكذلك محتوى البذور من البروتين. وقد تم التحصل على أعلى القيم من كل الصفات المدروسة عندما تم التلقيح بكل من بكتريا العقد الجذرية وفطر الميكروهيزا و الفسفورين معا. ويمكن أن نستخلص من النتائج أن تلقيح بذور الفول البلدى بكل من بكتريا العقد الجذرية وفطر الميكروهيزا و الفسفورين معا أو ببكتريا العقد الجذرية مع أى من فطر الميكروهيزا أو الفسفورين وإضافة السماد الأزوتى بمعدل ٢٠ كجم أزوت للفدان كجرعة منشطة يمكن أن ينصح به لزيادة إنتاجية الفول البلدى وكذلك تقليل التلوث البيئي نتيجة تقليل استخدام الأزوت تحت ظروف هذه الدراسة.