

Response of Faba Bean (*Vicia faba* L.) to Dual Inoculation with *Rhizobium* and VA Mycorrhiza under Different Levels of N and P Fertilization

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Abstract: Two field experiments were conducted during two successive seasons of 2003/2004 and 2004/2005 at the Experimental Farm of Faculty of Agric., Cairo Univ. at Giza, Egypt to investigate the effect of seed inoculation with *Rhizobium* and/or soil inoculation with the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae* under different levels of N and/or P fertilization on the nodulation, growth, productivity and nutritional value of two faba bean cultivars. There were significant differences between the cultivars in number and dry weight of nodules, plant height, branches and leaves number, shoot dry weight, leaf area, number and weight of pods and seeds, seed and straw yield, as well as 100-seed weight. Sakha 1 cultivar significantly surpassed Giza 40 cultivar in all previous tested parameters. Fungal infection and rhizobial inoculation either alone or in combination with 50% or 75% of the recommended doses of N and/or P fertilizers increased nodules formation, plant growth and yield as well as the concentration of N, P, K, Fe, Zn, Mn, Cu, protein, and total carbohydrate on both cultivars as compared to uninoculated plants. Biofertilizer has been identified as an alternative source for chemical fertilization to increase soil fertility and crop production in sustainable farming. It was concluded that dual inoculation was of significant importance, resulted in increase plant productivity, which could be attributed to enhancing mineral uptake, producing phytohormones and altering the microbial balance. Generally, results indicated the possibility of saving N and P fertilizers by 50% of the recommended dose without affecting on yield by *Rhizobium* and VA Mycorrhiza inoculation.

Key words: Faba bean cultivars; Biofertilizer; *Rhizobium*; Mycorrhiza; Nitrogen and phosphorous fertilization.

INTRODUCTION

Faba bean is one of the most important legume crops worldwide because it is a N₂ fixing leguminous plant, offering high quality protein, capable of returning atmospheric nitrogen to the soil. The crop area in Egypt was about 250000 feddan. As a consequence of inappropriate applications of chemical fertilizers during continuous crop cultivation, many countries suffer from problems such as pollution of agricultural lands, water resources, and soil salinization. In an attempt to reduce these chemical inputs and raise soil quality as well as improve crop production, biotechnological practices such as application of biofertilizers have been investigated. Increasing crop production is one of the major targets of agricultural policy, which could be achieved by several ways. Increasing the efficiency of atmospheric nitrogen fixation as well as the more organic phosphorus availability is considered as important ways to increase crop productivity. The nitrogen-fixing interaction between Rhizobia and legumes and the mycorrhizal association between

fungi and most of land plants are the most two commonly studied symbioses^[1]. Biofertilizers are products containing living cells of different types of microorganisms, which have an ability to convert nutritionally important elements from unavailable to available form through biological processes^[2,3].

The modern day intensive crop cultivation requires the use of chemical fertilizers but the relatively high cost of producing N fertilizer, as well as some of the problems associated with its application, such as runoff into soils and water sources, provide incentive for increased exploitation of biological nitrogen fixation (BNF). Rhizobia stimulate plant growth mainly by modifying root development, which improved macro- and micronutrients and water uptake, particularly in the early stages of plant development^[4]. The plant host organisms may be affected by one or more mechanisms such as nitrogen fixation, enhancing nutrient uptake, production of plant growth promoting substances, phytohormones, and organic acids, as well as prediction of plant from pathogens^[5,6,7,8] and the organism benefits by acquiring photosynthates from the plant^[9].

Egyptian soils are typically containing high levels of CaCO₃ and high pH. This type of soil requires high amounts of P fertilizer to support adequate plant growth^[10]. Moreover, sources of P fertilizer are in short supply, which may result in a crisis in plant nutrition in the 21st century^[11]. Thus, understanding the means by which AM fungi enable P acquisition and finding ways to employ this symbiosis for world-wide agriculture are of critical importance. Arbuscular mycorrhizae (AM) are symbiotic associations, formed between plants and soil fungi that play an essential role in plant growth, plant protection, and soil quality. The AM fungi expand their filaments in soil and plant roots. This filamentous network promote bi-directional nutrient movement where soil nutrients and water move to the plant and plant photosynthates flow to the fungal network Seguin *et al.*^[12]. VA Mycorrhizal symbiosis may benefit the host plant primarily by increasing the ability of the root system to absorb and translocate phosphorus through an extensive network of external hyphae^[13]. VAM fungi contribute to biological control when they interact with plant pathogens and reduce disease incidence^[14]. In this respect, Mott and Zuberer^[15] found a significant increase in plant dry weight, N and P content wherever the tripartite association of rhizobia, mycorrhizal fungi, and legumes were present. El-Ghandour *et al.*^[16], Ahmed and Elsheikh^[17], Rabie^[18], and Koreish *et al.*^[19] revealed that nodulation, growth, yield, and nutrient uptake of faba beans can be significantly enhanced by both *Rhizobium* and mycorrhizal inoculation. Moreover, Badr El-Din and Moawad^[20] and El-Ghandour *et al.*^[16] reported that the dual inoculation with both rhizobia and mycorrhizae induced more significant increases in plant dry weight, N and P content of faba bean than inoculation with either VA mycorrhizae or *Rhizobium* alone.

Indeed, utilization of *Rhizobium*-VAM association is a vital area for practical studies dealing with maximizing fertilizer efficiency to conserve energy, material resources, and reduce costs of food production. The objective of the present study is to evaluate the response of faba bean to inoculation with *Rhizobium* and/or VA mycorrhiza under different levels of N and/or P fertilization.

MATERIALS AND METHODS

Experimental Layout: Two field experiments were carried out at the Experimental Farm of Faculty of Agric., Cairo Univ. at Giza, Egypt, during the two successive seasons of 2003/2004 and 2004/2005 to evaluate the influence of seed inoculation with *Rhizobium* and/or soil inoculation with the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus mosseae*

at different levels of N and/or P fertilization on the nodulation, growth, yield, yield components, and nutritional value of two faba bean cultivars (*Vicia faba* L.).

Plant: Seeds of two faba bean cultivars, namely Sakha 1 and Giza 40 were obtained from Agronomy Res. Institute, Agric. Res. Centre, Ministry of Agric., Giza.

Soil: The experimental soil was a clay loam had a pH of 7.8 and EC of 2.1 mmhos/cm. Soil nutrient determination^[21] included 50 ppm available N, 19 ppm available P, and 391 ppm available K.

Inocula and Methods of Inoculation:

Rhizobium Inoculant: *Rhizobium* seed inoculation was done by using effective *Rhizobium leguminosarum* bv. *Vicia* ICARDA 441 which was provided from the Dept. of Agric. Microbiol., Agric. Res. Center, Giza. Slurry inoculation technique, in which the inoculant was mixed with moistened seeds in presence of Arabic gum as an adhesive, was followed.

Mycorrhiza Inoculant: Mycorrhiza soil inoculation was done by planting the seeds over a thin layer of mycorrhizal inoculum consists of rhizosphere soil from maize plants pot culture of *Glomus mosseae*, which contained spores, mycelium, and infected root fragments. *Glomus mosseae* was kindly provided from MERCEN, Ain Shams Univ.

Treatments: Each experiment included twenty treatments which were the combination of two faba bean cultivars i.e., Sakha 1 and Giza 40 and the treatments which were conducted as follows:

- Control (100%N+100%P "recommended doses").
- Inoculation with *Rhizobium*+75%N+100%P.
- Inoculation with *Rhizobium*+50%N+100%P.
- Inoculation with *Rhizobium*+25%N+100%P.
- Inoculation with VAM +100%N+75%P.
- Inoculation with VAM +100%N+50%P.
- Inoculation with VAM +100%N+25%P.
- Inoculation with both symbionts+75%N+75%P.
- Inoculation with both symbionts+50%N+50%P.
- Inoculation with both symbionts+25%N+25%P.

All treatments received 50 Kg/fed. potassium sulphate (48% K₂O). Nitrogen and phosphorus fertilizers were applied to the control treatment at the rate of 15 kg/fed. ammonium sulphate (20.5%N) and 150 kg/fed. superphosphate (15.5%P₂O₅), respectively. All additions were as recommended by Ministry of Agric.

Field Experiment: The treatments were arranged in a split plot design with four replicates, where faba bean cultivars were distributed in the main plots and the biofertilization treatments were occupied the sub-plots. The experimental unit consisted five ridges four meter in length and 60 cm apart (plot area = 4x3= 12m²). The distance between plants was 20 cm. Seeds were planted on November 5 in both seasons. Normal cultural practices were followed as usual in faba bean fields. After complete germination and at 20 days after sowing, plants were thinned leaving one plant per hill.

Measured Parameters: Plants of five replicates were taken 60 and 90 days old after sowing for evaluating number and dry weight of nodules.

Plant height "cm", branches and leaves number/plant, shoot dry weight /plant in "gm" were estimated at 60, 90 and 120 days from sowing by harvesting five guarded plants at random from each treatment. Total leaf area (LA) "cm² /plant" was measured by using leaf area meter model LI 3050A.

At full maturity, number of pods and seeds/plant, weight of pods and seeds/plant in "gm" and 100-seed weight were recorded using a sample of ten random guarded plants from the middle ridges of each plot, meanwhile, seed and straw yields "ton/fed" were determined from the three middle rows of every plot.

Chemical Analysis: Random samples of seeds and straw of each treatment were taken. N and P were determined according to Pregl^[22] and Jackson^[23], respectively. K was determined using the Flamephotometer. Fe, Zn, Mn and Cu were determined using an Atomic Absorption Spectro-photometer. The percentage of total carbohydrates was determined according to A.O.A.C.^[24]. Crude protein percentage was determined by multiplying the nitrogen percentage by 6.25.

Statistical Analysis: Data of nodulation, growth, yield, and its components were statistically analyzed according to Snedecor and Cochran^[25]. Combined analysis was made for the two growing seasons, whereas the results of the two seasons followed similar trend. For comparison between means, LSD test at 5% level was used.

RESULTS AND DISCUSSION

Nodulation: It is evident from Table (1) that number and dry weight of nodules were greater in Sakha 1 cultivar than Giza 40 cultivar. This increment may reflect the preferability of Sakha 1 cultivar as a good host-inoculum rather than Giza 40.

The data in the same table illustrate that the application of 75% or 50% of the recommended dose of N fertilizer with *Rhizobium* inoculant or the application of 75% or 50% of the recommended dose of P fertilizer with Mycorrhiza inoculant resulted in pronounced increases on the number and dry weight of nodules of the two cultivars compared with uninfected ones, which only received the recommended dose of N and P. A similar positive effect was observed by Badr El-Din and Moawad^[20], Ahmed and Elsheikh^[17] and Ahmed *et al.*^[26]. It is apparent that the inoculation with *Rhizobium* ensures the presence of a high density of these organisms in close proximity to the root systems of the seedlings causing the development of high numbers of nodules especially in the early stage of plant development. This is in agreement with the findings of other workers e.g., Antoun *et al.*,^[4] and Dileep-Kumar *et al.*,^[8], who found that rhizobia are capable of colonizing the root of legumes and produce plant growth-promoting substances of phytohormonal nature and exhibit antagonistic effects against many plant pathogenic fungi. It is worthy of note that seed inoculation with *Rhizobium* in presence of 50% of the recommended N induced more significant increase in nodulation. It is quite possible that excess N had reverse effect on nodulation since the available carbohydrates seeping from the shoots to the root system would be low causing feeble rhizobial growth resulting in lower nitrogen fixation. These results are in accordance with those obtained by Alexander^[27], who demonstrated that excess inorganic nitrogen reduced nodulation, root hair production, and leghaemoglobin synthesis. It is also evident that the favourable effect of Mycorrhiza on faba bean nodulation runs parallel with the findings by Carling *et al.*^[28], who found that the mycorrhizae aid in the uptake of P, S, and minor elements such as Co, Cu, and Zn, which in turn enhance nodulation and dinitrogen fixation.

Dual inoculation with both *Rhizobium* and VA mycorrhiza was a superior practice for nodules formation especially under 50% of the recommended rate of mineral N and P fertilization, compared to either alone or uninoculated ones as clearly shown in Table (1). This is in accordance with the findings of Barea and Azcon-Aguilar^[29], El-Hassanin and Lynd^[30] and Rabie^[18] who mentioned that the main effect of VA mycorrhiza on *Rhizobium* activity and N₂ fixation to be phosphate mediated mechanism and the synergism between both symbionts.

Growth Characters: The results presented in Tables (2a,b&c) show that there were significant differences between faba bean cultivars in plant height,

Table 1: Nodulation of the two cultivars of faba bean after 60 and 90 days of sowing as affected by inoculation with *Rhizobium* and/or Mycorrhiza in the presence of different levels of N and P fertilization (Average of the two seasons).

Character	Nodules number/plant						Nodules dry weight "mg/plant"					
	60			90			60			90		
Plant age (days)												
Varieties(A)	Sakha 1	Giza 40	Mean (B)	Sakha 1	Giza 40	Mean (B)	Sakha 1	Giza 40	Mean (B)	Sakha 1	Giza 40	Mean (B)
Control(100%NP)	45.0	28.7	36.8	67.3	46.0	56.7	300.2	185.0	242.6	673.8	440.1	557.0
Rh.+75%N+100%P	51.3	34.0	42.7	78.7	56.0	67.3	333.5	208.0	270.8	849.1	580.5	714.8
Rh.+50%N+100%P	55.3	38.0	46.7	85.7	63.0	74.3	380.0	231.6	305.8	889.9	643.1	766.5
Rh.+25%N+100%P	37.3	22.0	29.7	53.7	35.7	44.7	249.8	164.8	207.3	561.8	369.4	465.6
VAM+100%N+75%P	52.3	33.7	43.0	81.0	58.0	69.5	331.1	206.1	268.6	856.6	590.3	723.5
VAM+100%N+50%P	48.0	31.0	39.5	76.0	51.3	63.7	319.9	194.4	257.2	779.7	489.6	634.7
VAM+100%N+25%P	32.3	20.3	26.3	47.7	31.3	39.5	247.3	161.4	204.4	551.8	365.4	458.6
Rh.+VAM+75%NP	52.7	35.0	43.8	82.3	59.0	70.7	356.4	219.5	288.0	870.3	621.4	745.9
Rh.+VAM+50%NP	56.0	39.0	47.5	87.3	65.0	76.2	387.3	256.0	321.7	921.0	651.2	786.1
Rh.+VAM+25%NP	40.0	26.0	33.0	58.0	41.0	49.5	268.5	170.0	219.3	580.0	388.2	484.1
Mean (A)	47.0	30.8		71.8	50.6		317.4	199.7		753.4	513.9	
L.S.D.0.05 (A)	2.985			4.077			24.51			46.76		
L.S.D.0.05 (B)	6.674			9.117			54.81			104.6		
L.S.D.0.05 (AxB)	9.439			12.89			77.51			147.9		

Table 2a: Growth characters of the two cultivars of faba bean after 60, 90 and 120 days of sowing as affected by inoculation with *Rhizobium* and/or Mycorrhiza in the presence of different levels of N and P fertilization (Average of the two seasons).

Character	Leaves number/plant									Shoot dry weight "g/plant"								
	60			90			120			60			90			120		
Plant age (days)																		
Varieties(A)	Sakha 1	Giza 40	Mean (B)	Sakha 1	Giza 40	Mean (B)	Sakha 1	Giza 40	Mean (B)	Sakha 1	Giza 40	Mean (B)	Sakha 1	Giza 40	Mean (B)	Sakha 1	Giza 40	Mean (B)
Control(100%NP)	38.0	28.0	33.0	50.0	43.0	46.5	80.0	63.0	71.5	14.0	9.4	11.7	45.9	32.1	39.0	74.9	55.6	65.3
Rh.+75%N+100%P	46.0	37.0	41.5	70.0	58.0	64.0	104.7	86.0	95.3	16.1	12.8	14.5	58.1	43.0	50.6	96.8	77.3	87.1
Rh.+50%N+100%P	42.0	33.7	37.8	66.0	51.3	58.7	94.0	77.0	85.5	15.0	10.9	13.0	51.9	38.0	45.0	89.2	67.1	78.2
Rh.+25%N+100%P	31.0	22.0	26.5	36.0	31.0	33.5	50.0	39.0	44.5	10.2	6.8	8.5	32.0	22.2	27.1	46.1	35.1	40.6
VAM+100%N+75%P	44.3	34.0	39.2	70.0	55.0	62.5	100.0	81.0	90.5	15.7	11.7	13.7	55.2	40.1	47.7	93.7	72.0	82.9
VAM+100%N+50%P	41.3	32.0	36.7	64.3	50.0	57.2	90.0	71.0	80.5	14.8	10.5	12.7	49.9	36.3	43.1	86.3	65.0	75.7
VAM+100%N+25%P	32.0	24.0	28.0	38.7	35.0	36.8	61.0	45.0	53.0	11.3	7.3	9.3	36.3	24.6	30.5	54.0	38.3	46.2
Rh.+VAM+75%NP	48.0	40.7	44.3	74.0	64.0	69.0	112.3	93.7	103.0	18.7	13.2	16.0	63.3	45.3	54.3	106.0	81.9	94.0
Rh.+VAM+50%NP	46.0	35.0	40.5	68.0	55.0	61.5	108.0	88.0	98.0	16.8	11.6	14.2	60.0	40.0	50.0	98.6	74.1	86.4
Rh.+VAM+25%NP	30.0	24.0	27.0	38.3	34.0	36.2	56.0	43.0	49.5	10.8	6.8	8.8	35.0	23.0	29.0	50.0	38.0	44.0
Mean (A)	39.9	31.0		57.5	47.6		85.6	68.7		14.3	10.1		48.8	34.5		79.6	60.4	
L.S.D.0.05 (A)	4.050			5.769			6.367			1.299			3.773			5.800		
L.S.D.0.05 (B)	9.055			12.90			14.24			2.904			8.437			12.97		
L.S.D.0.05 (AxB)	12.81			18.24			20.13			4.107			11.93			18.34		

branches and leaves number/plant, shoot dry weight/plant, and LA /plant after 60, 90 and 120 days from planting. It can also be notice that Sakha 1 cultivar exceeded significantly Giza 40 cultivar in all previous growth characters. These results agreed with those of Ahmed and El-Abagy^[31], who mentioned that the differences among faba bean cultivars in

growth characters may be due to the differences in number of nodules formed on the root of the tested cultivar, consequently, the growth of each cultivar may depended mainly on dinitrogen fixation, also to the differences in partition and migration of photosynthates between cultivars and the endogenous hormones content.

Table 2b: Growth characters of the two cultivars of faba bean after 60, 90 and 120 days of sowing as affected by inoculation with *Rhizobium* and/or Mycorrhiza in the presence of different levels of N and P fertilization (Average of the two seasons).

Character	Plant height "cm"									Branches number/plant								
	60			90			120			60			90			120		
Plant age (days)	Sakha	Giza	Mean (B)	Sakha	Giza	Mean (B)	Sakha	Giza	Mean (B)	Sakha	Giza	Mean (B)	Sakha	Giza	Mean (B)	Sakha	Giza	Mean (B)
Varieties(A)	1	40		1	40		1	40		1	40		1	40		1	40	
Treatments(B)																		
Control(100%NP)	47.3	33.0	40.2	61.7	49.3	55.5	100.0	73.7	86.8	3.0	2.3	2.7	4.3	3.0	3.7	5.0	4.0	4.5
Rh.+75%N+100%P	62.3	51.0	56.7	84.3	76.3	80.3	135.3	110.3	122.8	4.0	3.0	3.5	5.7	4.0	4.8	7.3	6.0	6.7
Rh.+50%N+100%P	52.7	41.3	47.0	68.0	60.0	64.0	122.7	95.0	108.8	3.3	2.3	2.8	4.0	3.0	3.5	6.7	5.0	5.8
Rh.+25%N+100%P	40.0	28.3	34.2	50.0	41.3	45.7	71.7	59.7	65.7	2.0	1.7	1.8	3.0	2.3	2.7	3.3	2.3	2.8
VAM+100%N+75%P	57.7	48.0	52.8	78.7	71.7	75.2	131.3	103.3	117.3	3.7	2.7	3.2	5.0	3.7	4.3	7.0	5.0	6.0
VAM+100%N+50%P	50.7	37.3	44.0	66.0	56.7	61.3	113.7	91.7	102.7	3.0	2.3	2.7	4.0	3.0	3.5	6.7	4.7	5.7
VAM+100%N+25%P	41.7	30.3	36.0	53.7	40.3	47.0	89.3	61.3	75.3	2.3	2.0	2.2	3.3	2.7	3.0	4.0	3.0	3.5
Rh.+VAM+75%NP	65.7	53.7	59.7	89.0	80.0	84.5	141.3	115.3	128.3	4.3	3.3	3.8	6.0	4.7	5.3	8.0	6.7	7.3
Rh.+VAM+50%NP	55.0	44.3	49.7	74.0	64.7	69.3	130.3	114.0	122.2	3.7	3.0	3.3	5.3	4.0	4.7	7.3	6.7	7.0
Rh.+VAM+25%NP	41.0	30.0	35.5	52.3	41.7	47.0	85.7	61.7	73.7	2.3	2.0	2.2	3.0	2.7	2.8	3.7	2.7	3.2
Mean (A)	51.4	39.7		67.8	58.2		112.1	88.6		3.2	2.5		4.4	3.3		5.9	4.6	
L.S.D.0.05 (A)	2.711			3.308			6.425			0.456			0.544			0.596		
L.S.D.0.05 (B)	6.062			7.397			14.37			1.020			1.215			1.333		
L.S.D.0.05 (AxB)	8.573			10.46			20.32			1.443			1.719			1.885		

Table 2c: Growth characters of the two cultivars of faba bean after 60, 90 and 120 days of sowing as affected by inoculation with *Rhizobium* and/or Mycorrhiza in the presence of different levels of N and P fertilization (Average of the two seasons).

Character	Leaves area "cm ² /plant"								
	60			90			120		
Plant age (days)	Sakha	Giza	Mean (B)	Sakha	Giza	Mean (B)	Sakha	Giza	Mean (B)
Varieties(A)	1	40		1	40		1	40	
Treatments(B)									
Control(100%NP)	1816	1241	1528	4038	3179	3609	10010	6612	8311
Rh.+75%N+100%P	2372	1695	2034	5153	4004	4579	12481	9021	10751
Rh.+50%N+100%P	2153	1556	1855	4701	3612	4157	11904	8501	10203
Rh.+25%N+100%P	1306	885	1096	2803	2450	2627	7662	5093	6378
VAM+100%N+75%P	2161	1622	1892	4681	3788	4234	12301	8729	10515
VAM+100%N+50%P	2055	1443	1749	4511	3519	4015	11763	8255	10009
VAM+100%N+25%P	1431	989	1210	2955	2695	2825	8045	5139	6592
Rh.+VAM+75%NP	2703	1981	2342	5488	4368	4928	13331	9151	11241
Rh.+VAM+50%NP	2408	1706	2057	5056	3802	4429	12676	8946	10811
Rh.+VAM+25%NP	1400	976	1188	3002	2617	2810	7967	5195	6581
Mean (A)	1981	1409		4239	3403		10814	7464	
L.S.D.0.05 (A)	173.6			276.7			334.6		
L.S.D.0.05 (B)	388.2			618.7			748.2		
L.S.D.0.05 (AxB)	548.9			875.0			1058		

Also, it is evident that *Rhizobium* inoculation with 75% or 50% of recommended N and VAM inoculation with 75% or 50% of recommended P, both improved plant growth on both cultivars expressed as plant height, leaves number and LA /plant and shoot dry weight /plant at 60, 90 and 120 days after sowing compared with the uninoculated plants. These results are in harmony with those obtained by Badr El-

Din and Moawad^[20] and Ahmed and Elsheikh^[17]. This enhancement of plant growth could be referred to that rhizobia may augment plant growth by providing products of dinitrogen fixation, either by a direct bacterium-plant transport of fixed nitrogen or by a slow transfer due to a gradual death of the bacteria and their subsequent mineralization. In addition, the promotive effect of *Rhizobium* or VAM on plant growth might be

attributed to one or more of the following mechanisms, which include mobilization of insoluble nutrients followed by enhancement of uptake by the plant, production of toxic substances to soil-born pathogens, and production of plant growth regulators that stimulate plant growth^[32,4,8].

Data illustrated in the same tables show clearly that the dual inoculation with both symbionts under 75% or 50% of recommended dose of N and P fertilization induced more significant increases in all parameters than inoculation with either *Rhizobium* or VAM alone. Thus, dual infection was the most effective for both cultivars with regard to plant growth. These results are in agreement with those obtained by Mott and Zuberer^[15], El-Ghandour *et al.*^[16], Ahmed and Elsheikh^[17] and Rabie^[18], who found a significant increase in plant dry weight wherever the tripartite association of rhizobia, mycorrhizal fungi and legumes were present. It is quite apparent that the combination of VA mycorrhiza and *Rhizobium* was the most effective combination for plant growth, which could be due to the simulative effect of VA mycorrhizal fungi on N₂ fixation through its effect on P uptake^[33].

Yield and its Components: Faba bean cultivars significantly differed in number of pods and seeds/plant, weight of pods and seeds/plant, seed and straw yield/fed, as well as 100-seed weight (Tables 3a&b). Generally, Sakha 1 cultivar significantly surpassed Giza 40 cultivar in all previous characters.

Data on the rhizobial inoculation or fungal infection in presence of 50% of recommended rate of N or P fertilizers, respectively are presented in the same tables. Results indicate that there were appreciable increases in number of pods and seeds /plant, weight of pods and seeds/plant, as well as seed and straw yield /fed on both cultivars as compared with uninfected ones. In general, these increments were more significant in Sakha 1 cultivar as compared with Giza 40 cultivar. These favorable results of *Rhizobium* or VAM inoculation on faba bean yield run parallel with the findings of El-Ghandour *et al.*^[16]. Moawad *et al.*^[4] showed that rhizobial inoculation increased seed yield of faba bean plant, which could be attributed to the positive effect of *Rhizobium* on enhancement the nodulation, N content, and plant dry weight. It is worthy to notice that, in the *Rhizobium* inoculated treatments application of ammonium sulphate above 50% of the recommended dose showed a decrease in the yield of seeds but increasing the all growth characters as well as the straw yield. It means that addition of excess amounts of N is usually utilized for building more vegetative growth rather than giving high yield of seeds. Excess nitrogen will push the plant to grow vigorously deriving the carbohydrates to build

more vegetative growth. Similarly, fungal infection plus added 50% of the recommended dose of P fertilizer gave the highest yield of seeds. This may explain by the observation of Ahmed *et al.*^[26], who found that high phosphorus application completely inhibited the native VAM and suppressed mycorrhizal infection in the VAM-inoculated faba bean plants. Moreover, they attributed the increase in yield associated with VAM inoculations to the significantly increases in flower set and pod production as well as to the improvement in P uptake suggesting the possibility of using VAM as a low input technology in poor soils. There is well-documented evidence that arbuscular mycorrhizal fungi can assist the plant in obtaining mineral nutrients, primarily P, by using their extensive network of external hyphae, which branch and invade regions of the rhizosphere, i.e., VAM contribute to increasing availability and uptake of P and micronutrients^[35].

Also, it is clear that the dual infection plus added 50% of recommended dose of N and P fertilization showed the maximum significant increases in number of pods and seeds/plant, weight of pods and seeds /plant, as well as seed yield /fed on both cultivars as compared with the uninfected plants or those inoculated with *Rhizobium* or VAM alone. On the other hand, the increment in straw yield /fed by using dual inoculation plus 75% of recommended rate of N and P fertilization was more significant than that in case of 50%. These positive effect of the dual inoculation on yield coincide with those of El-Ghandour *et al.*^[16]. Hence, the maximum seed yield was observed in treatments inoculated with the both symbionts. This supports previous results indicating that *Rhizobium* enhances the mycorrhizal establishment by producing polysaccharides that leading to increase in the synthesis of polyglacturonase enzyme at the infection site, which facilitate the permeability of the root cell to the fungus^[36]. Moreover, inoculation with both symbionts increase the capacity of plants in utilizing light, water, mineral nutrients, and carbon dioxide in building great amount of metabolizes easily translocated from source to sink and finally accumulated in pods and seeds of faba bean plant.

Chemical Composition: Regarding the chemical changes, it is obvious from Tables (4a&b and 5a&b) that Sakha 1 cultivar showed the highest values of all the nutritional values except Mn concentration in the straw.

The data in the same Tables point out that the application of 75% or 50% of the recommended dose of N fertilizer with *Rhizobium* inoculant or the application of 75% or 50% of the recommended dose of P fertilizer with VAM inoculant resulted in considerable increases on the concentration of N, P, K,

Table 3a: Yield and its components of the two cultivars of faba bean as affected by inoculation with *Rhizobium* and/or Mycorrhiza in the presence of different levels of N and P fertilization (Average of the two seasons).

Character	No .of pods/ plant			Weight of pods"g"/plant			No. of seeds/ plant			Weight of seeds"g"/plant		
	Sakha	Giza	Mean (B)	Sakha	Giza	Mean (B)	Sakha	Giza	Mean (B)	Sakha	Giza	Mean (B)
Treatments(B)	1	40		1	40		1	40		1	40	
Control(100%NP)	28.0	20.0	24.0	130.4	60.3	95.4	120.3	70.3	95.3	114.2	48.0	81.1
Rh.+75%N+100%P	31.7	21.7	26.7	146.0	66.1	106.1	140.0	77.7	108.8	132.8	54.2	93.5
Rh.+50%N+100%P	37.0	27.0	32.0	182.5	93.6	138.1	174.0	105.0	139.5	163.6	74.1	118.9
Rh.+25%N+100%P	23.0	15.7	19.3	100.1	45.0	72.6	89.7	50.0	69.8	82.0	33.1	57.6
VAM+100%N+75%P	32.0	22.7	27.3	153.8	70.4	112.1	141.0	83.7	112.3	137.1	60.0	98.6
VAM+100%N+50%P	37.0	26.3	31.7	184.0	93.2	138.6	178.3	102.3	140.3	168.6	75.0	121.8
VAM+100%N+25%P	25.3	17.0	21.2	112.3	50.8	81.6	103.7	56.3	80.0	95.3	37.0	66.2
Rh.+VAM+75%NP	34.3	24.0	29.2	161.1	78.0	119.6	158.0	91.3	124.7	150.0	65.4	107.7
Rh.+VAM+50%NP	38.0	30.7	34.3	188.0	97.1	142.6	182.0	116.0	149.0	171.5	78.1	124.8
Rh.+VAM+25%NP	25.0	19.0	22.0	120.2	53.0	86.6	107.7	64.7	86.2	100.0	39.9	70.0
Mean (A)	31.1	22.4		147.8	70.8		139.5	81.7		131.5	56.5	
L.S.D.0.05 (A)	2.013			6.869			6.606			9.081		
L.S.D.0.05 (B)	4.502			15.36						20.30		
L.S.D.0.05 (AxB)	6.366			21.72			20.89			28.72		

Table 3b: Yield and its components of the two cultivars of faba bean as affected by inoculation with *Rhizobium* and/or Mycorrhiza in the presence of different levels of N and P fertilization (Average of the two seasons).

Character	100-seed weight			Seed yield "ton/fed"			Straw yield "ton/fed"		
	Sakha 1	Giza 40	Mean (B)	Sakha 1	Giza 40	Mean (B)	Sakha 1	Giza 40	Mean (B)
Treatments(B)									
Control(100%NP)	90.0	66.8	78.4	5.41	2.51	3.96	6.68	3.81	5.25
Rh.+75%N+100%P	93.9	74.8	84.4	6.37	3.00	4.69	8.53	5.01	6.77
Rh.+50%N+100%P	93.0	79.8	86.4	7.65	3.80	5.73	8.36	4.51	6.44
Rh.+25%N+100%P	85.0	65.9	75.5	3.88	1.69	2.79	4.50	2.36	3.43
VAM+100%N+75%P	94.3	80.8	87.6	6.54	3.04	4.79	8.38	4.88	6.63
VAM+100%N+50%P	93.0	81.0	87.0	7.81	3.88	5.85	8.26	4.38	6.32
VAM+100%N+25%P	85.8	66.0	75.9	4.29	1.92	3.11	5.00	3.00	4.00
Rh.+VAM+75%NP	96.8	79.0	87.9	7.00	3.27	5.14	9.21	5.30	7.26
Rh.+VAM+50%NP	95.4	80.0	87.7	8.00	4.02	6.01	8.61	4.70	6.66
Rh.+VAM+25%NP	83.8	63.8	73.8	4.58	2.11	3.35	5.10	2.77	3.94
Mean (A)	91.1	73.8		6.15	2.92		7.26	4.07	
L.S.D.0.05 (A)	4.370			0.274			0.320		
L.S.D.0.05 (B)	9.771			0.613			0.715		
L.S.D.0.05 (AxB)	13.82			0.867			1.011		

Fe, Zn, Mn, and Cu as well as the percentage of protein, and total carbohydrates in seeds and straw of the two cultivars comparable to those which only received the recommended dose of N and P, except P concentration in seeds and straw of Giza 40 cultivar

when plants inoculated with *Rhizobium* and fertilized with 50% of the recommended dose of N. Moreover, fungal infection was the most effective for both cultivars with regard to P, K, Fe, Zn, Mn, and Cu uptake as well as total carbohydrates percentage.

Table 4a: Chemical composition of faba bean seeds of the two cultivars as affected by inoculation with *Rhizobium* and/or Mycorrhiza in the presence of different levels of N and P fertilization (Average of the two seasons).

Character	N		P		K		Fe		Zn		Mn		Cu	
	Sakha	Giza	Sakha	Giza	Sakha	Giza	Sakha	Giza	Sakha	Giza	Sakha	Giza	Sakha	Giza
Varieties(A)	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Treatments(B)														
Control(100%NP)	43.2	37.1	11.8	10.3	15.4	15.2	362	341	91	80	26.0	25.0	20.1	19.0
Rh.+75%N+100%P	49.1	42.8	12.8	11.4	18.6	16.6	370	356	94	85	28.1	26.6	22.0	20.8
Rh.+50%N+100%P	44.0	38.0	11.8	10.3	16.1	16.0	367	351	94	84	26.9	25.5	20.7	19.7
Rh.+25%N+100%P	37.3	32.4	11.3	10.0	14.0	13.1	320	291	80	71	22.8	21.8	18.3	16.8
VAM+100%N+75%P	46.7	40.8	14.9	13.0	19.0	18.3	390	393	101	90	29.0	27.3	22.0	21.3
VAM+100%N+50%P	44.8	38.8	13.5	11.8	17.2	17.0	378	364	96	88	28.0	25.8	21.1	20.2
VAM+100%N+25%P	42.1	36.1	11.0	9.4	14.3	13.8	341	300	85	73	23.1	23.4	19.1	18.0
Rh.+VAM+75%NP	55.6	47.9	14.8	13.2	20.0	19.1	420	408	105	94	29.8	28.9	22.8	22.0
Rh.+VAM+50%NP	52.0	45.6	13.7	12.1	17.6	17.9	408	380	98	95	31.0	26.2	21.4	22.2
Rh.+VAM+25%NP	40.9	34.6	10.3	9.0	14.9	14.1	317	320	82	76	24.3	22.6	18.0	17.2

Table 4b: Chemical composition of faba bean seeds of the two cultivars as affected by inoculation with *Rhizobium* and/or Mycorrhiza in the presence of different levels of N and P fertilization (Average of the two seasons).

Character	Protein (%)		Total carbohydrate (%)	
	Sakha 1	Giza 40	Sakha 1	Giza 40
Varieties(A)				
Treatments(B)				
Control(100%NP)	27.0	23.2	56.0	47.1
Rh.+75%N+100%P	30.7	26.8	63.8	51.3
Rh.+50%N+100%P	27.5	23.8	59.0	49.0
Rh.+25%N+100%P	23.3	20.3	51.1	41.3
VAM+100%N+75%P	29.2	25.5	66.9	54.3
VAM+100%N+50%P	28.0	24.3	60.0	52.1
VAM+100%N+25%P	26.3	22.6	52.4	43.2
Rh.+VAM+75%NP	34.8	29.9	70.3	58.8
Rh.+VAM+50%NP	32.5	28.5	61.9	55.0
Rh.+VAM+25%NP	25.6	21.6	53.5	44.0

These increments were followed with parallel increases in pod and seed yields. It is worthy of note that rhizobial inoculation is of economical importance, it improved N concentration as a result of increasing N₂ fixation capacity. Moreover, inoculation with *Rhizobium* increased the nodulation of faba bean plant causing more N₂ fixation and consequently the nutrients (nitrogenous materials) secreted into the soil, it is also increased rhizospheric microflora especially the acid producers and phosphate dissolvers causing more available phosphorus, which agrees with the findings of Lipman and Conybeare^[37]. Similarly, Abd-Alla and

Omar^[38] found that *Rhizobium* inoculation significantly increased N and P contents of faba bean plant. Antoun *et al.*^[4] reported that rhizobia improve macro- and micronutrients uptake by modifying root development. It is also interesting to denote that the mechanism of increasing plant P concentration by VAM inoculation is based on the relatively high activity of VA mycorrhizal hyphae in absorbing soil P and then translocate onto the host roots through a specific efficient active translocation as mentioned by Cooper and Tinker^[39]. VAM may also lower the pH value of the soil, which increases the solubility of

Table 5a: Chemical composition of faba bean straw of the two cultivars as affected by inoculation with *Rhizobium* and/or Mycorrhiza in the presence of different levels of N and P fertilization (Average of the two seasons).

Character	N		P		K		Fe		Zn		Mn		Cu	
	Sakha	Giza	Sakha	Giza	Sakha	Giza	Sakha	Giza	Sakha	Giza	Sakha	Giza	Sakha	Giza
Control(100%NP)	20.0	14.7	1.8	1.6	24.8	22.8	796	653	107	100	35.5	38.9	23.0	21.3
Rh.+75%N+100%P	22.1	16.1	1.9	1.7	27.6	24.6	860	681	110	107	37.2	42.6	25.0	23.0
Rh.+50%N+100%P	20.8	14.8	1.9	1.6	26.5	23.8	831	670	113	107	36.6	40.0	23.7	21.8
Rh.+25%N+100%P	17.1	12.1	1.6	1.5	22.0	20.8	680	553	94	88	30.8	34.2	20.8	19.3
VAM+100%N+75%P	21.0	15.4	2.3	1.8	31.4	26.3	883	743	119	109	39.1	45.3	24.6	23.6
VAM+100%N+50%P	20.5	15.0	1.9	1.7	29.1	25.1	852	698	114	112	38.0	40.9	23.9	22.2
VAM+100%N+25%P	19.1	14.0	1.5	1.4	22.6	21.3	731	571	100	92	32.3	36.4	21.7	20.0
Rh.+VAM+75%NP	24.3	17.8	2.2	2.0	31.2	27.7	911	767	121	115	40.0	44.5	25.8	24.1
Rh.+VAM+50%NP	22.8	17.0	2.0	1.8	31.7	26.0	888	719	116	117	38.7	41.8	24.3	23.0
Rh.+VAM+25%NP	18.1	12.9	1.4	1.3	23.9	22.0	704	603	96	93	33.9	35.7	20.8	18.8

Table 5b: Chemical composition of faba bean straw of the two cultivars as affected by inoculation with *Rhizobium* and/or Mycorrhiza in the presence of different levels of N and P fertilization (Average of the two seasons).

Character	Protein (%)		Total carbohydrate (%)	
	Sakha 1	Giza 40	Sakha 1	Giza 40
Control (100%NP)	12.5	9.2	25.3	20.0
Rh.+75%N+100%P	13.8	10.1	27.0	21.4
Rh.+50%N+100%P	13.0	9.3	26.6	21.0
Rh.+25%N+100%P	10.7	7.6	22.3	17.0
VAM+100%N+75%P	13.1	9.6	29.0	22.0
VAM+100%N+50%P	12.8	9.4	27.8	21.6
VAM+25%P	11.9	8.8	24.0	18.4
Rh.+VAM+75%NP	15.2	11.1	30.1	23.9
Rh.+VAM+50%NP	14.3	10.6	31.2	22.6
Rh.+VAM+25%NP	11.3	8.1	23.4	18.0

phosphorus^[17]. In addition, Mycorrhizal fungi may increase the uptake of many other nutrients such as N, S, Ca, Zn, and Cu^[40]. In this respect, Seguin *et al.*^[12], reported that the AM fungi expand their filaments in soil and plant roots, this filamentous network promote bi-directional nutrient movement where soil nutrients and water move to the plant and plant photosynthates flow to the fungal network. VAM increased protein percentage, this is quite logic since phosphorus is an essential element in the synthesis of protein in the leguminous plants, which are well known to be rich in this ingredient. VAM also

increased total carbohydrates percentage, this could be explained by^[13], who revealed that VAM have been shown to increase the level of chlorophyll. The possibility of VAM to increase chlorophyll levels could be highly significant in terms of the consequent effect on photosynthesis. Moreover, Sivake and Walker^[41] indicated that VAM fungi have a role in the regulation of CO₂ fixation. Bethlenfalavy *et al.*^[42] noted that the stimulation of CO₂, N₂ fixation and the improvement of water and nutrient use efficiency may be induced by modification of plant water status and leaf gas exchange.

Also, it is evident that the dual infection in combination with 75% or 50% of the recommended dose of N and P fertilizers resulted in the greatest effect on the chemical composition of both cultivars compared with either the plants inoculated with *Rhizobium* or VAM alone or uninoculated ones. So, it is of interest to notice that the dual inoculation not only increased seed yield as mentioned before, but also improved seed nutritional value. Similar results were also reported by Rabie^[18], and El-Ghandour *et al.*^[16], they concluded that dual inoculants provided more balanced nutrition for the plants, and that the improvement in N and P uptake was the major mechanism involved. The close agreement of the present data with those reported in the literature indicates that the inoculation of legumes with VA mycorrhiza and *Rhizobium* was found to have synergistic beneficial effect on nodulation, nitrogen fixation, and legume growth. The organism benefit by acquiring photosynthates from the plant, and the plant benefits by obtaining nitrogen or phosphorus, which are both limiting in soil, i.e. mutually beneficial association^[9].

Summing up, the combined inoculation of *Rhizobium* with VAM enhanced nodulation, growth, yield and nutritional values of both studied faba bean cultivars under low-fertility conditions with the superiority of Sakha 1 as compared with Giza 40 cultivar. Moreover, VAM was more effective when plants were inoculated with *Rhizobium* than when they were fertilized with nitrogen, this indicated the interendophyte compatibility between *Rhizobium* and Mycorrhiza.

Increasing fertilizer costs and the increasing demand for food have emphasized the need for full exploitation of biosymbionts. Therefore, it is essential to evolve and adopt a strategy of integrated nutrient supply by using a judicious combination of chemical fertilizers and biofertilizers. The benefit to mankind and the economic potential makes it a worthwhile task.

ACKNOWLEDGMENT

The authors express their deep and heartfelt gratitude to Prof. B.T. Shawky, Professor of Microbial Chemistry, Department of Microbial Chemistry, National Research Centre for the outstanding technical advice of the microbiological part.

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