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Effects of Biological and Chemical Fertilizers on Growth and Symbiotic Properties of Faba Bean *(Vida/aha* L.) Under Salt Stress

Ahlam Elsammani Ahmed and Elsiddig Ahmed Elmustafa Elsheikh

Department of Biochemistry and Soil Science,
Faculty of Agriculture, Shambat, Sudan

Abstract: The effects of chemical (nitrogen and phosphorus) and biological fertilizers *(Rhizobium* and vesicular arbuscular mycorrhizae (VAM) *Glomus* sp.) on growth and symbiotic properties of faba bean under saline conditions were investigated in two pot experiments. Salinity significantly reduced the shoot fresh and dry weight, number of nodules, dry weight of nodules and percentage of mycorrhizal infection. Both VAM inoculation and phosphorus fertilization significantly increased the shoot and root fresh and dry weights, number of nodules and dry weight of nodules under normal and saline conditions. High levels of superphosphate (150 kg/ha) completely suppressed the enhancing effect of the VAM under normal conditions, and the effect was strong at 115 kg/ha under saline conditions. moculation of faba bean plants by *Rhizobium* significantly increased shoot and root fresh and dry weights, number and dry weight of nodules and pods fresh and dry weights, under saline and non-saline conditions. The dual inoculation increased the nodulation and dry matter under salt stress.

INTRODUCTION

In the Sudan, salt-affected soils occur in the desert and semi-desert zones, such as the high terrace of the River Nile and its tributaries, and in thr arid areas such as the central clay plain in northern Gezira. Most of these salt-affected soils have a relatively low nutrient status; they contain 0.01 - 0.02% organic nitrogen, whereas phosphorus usually occurs in 6organic form of calcium phosphate; and they are also low in trace elements. Salinity is known to decrease nodulation and nitrogen fixation through its harmful effect on nodule activity (Elsheikh 1992).

Faba bean is a very important pulse crop in the Sudan and its cultivation is generally limited to the comparatively cooler, arid climate zone mainly in the Northern and River Nile States. Adequate phosphorus was found to be vital for effective nodulation of legumes (Salem and Elmasri 1986). It is believed that at moderate P concentration, phosphorus plays a role in epidermal osmotic adjustment; possibly explaining the beneficial role of additional phosphorus on salt stress, however, at high level of P with salinity, phosphorus accumulates and causes damage. Vesicular arbuscular mycorrhizae (VAM) are important components of the rhizosphere and they have the ability to create a mutually beneficial root fungus association. Generally, legumes are quite responsive to VAM especially in soils with low available phosphorus. Mycorrhizal plants are very efficient in P absorption and accumulation, and have a greater tolerance to toxic heavy metals, root pathogens, drought, high temperature, saline conditions and adverse soil pH than non-mycorrhizal plants (Elsheikh 1993). Mycorrhizal research in the Sudan revealed that nodulation and growth of legumes can be significantly enhanced by both *Rhizobium* and mycorrhizal inoculation (Mahdi 1993; Mahdi and Atabani 1992).

Use of combined inoculation (*Rhizobium* and VAM) was suggested for establishing leguminous crops with minimum supplies of nitrogen and phosphorus fertilizers. Biofertilizers must receive more attention in countries like Sudan with a predominantly low-input agricultural system of production where chemical fertilizers, if available, may not be economically affordable (Mahdi 1993). Growth offaba bean is increased by dual inoculation but nutrient content, nodulation and root colonization are greatly variable in silt loam than in calcareous soils (Salem and Elmasri 1986). Salinity decreases mycorrhizal colonization, number of chlamydospores and nodulation by *Rhizobium* but dry weight and tissue P concentration increase in plants inoculated with VAM and *Rhizobium* in leucaena (Dixon *et at*. 1993). Biofertilization is steadily receiving increased attention and recognition from scientists because the microbial inoculants (including e.g. *Rhizobium* and mycorrhizal inoculant) introduced into soil or plant culture, directly or indirectly, enhance plant productivity. The main objective of this investigation was to fmd out whether it is possible to improve the performance and symbiotic properties of faba bean in a salt-affected soil by using chemical and biological fertilizers.

MATERIALS AND METHODS

Source of seeds, soil, *Rhizobium* and VAM strains

Seeds of faba bean cultivar "Basabeer" were obtained from Shambat Agricultural Research Station. The seeds were sterilized in sodium hypochlorite (chlorox) for five minutes and rinsed thoroughly in sterile distilled water. A clay soil from Shambat area was used throughout this investigation. The soil has the following characteristics: 17% silt, 31% sand and 48% clay; pH = 7.89, Ee = 1.10 dS/m, SAR = 4.02, and, Na, Ca, Mg and K were 5.83, 2.9, 1.3 and 0.26 (mg/l), respectively. The N and P contents were 0.04% and 0.011%, respectively. *Rhizobium leguminosarum* biovar. *viceae* strain TAL 1397 was supplied by the National Research Centre, Sudan. A locally isolated VA mycorrhizal strain from Shambat soil, identified as *Glomus* sp., was used. The VAM strain was multiplied and maintained in pot cultures of sudangrass (Garawya, *Sorghum sudanense)* grown in sterilized sand: clay (1:1 w/w). The seeds were inoculated with 50 g (per kg soil) of soil and root debris taken from sudangrass culture, and were placed 5 cm below surface of soil before sowing.

Pot experiments

All pot experiments were carried out at the Faculty of Agriculture, University of Khartoum, during 1994/95 in a randomized complete block design with three replicates. Treatments were assigned at random to pots using random numbers. Five sterilized seeds were planted in each pot, and the plants were thinned to two plants per pot 15 days later. *Rhizobium* inoculum and nitrogen fertilizer (in the form of urea; 46% N) were added where appropriate. Phosphorus fertilizer (triple super phosphate 48% P_2O_5) was added before sowing where required. Irrigation with tap water continued for three weeks after which irrigation with saline water was started as desired. Calcium and sodium chloride salts were used for preparing mixed-salt solution of different electrical conductivities (EC) to give a final electrical conductivity of 0.26 or 4.0 dS/m. Shoot, root and pod fresh weights and number of nodules and pods were determined immediately after harvest. Dry weights were determined after oven drying at 80°C for 48 hours. Nitrogen and phosphorus contents were determined according to Rowell (1994). The data obtained were analyzed statistically using the analysis of variance and regression models.

Experiment I

This experiment was carried to study the effect of salinity, phosphorus and VAM on the growth of faba bean. The following eight treatments were used:

i) control,
ii) inoculation with VAM,
iii) kg P_2O_5/ha,
iv) kg P_2O_5/ha and inoculation with VAM,
v) kg P_2O_5/ha,
vi) kg P P_2O_5/ha and inoculation with VAM,
vii) kg P_2O_5/ha, and
viii) kg P_2O_5/ha and inoculation with VAM. Each of these treatments was either irrigated with tap water or with saline water of EC 4.0 dS/m. Plants were harvested six weeks after sowing.

Experiment II

This experiment was conducted to study the effect of salinity and biological and chemical fertilizers on growth offaba bean. It included five treatments, namely:

i) control,
ii) kg N/ha and 120 kg P_2O_5/ha,
iii) inoculation with *Rhizobium* and VAM,
iv) inoculation with *Rhizobium* and fertilization with 120 kg P_2O_5/ha, and
v) inoculation with VAM and fertilization with 50 kg N/ha. Each of these treatments was either irrigated with tap water or with saline water of EC 4.0 dS/m. Plants were harvested eight weeks after sowing.

RESULTS

Experiment I

Effects of salinity and phosphorus on growth: Both VAM inoculation and phosphorus fertilization significantly ($P \leq 0.001$) increased the shoot and root fresh and dry weights (Table 1) and total plant weight (Fig. 1) under normal and saline conditions. Higher shoot and root dry weights were obtained when the plants were fertilized by 180 kg P_2O_5/ha in the presence or absence of VAM. Salinity significantly ($P \leq 0.001$) reduced shoot and root weights in all treatments. The response of plant growth to VAM, superphosphate and salinity followed linear regression models as follows (Fig. 1) :

Control	$Y = 1.76 + 0.019 X$	$r = 0.99$	(1)
VAM	$Y=2.27+0.016X$	$r = 0.95$	(2)
Salinity (4.0 dS/m)	$Y=1.17+0.012X$	$r = 0.99$	(3)
Salinity (4.0 dS/m) + VAM	$Y = 1.48 + 0.010 X$	$r = 0.94$	(4)

where Y is the plant dry weight (g) and X is the superphosphate level.

It is worth noting that the positive effect of VAM decreased with increasing the phosphorus level. Under normal conditions, in equations (1) and (2), the effect of VAM was completely suppressed at X value of 150 kg P_2O_5/ha, whereas, under saline conditions, in equations (3) and (4), the enhancing effect of the fungus was eliminated at X value of 115 kg P_2O_5/ha (Fig. 1).

The number and dry weight of nodules were significantly($P \leq 0.01$) increased by VAM inoculation and phosphorus fertilization (Table 2). Salinity significantly ($P \leq 0.001$) decreased the number and dry weight of nodules in all treatments. Phosphorus fertilization (180 kg P_2O_5/ha) resulted in an appreciable increase in dry weight of nodules in the presence or absence of VAM inoculation compared to untreated control plants (Table 2). The little mycorrhizal infection observed in uninoculated plants indicated that Shambat soil contains some mycorrhizal propagules. The VAM percentage of infection significantly increased with VAM inoculation and increased with phosphorus application up to 120 kg P_2O_5/ha and then declined.

Experiment II

Effect of salinity and biological and chemical fertilizers on growth: Salinity significantly ($P \leq 0.001$) reduced the shoot fresh and dry weights in all treatments (Table 3). Both biological and chemical fertilizers significantly ($P \leq 0.001$) increased shoot fresh and dry weights compared to untreated control plants (Table 3). However, differences between chemical and biological fertilizers were not significant. Similar effects were observed in root fresh and dry weights (Table 3).

Salinity significantly ($P \leq 0.001$) reduced the number and dry weights of nodules in all treatments (Table 4). Inoculation with *Rhizobium* produced higher number and dry weight of nodules with both mycorrhizal fungi and phosphorus fertilizer. Statistical analysis indicated that the dry weight of pods was significantly ($P \leq 0.001$) decreased by salinity and significantly ($P \leq 0.001$) increased by all treatments under saline and normal conditions (Fig.2).

Table 1. Effect of salinity, VAM and phosphorus fertilizer on shoot and root fresh and dry weights (g/plant) of faba bean cultivar "Basabeer".

Treatment	Fresh weight EC (dS/m)		Dry weight EC (dS/m)	
	0.26	4.0	0.26	4.0
		Shoot weight		
Control	4.30a	2.25a	1.02a	0.70a
VAM	5.01b	3.56b	1.61b	1.01b
60 kg P_2O_5/ha	5.15b	3.55b	1.78b	1. lIb
60 kg P_2O_5/ha + VAM	5.13b	3.97b	1.72b	1.10b
120 kg P_2O_5/ha	7.47c	5.02c	2.40c	1.48c
120 kg P_2O_5/ha + VAM	7.90c	5.12c	2.33c	1.41c
180 kg P_2O_5/ha	9.47d	6.80d	3.02d	1.95d
180 kg P_2O_5/ha + VAM	9.70d	6.91d	3.19d	1.98d
SE ±	LIB	0.90	0.30	-0.15
		Root weight		
Control	3.71a	2.40a	0.83a	0.54a
VAM	4.78b	3.15b	1.06b	0.71b
60 kg P_2O_5/ha	4.70b	3.11b	1.09b	0.74b
60 kg P_2O_5/ha + VAM	4.87b	3.04b	1.07b	0.71b
120 kg P_2O_5/ha	5.80c	4.50c	1.57c	1.00c
120 kg P_2O_5/ha + VAM	5.72c	4.43c	1.58c	0.96c
180 kg P_2O_5/ha	7.48d	5.80d	2.36d	1.47d
180 kg P_2O_5/ha + VAM	7.55d	5.80d	2.34d	1.45d
SE±	0.73	0.51	0.11	0.05

Means sharing the same superscript in a column (for each parameter) are not significantly different at $P \leq 0.05$.

Table 2. Effect of salinity, VAM and phosphorus fertilizers on number of nodules/plant, dry weight of nodules/plant and mycorrhizal infection percentage offaba bean cultivar "Basabeer"

Treatment	Number of nodules EC (dS/m)			Dry weight of nodules EC (dS/m)			Mycorrhizal infection (%) EC (dS/m)		
	0.26	4.0	Reduc-tion [1]	0.26	4.0	Reduc-tion	0.26	4.0	Reduc-tion
No VAM inoculation									
Control	23a	10a	56.5	37a	15a	59.5	36.7b	25.0b	31.9
60 kg P_2O_5/ha	34c	18c	21.7	55c	30c	18.9	40.1b	28.3b	22.9
120 kg P_2O_5/ha	34e	18c	21.7	55e	31c	16.2	36.3b	26.7b	27.2
180 kg P_2O_5/ha	38e	20c	17.4	60c	32c	13.5	20.3a	14.7a	59.9
VAM inoculation									
Control	29b	14b	39.1	41b	22b	40.5	70.3c	48.3c	-31.62
60 kg P_2O_5/ha	34e	180	21.7	55c	32c	18.9	70.0c	48.3c	-31.6
120 kg P_2O_5/ha	37c	19c	17.4	60c	32c	13.5	76.3c	50.1c	-36.5
180 kg P_2O_5/ha	36c	18c	21.7	59c	34c	10.8	48.3b	29.2b	20.4
SE±	4.3	3.7		5.0	2.5		15.0	7.8	

[1] All calculations were compared to uninoculated, unfertilized control.

[2]The negative sign indicates better performance under saline conditions compared to uninoculated unfertilized control.

Means sharing the same superscript in a coloumn are not significantly different at P≤ 0.05.

Table 3. Effect of salinity, biological and chemical fertilizers on shoot and root fresh and dry weight of faba bean cultivar "Basabeer"

Treatment	Fresh weight EC (dS/m)		Dry weight EC (dS/m)	
	0.26	4.0	0.26	4.0
		Shoot weight		
Control	3.88a	2.35a	1.40a	0.97a
Rhizobium + VAM	5.18b	3.30b	1.85b	1.30b
Rhizobium + 120 kg $P2O_5$/ha	5.22b	3.45b	1.89b	1.31 b
VAM + 50 kg N/ha	5.53b	3.35b	1.87b	1.30b
50 kg N/ha + 120 kg P_2O_5/ha	5.67b	3.51b	1.85b	1.30b
SE±	0.90	0.32	0.20	0.15
		Root dry weight		
Control	2.85a	1.81a	1. 10a	0.70a
Rhizobium + VAM	3.83b	2.49b	1.26b	0.85b
Rhizobium + 120 kg P_2O_5/ha	3.83b	2.43b	1.27b	0.86b
VAM + 50 kg N/ha	3.65b	2.48b	1.25b	0.83b
50 kg N/ha + 120 kg P_2O_5/ha	3.68b	2.48b	1.25b	0.85b
SE±	0.71	0.22	0.13	0.10

Means sharing the same superscript in a column (for each parameter) are not significantly different at $P \leq 0.05$.

Table 4. Effect of salinity, biological and chemical fertilizers on number of nodules/plant and dry weight of nodules (g/plant) of faba bean cultivar "Basabeer"

Treatment	Nodules number		Nodules dry weight	
	EC (dS/m)		EC (dS/m)	
	0.26	4.0	0.26	4.0
Control	Sa	1a	9a	Sa
Rhizobium + VAM	26c	16c	33c	20c
Rhizobium + 120 kg P_2O_5/ha	26c	14c	38c	20c
VAM + 50 kg N/ha	16b	9b	24b	12b
50 kg N/ha + 120 kg P_2O_5/ha	16b	8b	24b	12b
SE±				

Means sharing the same superscript in a column are not significantly different at $P \leq 0.05$.

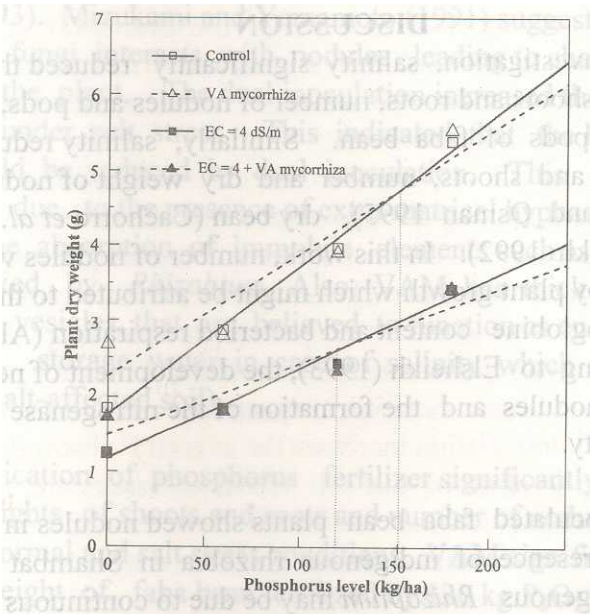

Fig 1. Effect of salinity, phosphorus and VA mycorrhiza on dry weight (g) of faba bean.

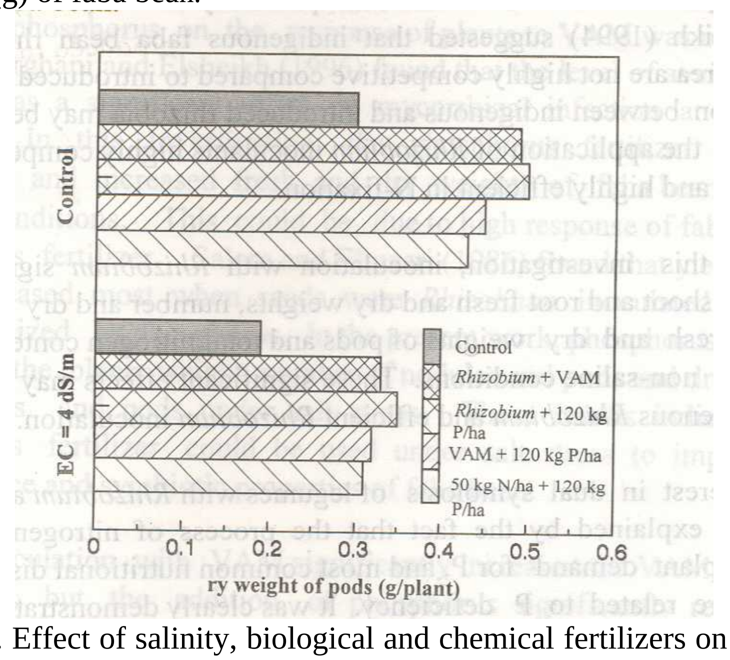

Fig. 2. Effect of salinity, biological and chemical fertilizers on dry weight of pods (g/plant) of faba bean cv. "Basabeer".

DISCUSSION

In this investigation, salinity significantly reduced the fresh and dry weights of shoots and roots, number of nodules and pods, dry weight of nodules and pods of faba bean. Similarly, salinity reduced the dry weight of roots and shoots, number and dry weight of nodules of faba bean (Elsheikh and Osman 1995), dry bean (Cachorro *et at*. 1993), and chickpea (Elsheikh 1992). In this work, number of nodules was affected by salinity than by plant growth which might be attributed to the reduction of the legheomoglobine content and bacteriod respiration (Abd-Elsamad 1993). According to Elsheikh (1993), the development of new nodules, the activity of nodules and the formation of the nitrogenase enzyme are reduced by salinity.

All uninoculated faba bean plants showed nodules in their roots, indicating the presence of indigenous rhizobia in Shambat soil. The presence of indigenous *Rhizobium* may be due to continuous addition of *Rhizobium* inoculum to legumes cultivated -in Shambat, specially faba bean and peas. Similar results were reported by Mahdi (1993). Osman and Elsheikh (1994) suggested that indigenous faba bean rhizobia in Shambat area are not highly competitive compared to introduced rhizobia. Competition between indigenous and introduced rhizobia may be partially solved by the application of *Rhizobium* inoculants highly competitive for nodulation and highly efficient in N-fixation.

In this investigation, inoculation with *Rhizobium* significantly increased shoot and root fresh and dry weights, number and dry weight of nodules, fresh and dry weights of pods and total nitrogen content, under saline and non-saline conditions. These significant effects may be due to weak indigenous *Rhizobium* and efficient *Rhizobium* inoculation.

Interest in dual symbiosis of legumes with *Rhizobium* and VAM has been explained by the fact that the process of nitrogen fixation increased plant demand for P, and most common nutritional disorders in legumes are related to P deficiency. It was clearly demonstrated, in the present investigation, that inoculation of faba bean with both VAM and *Rhizobium* significantly enhanced nodulation and dry matter production. Similar results were reported by many scientists for different crops

(Mahdi 1993). Mizukami and Yamamoto (1991) suggested that infection with VAM fungi interacts with nodules leading to change in hormonal balance of the plant. The dual inoculation increased the nodulation and dry matter under salt stress. This indicated that the harmful effect of salinity could be reduced by dual inoculation. This observation may possibly be due to the presence of extra-matrical hyphae of VAM which increased the absorption of immobile elements such as phosphorus and nitrogen fixed by *Rhizobium*. Also, VAM has thick-walled inter or intracellular vesicles that are believed to function as endophytic storage organs (e.g. storage water in case of salinity which is an important problem in salt-affected soil).

Application of phosphorus fertilizer significantly increased fresh and dry weights of shoots and roots and number of nodules and their dry weight in normal and salt stress conditions. VAM significantly improved the plant weight of faba bean fertilized by 120 kg P_2O_5/ha resulting in a higher dry matter than of plants fertilized by 60 kg P_2O_5/ha, whereas no effect of VAM on plants fertilized by 180 kg P_2O_5/ha was noted. The effect of phosphorus on the response of plants to VAM was reported to vary. Mirghani and Elsheikh (1996) found that the level of available P in the soil has a significant effect on mycorrhizal infection and on plant growth. In this investigation, the phosphorus fertilizer enhanced nodulation and increased fresh and dry weights of faba bean plants in normal conditions. This could be due to high response of faba bean to phosphorus fertilizer. Salem and Elmasri (1986) found that yield of faba bean increased most when seeds were *Rhizobium* inoculated and plots were fertilized by phosphorus. In the present work, phosphorus fertilizer increased the plant weight, number of nodules and pods and dry weights of nodules and pods under salt stress. These results indicated that phosphorus fertilizer could be used salt stress to improve the performance and symbiotic properties of faba bean.

Inoculation with VAM significantly increased the VAM infection percentage, but the addition of phosphorus significantly reduced it. Higher doses of phosphorus produced less VAM infection. Similarly, Mirghani and Elsheikh (1996) reported that infection of roots by VAM decreased dramatically with higher P doses. This may be due to the fact that the activity of VAM is reduced by the availability of nutrients specially phosphorus. Salinity significantly reduced the overall mycorrhizal infection percentage, and these results are in agreement with reports on leucaena (Dixon *et al.* 1993).

All chemical, biological and mixed fertilizers improved the dry matter production, produced higher number and dry weight of nodules and increased the weight of pods in faba bean plants under normal and saline conditions. *Rhizobium* and *Glomus* sp. exhibited similar positive results comparable to that of N and P. This indicated the efficiency of VAM and *Rhizobium* in P and N accumulation, respectively. According to Elsheikh (1993), the VAM inoculation increases the overall P absorption and may also lowers the pH value of the soil which increases the solubility of phosphorus. Chemical fertilizers affected the plant weight more than the dry weight of pods and number of nodules. This may be attributed to the limited supply of nitrogen and/or phosphorus. The results also indicated that 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 VAM.

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