

Effects of *Rhizobium meliloti* and VA-Mycorrhiza on Plant Density and Seed Production of Two Alfalfa Cultivars

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Summary

A field experiment was conducted at the Demonstration Farm of the Faculty of Agriculture, University of Khartoum in Shambat during the period December, 1997-June 1999 to investigate the effects of *Rhizobium meliloti*. Vesicular arbuscular mycorrhiza (VAM), and their combinations on plant density and seed production of two alfalfa cultivars (local Hegazi and imported Pioneer 5929). The treatments consisted of Rhizobium inoculation, VA-Mycorrhizal inoculation, a combination of R+VAM, and a reference check as a control. A split plot design, with cultivars in the main plots and microorganisms in the subplots, was used.

The microorganisms (bacteria and fungi) alone or in combination significantly improved plant density and seed yield of the two cultivars. Rhizobium increased total seed production over the control by 33%. Corresponding increments for the VAM alone and in combination with Rhizobium were 49.5% and 46%, respectively. The local cultivar Hegazi out-yielded the introduced Pioneer 5929 in seed yield components and total seed yield and significantly produced more plants per unit area compared to the introduced one. It produced 21% more seeds compared to the Pioneer 5929 variety.

Keywords: *Rhizobium meliloti*; Mycorrhiza; Alfalfa.

Introduction

Expenditure on fertilizer in agriculture is a major concern to farmers. With diminishing reserves of high quality raw materials and with escalating energy costs. The need to develop sustainable agriculture that requires low fertilizers input in the form of biofertilizer must receive more attention. This is especially true in countries like the Sudan with predominantly low input agricultural system of production.

The research covered in this paper is directed toward the use of biofertilizers through the use of beneficial microorganisms, mainly *Rhizobium meliloti* and VAM fungi in production of alfalfa fodder. In the Sudan alfalfa is thought to be the chief irrigated fodder crop. Now it is receiving more attention than ever because Saudi Arabia, the main producer of alfalfa in the Arab world and the main supplier of the Gulf countries with alfalfa, has decided to stop exporting forage due to limitations in its ground water supplies. As a result, Sudan is making attempts to bridge the gap. Many local and foreign investors are now seeking investments in the area of forage production in the Sudan. The Arab Authority for Agricultural Investment and Development (AAAID) is now establishing a 3000 feddans (one feddan=0.42 ha.) project in Elbagair and Orndom areas, south and east of Khartoum, for alfalfa production. And another 10000 feddans project in the Nile State is to be implemented in the coming years for this purpose (AAAID, personal communication).

The main problem with horizontal expansion in alfalfa production in the Sudan is the shortage of seeds of the indigenous cultivar Hegazi, which out-yields exotic cultivars (Agabawi, 1968 and Abusuwar and Mohamed, 1997). Currently, alfalfa seed prices are escalating and one ton of alfalfa seed costs 1.975.000 Sudanese Dinars, which is much higher than the cost of imported seeds of exotic cultivars. Several researchers, including Tomar, (1970) and Ojha *et al.*, (1977), reported that inoculation, in the absence of ni-

trogen and phosphorus, increased the level of nodulation five to six times. Pod yield showed a similar trend. Abusuwar and Mohamed (1997) reported a positive effect of Rhizobium inoculation on seed yield of alfalfa and Nayel and Khidir (1995) reported a significant effect of phosphorus and nitrogen fertilization on number of pods per plant and seed yield of alfalfa.

The objective of this research was to determine the effects of *Rhizobium meliloti* bacteria and VAM fungi, as biofertilizers, on plant density and seed production of two alfalfa cultivars.

Material and Methods

A field experiment was conducted during the period December 1997-June 1999 at the Demonstration Farm of the Faculty of Agriculture, University of Khartoum, in Shambat (Latitude 15°40'N and longitude 32°32'E). The climate of the area is semi-arid with low relative humidity, and the temperature ranges between 40°C maximum and 21°C minimum in winter (Oliver, 1965). The soil of the experimental site is alkaline (pH 8.0) cracking clay with about 50% clay content (Nayel and Khidir, 1995). It contains about 0.065 % Nitrogen (N), 23 meq/l potassium (K), and 0.194 meq./l available phosphorus (Elbasari, 1999).

Two alfalfa cultivars (local cultivar Hegazi and exotic cultivar Pioneer 5929) and four treatments for each cultivar were used. The treatments were inoculation with *Rhizobium meliloti* (R), inoculation with (VAM), inoculation with a combination of (R+VAM), and the control (C).

The experimental site was disc ploughed, cross-ploughed, disc harrowed, levelled and ridged up 70 cm apart. Sowing was done manually by broadcasting seeds and then lightly covered by a rake at a rate of 20kgs/feddan. Harvesting was also done manually using a sickle. The plot size was 5X5 metre, consisting of 6 ridges. The two outermost ridges were left as a guard area. The design used was a split-plot with four replications; the main plots were assigned to the cultivars and the subplots to the microorgan-

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ism treatments. An initial dose of phosphorus fertilizer in the form of triple- super phosphate (46% P), was applied at a rate of 50 g/a POs frall treatments before planting. *Rhizobium meliloti* strain TAL380, isolated from Niftal- Hawaii-USA mother legume (*Medicago sativa* L.), was prepared by the National Council for Research (NCR), Khartoum. VAM (*Glomus manihotis*) was isolated according to the wet sieving and decanting method described by Gerdemann and Nicison (1963), in which a considerable amount of soil was collected from different parts of Shambat Demonstration Farm including alfalfa, maize, and sorghum fields. The collected VAM was cultured and multiplied in a glass- house with sterilized Sudan grass seeds. Irrigation was applied immediately after planting and at intervals of 7 days during summer and 10 days during winter.

Plant density was monitored in an area of 0.7 sq. metres in the middle of each plot in a permanently marked area throughout the experimental period at 15 days from planting, one month and two months after planting and after each cut thereafter. Data on seed production was collected during the period March -June 1999. These included number of racemes per plant, number of pods per raceme, number of seeds per pod, total seed yield and 1000-seed weight. After the last cut in March 1999, when environmental conditions were favorable, the crop was left for seed production. An area of 0.7 sq. metres in the centre of each plot was used for counting the number of racemes at 10 days interval starting from the appearance of the first floral bud. Five plants were randomly chosen from the above- mentioned area and tagged for counting the number of racemes per plant. The average number of racemes per plant in each count was multiplied by the plant density in the 0.7-sq. metre area to obtain the number of racemes per unit area. When pods of the tagged plants were mature, the number of pods per raceme was counted. Seed yield was

determined when 70 to 80% of the pods turned brown to dark brown, as a sign of maturity, according to Marble (1989). Mature pods were picked manually three times at 10 days interval. Pods were dried and threshed to obtain total seed yield in kg/ha. A sample containing 1000 seeds was counted randomly from each experimental unit to determine 1000 seed weight.

Results and Discussion

Effect of treatments on plant density

Plant density was monitored 15 times throughout the experimental period; three times before the first cut and at each cut thereafter. Rhizobium inoculation increased plant density in almost all counts throughout the experimental period with significant differences in 11 out of 15 counts (Table 1). Similarly, VAM significantly increased plant density in 11 out of 15 counts when compared to non-inoculated plants and it resulted in more plants per unit area when compared to Rhizobium inoculated plants and the control.

The effect of tripartite symbiosis between alfalfa, Rhizobium and VAM, as demonstrated in Table I, resulted in an increased plant density in all counts as compared to VAM plants alone and Rhizobium plants alone. This increase in plant density resulting from the joint effect of the bacteria and the fungi was significant in 11 out of 15 counts. The vital role of *Rhizobium meliloti* is the fixation of atmospheric nitrogen that is available to the host plant (alfalfa). In addition, VAM is known to accelerate water uptake and phosphorus absorption by the plant. This way, the two microorganisms can work in concert to increase food reserves in alfalfa crowns and roots to enhance tillering ability of the crop, to result in more tillers per unit area (Bishoni and Dut, 1983 and Peterson *et al.*, 1994 and Abu-suwar and Mohmed, 1997).

Table 1. Effect of *Rhizobium meliloti* and VA-mycorrhiza inoculation on plant density (population count) 0.7 m².

Treatment	Date and number of samplings (cuttings)														
	Before harvest			1st	2st	3st	4st	5st	6st	7st	8st	9st	10st	11st	12st
	25/12/97	9/1/98	9/2/98	25/2/98	28/3/98	5/5/98	5/6/98	11/7/98	10/8/98	1/9/98	15/10/98	25/11/98	25/12/98	25/1/99	2/3/99
C	a	d	d	b	c	d	b	c	c	c	c	a	d	a	a
R	138.50	102.55	128.25	125.90	143.65	224.38	190.03	203.80	251.50	256.25	22.75	273.55	274.38	222.25	293.65
VAM	a	bc	c	b	abc	c	b	bc	abc	abc	abc	a	abc	a	a
VAM+R	148.15	12.34	151.10	127.90	178.65	241.88	235.25	245.80	324.63	339.75	276.63	328.80	353.13	251.25	333.65
	a	b	b	a	ab	a	a	ab	ab	a	a	a	a	a	a
	173.05	129.65	161.90	158.75	188.90	310.26	258.00	267.75	360.50	417.75	354.63	314.25	283.63	238.00	313.15
	a	a	a	ab	a	a	a	a	a	ab	ab	a	ab	a	a
	144.55	148.05	167.06	144.90	195.40	284.00	240.00	275.50	361.30	377.75	304.38	34390	369.25	237.88	358.65
S.E	9.39	2.74	1.34	7.59	12.80	12.41	3.21	16.13	25.03	31.60	26.56	18.81	23.29	10.94	19.17
C.V	17.50	6.2	2.50	15.40	20.00	15.20	2.40	18.00	21.00	25.70	25.80	16.80	19.00	13.40	16.07

C=Control; R=Rhizobium; VAM=mycorrhiza; VAM+R=VA-VAM-mycorrhiza+Rhizobium

Means followed by the same letter (s) in a given column are not significantly different at 0.05 level according to Duncan's Multiple Range Test.

Table 2. Effect of cultivars on plant density (population count) 0.7 m².

Cultivar	Date and number of samplings (cuttings)														
	Before harvest			1st	2st	3st	4st	5st	6st	7st	8st	9st	10st	11st	12st
	25/12/97	9/1/98	9/2/98	25/2/98	28/3/98	5/5/98	5/6/98	11/7/98	10/8/98	1/9/98	15/10/98	25/11/98	25/12/98	25/1/99	2/3/99
CV ₁	a	a	b	a	a	a	a	a	a	a	a	a	a	a	b
	145.35	124.60	146.95	143.60	183.90	258.50	230.44	230.40	310.81	355.19	293.75	265.10	317.58	216.63	292.16
CV ₂	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
	156.78	127.53	157.20	135.13	169.40	271.69	232.15	276.03	338.19	340.56	287.94	365.15	371.81	258.06	357.42
S.E	6.85	2.55	0.30	4.17	9.79	10.23	6.28	19.12	22.75	11.12	15.72	27.34	29.52	11.67	9.39
C.V	18.20	8.10	8.00	12.10	22.00	15.50	10.90	30.00	28.00	12.80	21.16	34.70	34.40	19.60	11.60

CV₁=Pioneer (5929) cultivar; CV₂=Hegazi cultivar

Means followed by the same letter (s) in a given column are not significantly different at 0.05 level according to Duncan's Multiple Range Test.

The local cultivar Hegazi recorded higher number of plants per unit area compared to the introduced cultivar Pioneer 5929 in 9 out of 15 counting occasions (Table 2) with statistical differences observed only in count 2 before harvest and count 12 after harvest. The local cultivar Hegazi, which is being cultivated in Sudan for so many years, has become more adapted to the environmental conditions, and therefore it is expected to outyield exotic varieties. Similar conclusion was reached by Agabawi (1968) and by Abusuwar and Mohmed (1997).

Effect of treatments on seed production

The effect of microorganisms on seed production is presented in Table 3. Rhizobium inoculation increased the number of racemes per plant and the number of racemes per unit area and it was significant over the control in three out of four sampling occasions. On the other hand, VAM doubled the number of racemes per plant and it was significant in 3 out of 4 sampling dates. VAM+R resulted in a significantly higher number of racemes per plant and per unit area in all counting occasions. The single effect of

Rhizobium meliloti on number of racemes per plant exceeded that of the joint effect of VAM+R in 3 out of 4 counting dates (2,3,4). Moreover, it exceeded the joint effect of VAM+R in number of racemes per unit area in 2 out of 4 counting dates. It is expected that both microorganisms will benefit the host plant through atmospheric nitrogen fixation by the bacteria and acceleration of phosphorus and water uptake by the host plant. This comes through the external hyphae produced by the fungus, which increases the root surface area to increase their absorption capacity for water and nutrients (Abbott *et al.*, 1979 and Hayman, 1983). Fredeen and Terry (1988) suggested that VAM infection appeared to increase total leaf surface by increasing leaf tissue and changing leaf structure. The results reported in this paper confirmed the observation of Alien (1989), Mada and Baggara (1993) and Douglass (1994) who stated that VAM inoculation improves plant growth by improving water and nutrient uptake capacities. Smith and Daft (1997) proposed that the increase in P-uptake is due to the extension of VAM hyphae beyond the zone of phosphorus depletion. The role of

Table 3. Effect of *Rhizobium meliloti* and VA-mycorrhiza inoculation on seed production.

Treatment	Number of racemes/plant				Number of racemes per unit area				No. of pods/raceme	No. of seeds/pod	1000 seeds weight (g)	Total seed yield (kg/ha)
	1	2	3	4	1	2	3	4				
	9.4.99	19.4.99	29.4.99	9.5.99	9.4.99	19.4.99	29.4.99	9.5.99				
Control	a	d	d	c	b	b	d	c	a	d	c	d
	4.25	7.80	11.30	12.45	13.03.35	23.12.00	3245.15	3620.70	13.90	6.30	2.1299	218.15
Rhizobium	a	a	a	a	abc	ab	a	a	a	abc	ab	abc
	5.40	12.25	17.35	19.60	1852.05	3646.95	5735.30	6403.83	16.40	7.85	2.3229	289.65
VA-mycorrhiza	a	abc	bc	abc	ab	ab	bc	abc	a	a	a	a
	4.95	10.20	13.90	17.20	1625.65	3218.45	4505.65	5382.05	16.45	8.00	2.3991	326.05
VAM+Rhizobium	a	a	ab	ab	a	a	ab	ab	a	ab	abc	ab
	6.40	11.20	15.95	18.95	2404.80	4128.80	5646.80	6159.40	16.90	2.2651	2.26551	318.10
S.E	0.63	0.73	0.78	1.59	233.57	442.05	345.12	589.20	0.91	0.28	0.06	16.55
CV%	34.30	20.5	15.10	26.50	36.70	37.50	37.50	30.90	16.20	10.80	7.70	16.30

C=Control; R=Rhizobium; VAM=mycorrhiza; VAM+R=VA-VAM-mycorrhiza+Rhizobium

Means followed by the same letter (s) in a given column are not significantly different at 0.05 level according to Duncan's Multiple Range Test.

Table 4. Effect of cultivar inoculation on seed production.

Cultivar	Number of racemes/plant				Number of racemes per unit area				No. of pods/ raceme	No. of seeds/ pod	1000 seeds weight (g)	Total seed yield (kg/ ha)
	1	2	3	4	1	2	3	4				
	9.4.99	19.4.99	29.4.99	9.5.99	9.4.99	19.4.99	29.4.99	9.5.99				
Pioneer 5929	*b	a	a	a	b	a	a	a	a	d	b	a
	2.70	11.30	16.80	21.10	769.76	3124.08	5117.08	6319.79	16.23	7.43	2.0181	260.95
Hegazi	a	a	a	a	abc	a	a	a	a	a	a	a
	780	9.43	12.45	12.95	2823.15	3529.03	4499.10	4463.21	15.60	7.75	2.5404	315.03
S.E	0.60	1.40	1.69	2.06	301.60	567.60	703.45	794.12	0.62	0.26	0.0200	20.73
CV%	45.50	55.30	46.40	48.40	67.10	68.20	18.60	59.20	15.50	14.00	3.4000	28.80

CV1=Pioneer (5929); cultivar CV2=Hegazi cultivar

Means followed by the same letter (s) in a given column are not significantly different at 0.05 level according to Duncan's Multiple Range Test.

VAM in accelerating P-uptake is of a particular importance in clay soils like the one where the present experiment was conducted. Clay soils, especially alkaline ones, are known to fix phosphorus and make it unavailable to plants. However mycorrhizal fungi can play a great role in freeing this phosphorus and accelerating its uptake by plants.

The effect of microorganisms on number of pods per raceme was not significant (Table 3), however the Rhizobium and VAM treatments showed more pods per raceme, alone or in combinations compared to the control. The two microorganisms significantly increased the number of seeds per pod, 1000 seed weight and total seed yield compared to the control (Table 2). The Rhizobium treatment increased total seed yield over the control by 33%. Corresponding increments for VAM and R+VAM in total seed yield over the control were 46% and 49.5%, respectively. Grime *et al.*, (1988) confirmed that inoculation of alfalfa with effective VAM fungi, not only improves plant growth and nutrient uptake, but also enhances the activity of Rhizobium meliloti when applied as inoculum.

The effect of cultivars on seed production is presented in Table 4. The local cultivar Hegazi produced significantly more racemes per plant and per unit area than Pioneer during the first count, but this trend was reversed in the later counts. This might be an indication that the local culti-

var has an early maturing habit compared to the introduced Pioneer. This is in harmony with the finding of Marble (1989) that Pioneer cultivar has a late flowering habit. With respect to seed yield components, the Hegazi variety produced significantly more seeds per pod and heavier seeds (1000 seed weight) than the introduced Pioneer. This was reflected in the production of higher seed yield by the Hegazi, which exceeded the introduced Pioneer in total seed production by 21%.

Alfalfa seed production is highly dependent on normal growth of adapted varieties (Bolton, 1962). In the present experiment Hegazi plants grew larger and taller than the Pioneer plants which have a prostrate growth habit. This gives Hegazi an advantage of having flowers readily exposed to pollinating insects. Agabawi (1968) stated that Hegazi variety outyielded American ones in forage and seed production.

The total seed yield was positively correlated with yield components (number of racemes per plant, number of pods per raceme, number of seeds per pod and 1000 seed weight) as shown in Table 5. These observations are in line with the results of Taylor (1987), who found that seed yield was positively correlated with number of stems per unit area, number of racemes per plant, and number of seeds per pod. In addition, Dovart and Mariam (1969) stated that seed yield is positively associated with carbohydrates percentage of the root, number of secondary branches, number of racemes per stem and number of pods per raceme.

It can be concluded, from the results of this study, that beneficial soil microorganisms such as VAM fungi and Rhizobium meliloti bacteria have a significant positive effect on plant density of alfalfa and on its seed yield components. Furthermore, the local cultivar Hegazi showed superior performance with respect to plant density and seed production compared to the exotic variety Pioneer 5929.

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Table 5. Correlation coefficient between the effect of *Rhizobium meliloti*, VA-mycorrhiza in inoculation and cultivars on yield components and seed yield: Field experiment.

	Number of Raceme/ plant	Number of pods/ raceme	Number of seeds/ pod	1000 seed weight (g)
Number of pods/raceme	0.733 NS	-	-	-
Number of seeds/pod	0.729 NS	0.911**	-	-
1000 seed weight	0.814 *	0.961***	0.953***	
Total seed yield kg/ha	0.747 NS	0.921**	0.886*	0.882*

NS Not significant at P=0.05.

* significant at P=0.05

** significant at P=0.01

*** significant at P=0.01

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تأثير بكتريا الرايزوبيوم وفطر الماكورايزا على الكثافة النباتية وإنتاج البذور لصنفين من البرسيم

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الخلاصة

أجريت تجربة بالمزرعة الإيضاحية بكلية الزراعة، جامعة الخرطوم، خلال الفترة ديسمبر 1997 وحتى يونيو 1999م لدراسة تأثير إضافة بكتريا الرايزوبيوم وفطر الماكورايزا على الكثافة النباتية وإنتاج البذور لصنفي البرسيم الحجازي والبايونير 5929. اشتملت المعاملات على إضافة بكتريا الرايزوبيوم ميليتوس، فطر الماكورايزا، وإضافة الفطر والبكتريا سوياً بالإضافة إلى الشاهد. استخدم تصميم القطع المنشقة حيث استعملت القطع الرئيسية لزراعة صنفي البرسيم والقطع الفرعية على معاملات الأحياء الدقيقة في أربع مكررات.

إضافة الأحياء الدقيقة (بكتريا وماكورايزا) كل بمفردها أو كخليط أدت إلى زيادة الكثافة النباتية وزيادة البذور على صنفي البرسيم وكانت الضروقات معنوية. وإضافة بكتريا الرايزوبيوم بمفردها أدت إلى زيادة إنتاج البذور بنسبة 33% مقارنةً بالشاهد. أما فطر الماكورايزا بمفرده فقد زاد إنتاجية البذور بنسبة 49.5%، وعند خلط الفطر والبكتريا كانت الزيادة 46%.

تفوق الصنف المحلي (حجازي) على الصنف المستورد (بايونير 5929) في الكثافة النباتية وإنتاج البذور وكانت الزيادة في إنتاج البذور 21%.

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