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Effect of *Rhizobium melilotti* and VA-mycorrhizae on Forage Yield and Quality of Two Alfalfa Cultivars

Awad O. Abusuwar and Sulaiman A. Ahmed
Department of Agronomy, Faculty of Agriculture, University of Khartoum
Shambat, Sudan

أثر إضافة بكتيريا الرايزوبيوم وفطر الماكورايزا على إنتاجية ونوعية علف صنفين من البرسيم

عوض عثمان أبو سوار وسليمان آدم احمد
قسم المحاصيل الحقلية ، كلية الزراعة
جامعة الخرطوم، السودان

أجريت تجربة حقلية فى المزرعة التجريبية بكلية الزراعة جامعة الخرطوم خلال الفترة من ديسمبر ١٩٩٧ الى يونيو ١٩٩٩ لدراسة اثر بكتيريا الرايزوبيوم وفطر الماكورايزا على إنتاجية ونوعية صنفين من البرسيم (الحجازي وبايونير ٥٩٢٩) . اشتملت المعاملات على بكتريا الرايزوبيوم وفطر الماكورايزا بالإضافة الى الشاهد . استعمل تصميم الأحواض الانشطارية بثلاث مكررات . وضعت أصناف البرسيم فى الأحواض الرئيسية ومعاملات الأحياء الدقيقة فى الأحواض الفرعية .

أظهرت معاملات إضافة البكتيريا ، الفطر ، و البكتيريا + الفطر زيادة معنوية فى معايير النمو والتى انعكست فى زيادة الإنتاجية ونوعية العلف . وقد زاد الوزن الرطب للعلف بمقدار ٤٢ % بإضافة البكتيريا بينما كانت الزيادات ٦٠% و ٦٥% نتيجة لإضافة الفطر ، والفطر + البكتيريا على التوالي .

إضافة البكتيريا أدت الى تحسن القيمة الغذائية للعلف حيث زاد معدل البروتين الخام بنسبة ٢٧% والفسفور بنسبة ٣٢% . وكانت الزيادات المقابلة نتيجة لإضافة الفطر والبكتيريا ٢٣% للبروتين الخام و٣٦% للفسفور. تفوق الصنف المحلى حجازي على المستورد بايونير فى معايير النمو والتى انعكست على الإنتاجية العالية وتحسن القيمة الغذائية حيث زادت نسبة البروتين الخام فى الصنف حجازي بنسبة ٢٦% مقارنة بالبايونير .

Key words: *Alfalfa yield and quality, Nodulation, Nitrogen fixation, Rhizobium melilotti, VA-mycorrhizae.*

ABSTRACT

An experiment was conducted at the Demonstration Farm of the faculty of Agriculture, University of Khartoum at Shambat during the period December 1997 to June, 1999, to investigate the effect of *Rhizobium meliloti* and VA-mycorrhizae on growth, forage yield and quality of two alfalfa cultivars (local Hegazi and imported Pioneer 5929). The treatments consisted of inoculation with *Rhizobium melilotti*, VA-mycorrhizae, and (VAM+R) in addition to a reference control. A split- plot design with three replications was used in which the two alfalfa cultivars were assigned to the main plots and the microorganisms to the sub-plot treatments. *Rhizobium melilotti*, VA-mycorrhizae, and their combinations significantly improved growth parameters, which were reflected in higher yields of good quality forage. *Rhizobium melilotti* increased forage fresh yields by 42%, whereas the increments in forage fresh yields by VAM alone and VAM+R were 60 % and 65%, respectively. The forage nutritive value was improved by Rhizobia inoculation in the winter samples by 26.9% for the crude protein and by 32.3% for phosphorus content. Corresponding increments for the joint effect of VAM+R were 23.2% for crude protein and 36.1% for phosphorus compared to the control. The local cultivar Hegazi outscored the introduced Pioneer in the growth parameters which were reflected in higher forage yield of good quality compared to the introduced one. It produced 25.7% more protein than the Pioneer variety.

Introduction

Sudan natural rangelands, which occupy two-thirds of the country area, are the main source of feed for livestock in the country. Despite this fact their contribution in terms of forage supply is limited due to overgrazing, overcutting and man misuse. This leads to desertification that is aggravated by frequent drought spells. Currently the deficit in forage supplies is estimated at 23 million tons of total digestible nutrients (TDN) annually as reported by [1].

Rangelands in Sudan are diminishing due to the expansion of rainfed agriculture and drought. The large animal wealth, which is estimated as 113 million heads, as reported by [1], calls for a continuous supply of forage, to improve dairy and meat production for local markets and for export. This necessitates more attention being given to irrigated forages to bridge the gab between current forage supply and expected feed demands by livestock. Moreover, Sudan now with its rich animal wealth, fertile soils and good quality irrigation water is expected to bridge the gab in animal proteins and forage supplies to Arab countries and worldwide.

Alfalfa (*Medicago sativa* L.) is considered the Queen of forages due to its high yielding abilities with high quality forage. It is the most widely forage grown worldwide and is considered the main forage crop

in Argentina and in the United States. In the Sudan it ranks top among the forage crops grown under irrigation. The crop, beside its high yielding ability of good quality forage, it also increases the storage level of soil nitrogen, improves soil tilth, and reduces soil erosion [2] and a good pasture crop for bees in honey production.

The main factor limiting production in the Sudan is the cost of chemical fertilizers. This is particularly true with a predominantly low input system of production in the Sudan. This necessitates looking into alternatives that are ecologically sound and financially feasible. The use of biofertilizers through making use of beneficial bacteria (*Rhizobium melilotti*) and fungi (VA-mycorrhizae) could be a sound solution. These two organisms could reduce cost of fertilizers substantially, in addition to having a clean and healthy environment free of chemical pollution [3]. Consequently one of the objectives of this study was to evaluate the effects of VAM and *Rhizobium melilotti* on forage yield and quality of two alfalfa cultivars, namely Hegazi and Pioneer 5929. Little research and scanty information are available on these two organisms on alfalfa in Sudan [4], which justifies more research in this area.

Materials and Methods

An experiment was conducted during the period December 1997 to June 1999 at The Demonstration Farm of the Faculty of Agriculture, University of Khartoum, at Shambat (Latitude 15° 40′ N and longitude 32 °32′ E). The climate of the area is semi-arid with low relative humidity, temperature ranges between 40°c maximum and 21°c minimum in winter [5]. The soil of the experimental site is alkaline (pH 8.0) cracking clay with about 50% clay content [6]. It contains about 0.065 % Nitrogen (N),). 23 meq/l potassium (K), and 0.194 meq./l available phosphorus [7].

The treatments consisted of two alfalfa cultivars (Local cultivar Hegazi and exotic cultivar Pioneer 5929). Four treatments were prepared for each cultivar. They included inoculated *Rhizobium melilotti* (R), inoculated VA-mycorrihza (VAM), and a combination of R+VAM, in addition to the control (C).

The experimental site was disc ploughed, cross-ploughed, disc harrowed, levelled and ridged up 70 cm apart. Plot size was 5X5 meters, 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 sub- plots for the microorganism treatments. An initial doze of phosphorus fertilizer in the form of triple super phosphate (46% P) was applied at a rate of 50 kg /ha P_2O_5 for all treatments before planting. *Rhizobium melilotti* strain TAL380 isolated from Niftal –Hawaii-USA mother legume (*Medicago sativa* L.) was prepared by the National Council for Research (NCR), Khartoum. VAM was isolated according to The Wet Sieving and Decanting method described by [8], in which a considerable

amount of soil was collected from different areas of Shambat Demonstration Farm including alfalfa, maize, and sorghum fields. The VAM collected was cultured and multiplied in a glasshouse with sterilized Sudangrass seeds. Irrigation was applied immediately after planting and at an interval of 7days during summer and every 10 days during winter.

The following parameters were measured during the course of the study: Plant height, number of leaves per plant, leaf area index, leaf to stem ratio, and forage yield and nutritive value. Before each cut, five plants were randomly taken to measure plant height in each treatment. Plant height was measured from the first node to the apical bud of the plant. The same five plants that were taken for plant height were used to determine the number of trifoliate leaves per plant. Leaf area index was determined one day before each harvest using the Punch Method described by [9]. The five plants that were used in determination of plant height and number of leaves per plant were clipped and partitioned into leaves and stems. Leaf to stem ratio was determined on dry matter basis after drying the samples to a constant weight.

Using a spring balance, the entire plot in each treatment was cut separately and weighed to get fresh yield in tons/ha. For dry matter production a sampled area of 0.7 sq. meters was cut, air-dried and dry matter was then determined in tons/ha. Twelve harvests were made during the experimental period for fresh and dry yield determinations. From the samples of alfalfa dry matter, samples were ground for three harvesting dates to represent summer (May), fall (August), and winter (January) to determine forage nutritive value in terms of crude protein and phosphorus content. Crude protein was determined using the Microkejeldhal technique according to [10]. Similarly phosphorus content was determined according to the method described by [10].

Results and Discussion

Effects of treatments on growth attributes

Rhizobia inoculation significantly increased plant height in 5 out of 12 sampling occasions (Table 1). Mycorrhizal plants grew better and taller than the controlled plants and the increase in plant height was significant in 5 out of 12 sampling dates. The combination of R+VAM, in most of the sampling dates, was more effective on plant height than either treatment applied alone. The local cultivar Hegazi was taller than the introduced Pioneer 5929. However significant increase was detected in 5 out of 12 sampling dates. The local cultivar Hegazi showed a quick recovery after cutting compared to Pioneer which takes relatively longer time to recover.

Rhizobia inoculation increased number of leaves per plant throughout the different sampling dates and it was significant in 7 out of 12 sampling dates (Table 2). Mycorrhizal plants, on the other hand,

exhibited, not only larger leaves, but also produced more leaves per plant compared to the control, and it was significant in 7 out of 12 sampling dates.

The joint effect of R+VAM on number of leaves per plant was greater than either treatment applied alone. With respect to the effect of cultivars, it was found that the introduced cultivar Pioneer 5929 had produced higher number of leaves compared to the local one in 10 out of 12 sampling dates. Significant differences between the two cultivars were detected in 4 out of 12 sampling occasions (Table 2).

The effect of treatments on leaf area index was significant in 7 out of 12 sampling dates (Table 3). Rhizobia-infected plants showed a significant difference compared to their corresponding control in 7 sampling occasions. Moreover, VAM fungi statistically increased leaf area index over non-mycorrhizal plants. The association of VAM+R showed significant differences among some treatments, while an increase in leaf area index was observed in all sampling occasions. The local cultivar Hegazi exhibited an increase in leaf area index in 7 sampling occasions over the Pioneer variety.

The effect of treatments on leaf to stem ratio is presented in table 4. Rhizobia inoculation increased leaf to stem ratio in 10 out of 12 sampling dates. Statistical differences, however, were detected in 5 sampling dates. The mycorrhizal infected plants exhibited higher leaf area index compared to the control and the Rhizobia-infected plants in 7 out of 12 sampling dates throughout the experimental period, but significant differences were detected in 5 sampling dates. The joint effect of VAM+R increased leaf to stem ratio in 8 counts out of 12. In most of the sampling dates plants inoculated with VAM+R have had higher leaf to stem ratio than either treatment applied alone. With respect to the effect of cultivars on leaf to stem ratio, the local cultivar Hegazi scored higher leaf to stem ratio compared to the introduced one in almost all sampling dates (Table 4).

The increase in growth parameters resulting from the inoculation of mycorrhizal fungi and the Rhizobium bacteria is expected. Both microorganisms benefit their host by providing nutrients (nitrogen and phosphorus) in addition to the increase in root surface area (mycorrhizal effect) which accelerates and increases capacity of roots in absorbing water and nutrients. Nitrogen is an essential element for plant growth and development. It is tied up in proteins, chlorophyll, amino acids and nucleic acid. Nitrogen deficiency is always accompanied by slow growth, decreased branching at maturity and lower yields. The vital role of Rhizobium bacteria is the provision of this essential element and making it available to plants. The plant benefited from this association with Rhizobium by increasing the amount of food reserves in the crowns and roots of alfalfa (11; 3). On the other hand, phosphorus, which is provided by the fungus mycorrhizae, is a constituent of nucleic acid and intimately concerned with the utilization of nitrogen and vital functions of plants (photosynthesis, amino acids, etc...) and it stimulates root development (12; 13).

Effects of treatments on forage yield

Forage fresh yield

Rhizobium meliloti significantly increased forage fresh yield in 11 out of 12 harvesting dates (Table 5). It increased forage fresh yield over the control by 42%. Maximum forage fresh yield was reported in harvest number 4 and it amounts to 10.34 tons/ha. Similar to the results obtained in Rhizobium, VAM inoculation increased forage fresh yield in 11 out of 12 harvesting dates. VAM inoculated plants increased forage fresh yields by 65% over the control and it was significant in 11 out of 12 harvesting dates. As evident from the results of growth parameters that were improved by the microorganisms, it is expected to be reflected in forage yield since, at the end, yield is the resultant of growth parameters.

Despite the fact that no statistical differences were detected between cultivars with respect to forage fresh yield, the local variety Hegazi outyielded the introduced one in 11 out of 12 harvesting dates (Table5). As seen from the growth parameter data, Hegazi variety was always outscoring Pioneer , and it is logical to have this reflected in the final yield.

Forage Dry Matter

A similar trend that was observed with forage fresh yield was reflected on forage dry matter. Rhizobia and VAM inoculated plants significantly increased dry matter over the control in 8 out of 12 harvesting dates (Table6). Inoculation of plants with both VAM and Rhizobia increased dry matter in all harvesting dates and it was significant in 8 out of 12 harvesting dates. Generally, plants inoculated with both VAM and Rhizobia produced higher dry matter than the their corresponding controls and when each microorganism was used separately. The total dry matter increment by the joint effect of VAM+R was 60% over the control.

The local cultivar Hegazi outyielded the introduced one in dry matter production. However significant differences were detected in 2 harvesting dates (Table 6).

Effect of treatments on forage nutritive value

Alfalfa forage nutritive value was estimated in three harvesting dates representing summer, fall, and winter (Tables 7 and 8) for crude protein and phosphorus content, respectively. In summer samples there were no significant differences observed between treatments both for the crude protein and phosphorus content, though Rhizobia and VAM+R increased protein and phosphorus contents by 22% and 24%, respectively compared to the control.

No statistical difference for crude protein was observed between the two cultivars (Table7), however the local cultivar Hegazi significantly accumulated more phosphorus than the exotic Pioneer 5929 did

(Table 8). In fall samples, although plants inoculated with VAM+R gave higher crude protein (28.6%) and higher phosphorus content (4 mg/gm), no significant differences were detected for either crude protein or phosphorus. In winter samples, Rhizobia inoculation significantly increased plant protein by 26.9% and plant phosphorus by 32.3% over the control. VAM inoculated plants statistically gave crude protein similar to those of the control, but significantly absorbed more phosphorus in their tops by 43.6% over their corresponding control. The joint effect of VAM+R increased crude protein and phosphorus by 23,3% and 36.1% over the control, respectively. These results are in line with those reported in [2, 3, 14]. They reported that Rhizobium inoculation improved forage quality through increasing plant protein contents. Positive effect VAM fungi on forage nutritive value, especially phosphorus content, were reported in [4, 15, 16].

The local cultivar Hegazi significantly produced more protein amounting to 25.7% greater than the introduced Pioneer, while no significant differences were detected between the two cultivars for the phosphorus content. The outstanding performance of Hegazi with respect to forage nutritive value is expected since it resulted in more leaves per plant, higher leaf to stem ratio and higher leaf area index compared to Pioneer and all these parameters contribute to the quality of the forage produced.

It can be concluded from the results of this study that microbial inoculation of alfalfa seeds by VAM and *Rhizobium meliloti* improved the growth parameters of the crop, which was reflected in higher fresh and dry yields of high quality. Moreover, the local cultivar Hegazi proved superior over the exotic Pioneer in growth parameters, forage yields and nutritive value. It can be assumed that production cost inputs can be reduced through the use of biofertilizers to enhance production in systems of low production inputs as in the Sudan.

Table 1. Effect of *Rhizobium meliloti* , VA-mycorrhiza and cultivar on plant height (cm)

Treatment	Date and Number of Sampling (cuttings)											
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
	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
control	55.20b	41.02c	47.98c	44.85a	45.80a	39.09d	44.15d	45.50c	35.40a	48.79a	53.88a	51.60a
Rhizobium	59.05ab	46.47abc	54.04ab	54.40a	50.50a	52.46abc	53.45ab	53.83a	38.05a	54.54a	58.43a	57.30a
VAM	58.15a	48.60ab	52.64bc	54.10a	47.60a	56.17ab	52.60abc	52.70ab	39.73a	53.33a	58.84a	56.05a
VAM+R	59.00ab	49.95a	58.93a	51.85a	48.65a	53.04a	56.59a	50.17abc	39.40a	53.30a	61.01a	55.36a
S.E	1.63	2.02	1.87	55.50	2.62	3.09	1.91	1.50	1.37	2.34	2.23	2.59
C.V	7.80	12.20	9.90	2.44	15.40	17.40	10.51	8.70	10.10	12.60	10.90	13.30
Pioneer	59.43a	42.55a	52.14a	48.25b	50.00a	49.44a	50.80a	46.96a	30.65b	46.35b	51.95b	50.50b
Hegazi	56.28a	50.50a	54.55a	54.88a	46.43a	50.94a	52.50a	51.14a	46.21a	58.73a	64.13a	59.65a
S.E	1.78	1.95	2.93	0.83	1.63	1.23	2.14	1.23	3.11	2.68	0.84	1.43
C.V	12.10	16.80	21.90	6.40	13.50	9.70	16.60	10.00	32.40	20.40	5.80	10.40

VAM= VA-mycorrhiza; VAM+R= VA-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 *Rhizobium meliloti* ,VA-mycorrhiza and cultivar on number of leaves per plant.

Treatment	Date and Number of Sampling (cuttings)											
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
	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
Control	58.75a	42.15c	57.39c	51.95c	49.40a	40.34a	42.85d	40.72c	37.20a	42.69a	41.35 a	45.40c
Rhizobia	68.65a	55.95ab	62.84bc	71.20a	54.95a	63.92a	49.40abc	54.42a	41.75a	50.38a	45.90 a	54.85a
VAM	72.65a	50.35abc	67.95b	60.60abc	52.10a	55.71bc	52.90ab	52.25ab	39.25a	49.64a	46.15 a	47.85bc
VAM+R	64.75a	59.15a	81.81a	67.05ab	55.65a	60.34ab	50.30a	48.59abc	40.65a	51.12a	48.35 a	50.30b
S.E	11.78	3.70	4.26	4.19	9.37	2.61	1.40	1.98	2.16	2.12	1.62	1.33
CV	49.00	19.90	17.80	18.90	50.00	13.30	11.60	11.40	15.40	12.60	10.10	7.60
CV_1	78.90a	59.95a	71.03a	57.53a	66.83a	58.79a	50.30a	51.11a	47.46a	47.50a	51.08a	51.13a
CV_2	59.58b	43.85a	63.97a	67.83a	39.18b	51.61a	47.43a	46.88a	42.63b	49.40a	39.80b	48.08b
S.E	4.54	4.30	3.55	2.67	5.34	2.16	1.76	1.13	1.09	2.90	1.44	1.64
C.V	27.40	33.00	21.00	17.00	40.00	15.60	14.40	9.20	10.90	23.90	12.70	13.20

VAM= VA-mycorrhiza; VAM+R= VA-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 3. Effect of *Rhizobium meliloti* ,VA-mycorrhiza and cultivar on leaf area index (L.A.I).

Treatment	Date and Number of Sampling (cuttings)											
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
	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
Control	1.20b	1.53a	3.08a	1.93d	1.66a	2.15a	1.87c	1.39d	1.46c	2.34d	1.90a	2.05 d
Rhizobia	2.70a	1.71a	3.80a	3.19bc	1.92a	4.13a	2.43c	2.17ab	2.01ab	3.23ab	1.85a	2.75 b
VAM	2.00ab	1.23a	3.90a	2.73b	2.10a	4.06a	3.10ab	2.45a	1.82abc	3.61a	1.95a	2.70 bc
VAM+R	1.75a	1.90a	3.90a	3.43a	2.76a	3.78a	3.39a	2.15abc	2.03a	3.14a	2.05a	3.15a
S.E	0.27	0.25	0.52	0.20	0.31	0.64	0.19	0.21	0.15	0.23	0.11	0.18
C.V	4.50	45.00	39.00	20.00	41.10	51.00	19.80	29.10	22.60	20.80	16.70	18.17
Pioneer	2.23a	1.56a	3.67a	2.52b	2.89a	3.53a	3.01a	2.23a	1.40a	2.79a	1.95a	2.48a
Hegazi	1.60a	1.63a	3.66a	3.12a	1.83b	3.53a	2.38a	2.07a	2.25a	3.37a	1.93b	2.85a
S.E	0.37	0.18	0.36	0.28	0.12	0.39	0.18	0.29	0.20	0.47	0.10	0.21
C.V	77.00	44.00	38.00	40.00	19.90	44.00	26.50	57.40	44.00	6.10	19.70	30.00

VAM= VA-mycorrhiza; VAM+R= VA-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 *Rhizobium meliloti* ,VA-mycorrhiza ana cultivar on leaf to stem ratio

Tretment	Date and Number of Sampling (cuttings)											
	Ist	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
	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
Control	1.16a	1.38d	1.44d	1.29a	0.88d	1.27a	0.93d	1.37a	1.18a	1.61a	1.13d	0.95a
Rhizobia	1.30a	1.97a	2.41a	1.37a	1.81ab	1.38a	2.02abc	1.70a	1.22a	1.89a	2.18ab	1.16a
VAM	1.34a	1.85ab	2.13ab	1.71a	1.62abc	1.68a	2.04ab	1.62a	1.45a	2.01a	1.95abc	1.05a
VAM+R	1.20a	1.83abc	2.09abc	1.51a	1.83a	1.46a	2.46a	2.02a	1.21a	1.83a	2.37a	1.00a
S.E	0.06	0.08	0.14	0.13	0.14	0.12	0.25	0.16	0.10	0.17	0.08	0.06
C.V	13.30	11.70	19.90	25.50	26.50	23.00	38.00	27.10	23.50	25.50	27.20	16.17
Pioneer	1.35a	1.72a	2.04a	1.53a	1.46a	1.43a	1.79a	1.66a	1.44a	2.21a	1.88a	1.25a
Hegazi	1.15b	1.80a	1.99a	1.41a	1.61a	1.46a	1.93a	1.69a	1.09a	1.46a	1.93a	0.83b
S.E	0.02	0.25	0.13	0.07	0.09	0.03	0.51	0.09	0.15	0.35	0.26	0.05
C.V	6.60	56.00	26.30	17.90	23.30	8.00	10.00	20.00	48.00	57.00	54.00	15.20

VAM= VA-mycorrhiza; VAM+R= VA-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 5. Effect of *Rhizobium melilotti* and VA-mycorrhiza inoculation on forage fresh yield (tons/ha)

Treatments	Date and Number of Sampling (cuttings)												
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	Total Fresh yield
Treatment	25/2/ 98	28/3/ 98	5/5/ 98	5/6/ 98	11/7/ 98	10/8/ 98	1/9/ 98	15/10/ 98	25/11/9 8	25/12/ 98	25/1/ 99	2/3/ 99	
Control	4.30c	6.44d	6.19d	6.44d	7.28d	5.33a	4.29d	3.12d	5.57a	4.98b	5.71a	3.81d	5.29a
Rhizobia	5.25bc	9.94abc	10.24abc	10.34abc	10.32abc	7.59a	7.12bc	5.62abc	5.82a	6.37ab	5.84a	5.85ab	7.56bc
VAM	6.15ab	10.85ab	12.28a	11.19ab	11.24ab	7.83a	9.65a	6.19a	6.77a	6.80ab	6.86a	5.70abc	8.45ab
VAM+R	6.45a	13.14a	11.74ab	12.02a	11.74a	8.03a	7.35ab	5.99ab	6.74a	7.94a	6.91a	6.45a	8.72a
S.E	0.40	1.08	1.14	0.93	0.51	0.79	0.79	0.60	0.51	0.64	0.55	0.39	0.33
C.V	20.40	30.00	31.90	26.00	14.20	31.00	31.00	25.00	22.90	27.50	24.40	20.60	12.50
Pioneer	5.43a	10.12a	9.79a	9.84a	10.09a	6.18a	6.38a	4.46a	5.65a	5.38a	6.12a	4.70a	7.03a
Hegazi	5.65a	10.07a	10.43a	10.16a	10.20a	8.21a	7.82a	6.00a	6.79a	7.66a	6.54a	6.20a	7.98a
S.E	0.35	0.72	0.37	0.80	0.06	0.94	0.91	0.44	0.82	0.73	0.54	0.46	0.25
C.V	25.20	28.40	14.50	32.00	2.30	35.00	32.00	33.60	52.40	44.80	34.00	33.90	13.20

VAM= VA-mycorrhiza; VAM+R= VA-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 6. Effect of *Rhizobium melilotti* ,VA-mycorrhiza and cultivar on dry matter production (Tons/ha)

Treatments	Treatment / Date and Number of Sampling (cuttings)												
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	Total dry matter
	25/2/ 98	28/3/ 98	5/5/ 98	5/6/ 98	11/7/ 98	10/8/ 98	1/9/ 98	15/10/ 98	25/11/9 8	25/12/ 98	25/1/ 99	2/3/ 99	
Control	1.26c	1.26c	1.54a	1.37b	1.18d	1.49a	1.47a	1.15c	0.85c	1.20b	1.12a	1.90a	1.31d
Rhizobium	1.43bc	1.60c	1.72a	2.08b	1.51c	2.11a	1.51a	1.61abc	1.08abc	1.50ab	1.34a	2.40a	1.68bc
VAM	1.56ab	2.91a	1.98a	2.44ab	2.42ab	2.07a	1.84a	1.87ab	1.34a	1.57ab	1.39a	2.10a	1.94ab
VAM+R	1.80a	2.37b	2.02a	2.86a	2.65a	2.14a	1.79a	1.90a	1.24ab	1.92a	1.43a	2.60a	2.09a
S.E	0.08	0.18	0.16	0.23	0.09	0.21	0.15	0.18	0.90	0.14	0.12	0.24	0.09
C.V	14.50	28.70	25.20	23.50	12.90	26.40	26.40	23.00	27.70	25.80	25.80	29.90	13.80
Pioneer	1.49a	1.70a	1.81a	2.18a	1.93a	1.77a	1.51a	1.47a	0.98a	1.31a	1.16a	1.70b	1.60a
Hegazi	1.55a	1.97a	1.81a	2.37a	1.94a	2.13a	1.79a	1.80a	1.27a	1.78a	1.47a	2.80a	1.91a
S.E	0.17	0.16	0.12	0.15	0.23	0.22	0.14	0.19	0.19	0.12	0.11	0.05	0.10
C.V	46.20	34.00	27.20	26.90	47.00	45.00	33.00	47.00	66.00	31.80	32.90	9.70	22.90

VAM= VA-mycorrhiza; VAM+R= VA-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 RangeTest.

Table 7. Effect of *Rhizobium melilotti*, VA-mycorrhiza inoculation and cultivars on plant crude protein %.

	Date and number of sampling		
Treatments	Summer (1) 5.5.1998	Fall (2) 10.8.1998	Winter (3) 25.1.1999
Control	22.02a	21.00a	21.93c
Rhizobium	26.94a	25.77a	27.84ab
VA-mycorrhiza	24.99a	24.00a	22.00c
VAM+Rhizobium	27.37a	28.62a	28.13a
S.E	2.42	2.43	1.5
CV%	19.23	19.57	12.8
Cultivars			
Pioneer 5929	25.7a	25.25a	24.23b
Hegazi	24.96a	24.44a	25.72a
S.E	2.35	3.98	0.1
CV%	26.5	45.00	1.2

Means followed by the same letter(s) in a given column are not significantly different at 5% level according to Duncan Multiple Range Test (DMRT).

Table 8. Effect of *Rhizobium melilotti*, VA-mycorrhiza inoculation and cultivars on plant phosphorous content (mg/gm sample)

	Date and number of sampling		
Treatments	Summer (1) 5.5.1998	Fall (2) 10.8.1998	Winter (3) 25.1.1999
Control	3.17a	3.44a	2.94d
Rhizobium	3.78a	3.73a	3.89abc
VA-mycorrhiza	3.88a	4.02a	4.34a
VAM+Rhizobium	3.57a	4.00a	4.00ab
S.E	0.01	0.03	0.02
CV%	7.67	17.80	14.80
Cultivars			
Pioneer 5929	3.50b	4.04a	3.83a
Hegazi	3.64a	3.56a	3.75a
S.E	0.001	0.01	0.01
CV%	1.40	8.18	9.24

Means followed by the same letter(s) in a given column are not significantly different at 5% level according to Duncan Multiple Range Test (DMRT).

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