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The Efficiency of Local Inoculation Carriers Used with Indigenous and Exotic *Sinorhizobium Meliloti* Strains in Fixing Atmospheric Dinitrogen with Local Alfalfa (*Medicago Sativa L.)* Cultivars under Omani Conditions

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Abstract-A growth chamber experiment was conducted at the Agricultural Research Center -Oman to investigate the need for inoculating alfalfa, the major fodder crop of the country.Three local alfalfa cultivars representing three distinct geographical regions with variable environmental conditions were selected for inoculation with four *Sinrhizobium meliloti* strains.The selection was based on the relative efficiency in fixing atmospheric nitrogen with the tested local cultivars. A pot experiment was conducted to investigate the performance of the two selected local *Rhizobium* isolates, their mixture together with the introduced *Sinorhizobium* strain obtained from Australlia as a commercial inoculum when added to local inoculation carriers ; date palm leaves, dates by-products & a peat carrier.Two local alfalfa cultivars were included in the experiment, namely; Batini and Dakhlia.Two types of irrigation waters were used i.e.EC 0.8 & 4 DS/m to cater for the creeping water salinization in the country.At the end of the experiment, which continued for two month,data associted with nitrogen fixation parameters were colleted including nodulation, tissue dry weight, and nitrogen percentage. The results indicated that all the tested strains were not efficient in supplying the alfalfa with the required nitrogen for optimum growth. The strains, however interacted differently with the tested cultivars.The Australian and the Sharquia strains were highly infective on Batinah cultivar, while giving poor nodulation on the Interior cultivar. Also the strain from Sharquia gave the highest tissue dry weight with the Batinah cultivar thus indicating relatively effective nodulation.No significant differences were encountered upon irrigation with either saline waters. The mixture of the two local *Sinorhizobium* isolates was promising when compared with the addition of the single local isolates, or with the addition of the introduced Australlian *Sinorhizobium.* This was evident in the imrovement of tissue dry weights of all cultivars at all levels of salinity. The local inoculation carriers consistently increased tissue dry weights with all the added *Sinorhizobium* cultures when used with brackish waters, which showed future prospects for their use.The percent tissue nitrogen, however improved with both cultivars upon addition of all inoculant carriers to the cultivars irrigated with sweet water. These results are of immense importance for the productivity of alfalfa in Oman by using cheap local source of inoculation.

Keywords:*Sinorhizobium*,Inoculation carriers, Indigenous, Exotic, *Medicago sativa*, nodulation, tissue nitrogen.

Introduction

Legumes are the major source of protein, oil, and forage for humans and animal consumption and are among the worlds most important crops. About 85% of nitrogen fixation in agricultural soils comes from pasture and forage legumes. Biological nitrogen fixation contributes an estimated 140 TG (million tons) of nitrogen annually to the earth of which 80% comes through symbiotic associations and 20% by free living organisms (Barnes et.al.,1980).

Alfalfa gained recognition because of its high yield and superior palatability. The capacity for nitrogen fixation in association with *Sinorhizobium melilot* has been a primary factor contributing to the excellence of alfalfa. It consistently shows greater amounts of nitrogen fixation and percentage nitrogen derived from symbiosis than most other legumes on a seasonal basis. Estimates of nitrogen fixation in alfalfa vary from 50-463 kg nitrogen fixed/ha/yr with about 200 kg/ha/yr (Gutschick,1980). This variability, however , is a reflection of a variety of factors, the most important of which are the interaction between the macrosymbiont (cultivar) and the microsymbiont (Sinor*hizobium* strain).

The aim of legume inoculation is to ensure that the rhizobia applied are sufficient to ensure rapid and abundant nodulation of the host legume. The number of the *Sinorhizobium* cells needed for sufficient nodulation vary with the host as well as the prevailing environmental conditions. However, at least 5000 Rhizobium cells should be available per seed for reasonable inoculation results to be attained. (Spediel & Wollum, 1980). To cater for the harsh environmental stresses that might interfere with the inoculation process by killing the rhizobium cells and/or reduce their efficiency, the technology of inoculant carriers was adopted in the early seventies (Burton et.al. 1978). Peat was used extensively as a carrier and proved to be efficient. In developing countries located in the arid regions of the world, however, peat is not available and if found it is a little bit expensive. Therefore, many local carriers were tried and proved to be similar to peat as inoculant carriers e.g. Nile silt, charcoal, oil-based inoculant(Hadad et.al 1986).

Alfalfa is the most important forage crop in Oman occupying an approximate area of 25,0000 feddans(MAF,1997)..No research in this field was ever conducted in Oman, except for the one trial with imported inoculum brought from Australia conducted by Parkash(1989),who reported an increase in alfalfa productivity in inoculated plots compared to control treatments. Therefore, studies dealing with the Omani soils inhabiting nitrogen fixation organisms is of high significance to the alfalfa growers in Oman.Investigations dealing with indigenous isolates from different regions of

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potential in growing alfalfa revealed that although the Omani soils are populated with alfalfa rhizobium, their numbers were very low probably due to the harsh environmental conditions prevailing (Hadad and El-Hashmi, 1999). The search for a suitable inoculant carrier is therefore of high significance to the alfalfa growers.

Date palm is the main grown crop all over the country occupying 50% of the total cultivated area(MAF, 1993). The leaves of dates (Khos) are thus available at no cost. Also, the by-products of the date industry could be obtained free of charge. This might justify their use as carriers for rhizobium inoculum if proved to be suitable for the persistence of the *Sinorhizobium* cells added as inoculum.

This work was therefore carried out to avail the soils of Oman with respect to the native inhabiting rhizobia and study their possible interactions with the local alfalfa cultivars , commonly grown in different regions of Oman and to investigate the outcome of formulating inoculant carriers from the available local material suitable for application by the farmers in the field.

Materials & Methods

Experiment 1

A growth chamber experiment was established at Rumais ARC (Agricultural Research Center) .Three alfalfa local cultivars representing three growing areas in oman; namely Batinah, Sharquia, and the Interior of Oman (Dakhylia) were included in the trial. The seeds obtained from the local farmers, were pregerminated in petri dishes after surface sterilization with 95% ethyl alcohol followed by three successive washes with sterile tap water. A mixture of Vermiculite and sand (1:1 by volume) weighing half kilogram was used in growing the seeds(Hadad et.al.1982). The mixture was transferred to autoclavable plastic bags & sterilized for one hour at 121°C and 15 lbs. of pressure. The sterile mixture contained in the autoclavable bags was transferred to metal cans. The alfalfa germinating seeds were aseptically added to the sterile mixture at 2-cm depth. Methods of rhizobia isolations, purification, authentication, and preparation for inoculation were as described earlier by Vincent (1972).Four rhizobium strains were selected , two indigenous to Oman; Batinah & Sharquia, and two alien strains from Canada & Australia.One ml of each of the specific selected *Sinorhizobium melioti* strain was aseptically added to the sown seeds in each pot and were irrigated immediately with sterile tap water to avoid contamination. A nitrogen control was included in the trial to which nitrogen was added in the form of ammonium sulfate solution three times during the growth period; at sowing, 2 weeks from sowing, and at the flowering stage i.e. 35 days from sowing. A complete nutrient solution lacking nitrogen (Speidel & Wollum, 1980) was added to all the pots interchangeably with sterile tap water during the whole growth period. A noninoculated control was included.The whole set was transferred to a growth chamber, where the temperature,humidity , and light intensity were adjusted to suit the growing conditions of alfalfa. (Vance,1978).With the three alfalfa cultivars used in the trial, 6 treatments, and 4 replication, a total of 72 pots were laid out in a completely randomized design. The pots were weekly rerandomized. The growth period continued till the flowering stage i.e. 35 days from sowing. Upon harvest, fresh top weights were taken and the nodules were counted. The fresh tissue was dried in an oven at 72 hours for 48 hours and the dry weights were taken. Mstatc was used for statistical analysis.

Experiment 2

For the experimentation with the inoculation carriers, a pot experiment was conducted on two Omani local alfalfa cultivars; Batinah and Dakhylia obtained from the local farmers in the respective regions in Oman were used in this trial. Irrigation water with different salinities (EC= 4 DS/m and 0.8 DS/m) were used. The soils were chosen with low EC=2.6 DS/m. Pots with 4 kg- capacity were used in this study. Chemical and physical properties of the soils used are presented in (table 1). The pots were irrigated with 1 liter of sweet water before sowing (2 days before planting). Twenty alfalfa seeds from each of the two cultivars were treated with ethanol (95%) for surface sterilization and were sown 2-cm below the soil surface early January. Immediate irrigation with one liter of sweet water followed.

Rhizobium strains

Four *Sinorhizobium meliloti* strains were used for inoculating the alfalfa seeds. The Batinah strain and the strain from Sharquia were isolated earlier at the Soil Microbiology laboratory at Rumais using standard isolation methods as described by Vincent(1972).An exotic Australian strain , isolated from a commercial inoculum was used as a 3rd treatment. For rhizobia propagation, YEMB was used. Plate counts were made after 7 days growth in an orbital shaker adjusted at 1200 rpm. Both strains from Batinah and Sharquia were mixed in equal numbers 1:1 and included as a treatment. All the used strains were adjusted to give 10^8 rhizobium cells /ml using standardad procedures.

Inoculantion Carriers

Three inoculation carriers; date palm ground leaves, date palm industry by-products(both local material), and peat, were used in this study. Methods for inoculum preparation followed what had been documented earlier (Bohlool,1990; Vincent,1982).

Each of the designated rhizobium strains treatments was mixed with the three inoculum carriers which made a total of 12 treatments. A noninoculated control and a nitrogen control were included. To the nitrogen control, urea was added after calculations based on the Research center recommendations (200 kg/ha). Calculations were made for each of the nitrogen control treatment pots.Sowing started with both controls to avoid contamination that could be resulting through adding the inoculum to the pots. One gram of each of the rhizobium treatments containing 10^8

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rhizobium cells packed in Aluminum foil was added to each of three replications designated for each treatment. Immediate irrigation followed with sweet water to allow for normal germination.

A basal dose of macronutrients (P&K) was added to all the pots at the rates recommended by the research center.The nitrogen treatment doses were added as three splits . Irrigation with saline water started after 15 days from sowing.The growth continued for 2-month from sowing. Upon harvest, the pots were irrigated and left for 2 hrs. The roots were freed from the attached soil.. Ten plants were selected randomly. The nodules were counted the tissue was dried at 72 C for 48 hours. Tissue nitrogen was determined using Kjeldahl nitrogen.

Results & Discussions

1. **Growth Champer Experiment**

The data are presented in table 1.

Significant differences were encountered between treatments at the 0.05 level of propability. The strains from Sharquia and Australia showed a consistent trend twards increasing nodulation with the local cultivars from Batinah and Sharquia... This trend was completely reversed with the Dakylia cultivar, where both the Australian and the Sharquia strain gave the lowest nodulation. The strain from the Batinah , on the other hand, gave the highest nodulation with the Dakhlia cultivar followed by the Canadian strain.The interaction of the rhizobium strains with the local cultivars tested is therefore different.

It could therefore be concluded that both the Australian and the Sharquia strain are relatively infective. Single degrees of freedom contrasts revealed that the Australian rhizobium strain is highly infective on both cultivars from Sharquia and Batinah, while showing poor infection on the Dakhylia cultivar. The high capability of both strains, however, was not reflected in tissue dry weights with the Sharquia cultivar showing even lower tissue dry weight than both controls; uninoculated and nitrogen.. This is an indicator of inefficiency in fixing atmospheric nitrogen.Hadad et.al ,1982 showed a high correlation between tissue dry weight and the amount of nitrogen fixed.

With the Batinah cultivar, however, the Sharquia strain gave the highest tissue dry weight and even outyielded the nitrogen control. It is worth noting that the strain from Canada gave the lowest nodulation and the lowest tissue dry weight when inoculated with the Batinah cultivar. The Batina strain showed high infectivity, which is reflected, in the high tissue dry weight of the Dakhlia cultivar. This seems to be a promising strain for future inoculation of the Dakhylia cultivar.

It is apparent from the data presented above that local rhizobia from Oman are not supplying the alfalfa crop with the required nitrogen for optimum growth. The data clearly showed that rhizobia isolated from the different regions are not necessarily the best compatible for the local alfalfa cultivars.This could be attributed to the wide variability in the environmental conditions prevailing in the different regions of Oman. This in turn may have influenced the alfalfa cultivars as well as the rhizobium strains. Hadad & Loynachan(1985) could not find significant interactions between their tested Rhizobium strains & the groundnut cultivars, which they attributed to the similarity of the geographical origin of the cultivars.The set environment in the growth chamber may have influenced the overall performance of the tested Rhizobium strains , and consequently their interaction with the tested alfalfa cultivars. The interaction between the Batinah Rhizobium strain with the local Batinah alfalfa cultivar presented in table 1 clearly demonstrates that local alfalfa cultivars from a specific geographical region are not necessarily best nodulated with rhizobium strains isolated from the cultivars in different geographical sites.

Although general conclusions could be drawn from the presented data regarding the significance of the urgent need for the search for more infective and efficient Rhizobium strains for the local alfalfa cultivars in Oman.Both exotic as well as indigenous strains should be sought. Further testing needs to be conducted in the field, where the prevailing environmental conditions might have a great impact on the outcome due to the variability in climatic conditions. Both the Sharquia and the Dakhylia regions are hot and dry, while in the Batinah region, hot and humid climate dominates (representing the coastal areas in Oman). This could have a markedly influenced the survival of rhizobia, their ability to invade the alfalfa roots, and consequently their symbiotic ability in fixing atmospheric nitrogen.

Further research in this field is stressed to verify these results using suitable inoculant carriers in the field.Also, the identities of the isolated indigenous rhizobia needs to be thoroughly investigated using serological tests to confirm that the Omani soils are not invaded by alien imported inoculum strains of Rhizobium.

2. **Greenhouse Experiment**

Table 2 presents the characteristics of the soils used in this study. The data are presented in tables 3 through 5 .

Cultivar Effect

High nodulation was obtained with the Batinah cultivar over the Dakhlia cultivar irrespective of the strain, inoculum carrier, or the level of salinity used (table 2).This may have been a genetic character.Janice et al (1991), reported differential specifities on the cowpea cultinvars they tested . They concluded that homologous rhizobial indigenous populationsis one of the primary determinants of legume inoculation. Vance,1978 also reorted similar results with the alfalfa cultivars they tested.

Effect of irrigation water salinity

Variance in nodulation and tissue dry weights, or tissue nitrogen with water salinity is rather erratic. Inconsistent results were obtained with either cultivar at the two salinity levels tested. The low level of salinity could have

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been the reaon. The high infection percentage obtained with the Batinah strain, however may have been due to the inherent tolerance of both the cultivar from Batinah and the *rhizobium* strain isolated from Batinah, which represent the saline areas in the Sultanate.

Sinorhizobium strain Effects

The performance of the *rhizobium* strains tested consistently showed superiority of the Australlian strain together with the mixture of the local strains over the strains from Batinah and Sharquia (added as single strains) on the Batinah cultivar. However, with the cultivar from Dakhylia, the *rhizobium* strain performance was not reflected positively in either the nodulation percentage , or the nitrogen fixation parameters. The reason being that the cultivar from Dakhlia was never exposed to inoculation with *Rhizobium* as compared to the Batinah cultivar, which was subjected to inoculation with intoduced inoculum from Australia . Also, the rhizobium strain isolated from Bainah could have interacted positively with the Batinah cultivar.

Inoculum carriers

The inoculum carriers seems to have preserved the rhizobium cells since all strains in the different carriers consistently tended to increase nodulation at higher water salinity levels in all the cultivars tested. The local inoculum carriers, however improved tissue dry weights when the salinity was raised. This is reflected in the trend twards increasing tissue dry weights of both tested cultivars.Also, all carriers improved tissue nitrogen at the lower salinity level in all cultivars.This agrees with the findings of Singleton, 1992.

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Table1.The response of three local alfalfa cultivars to inoculation with three indigenous and an exotic strain of *Sinorhizobium meliloti* , and to nitrogen fertilization under Growth Chamber conditions

Alfalfa Cultivar	(Sharquia)		(Batinah)		(Dakhylia)	
Growth Traits	Nodule No.	Tissue Dry.Wt(g)	Nodule No.	Tissue Dry.Wt(g).	Nodule No.	Tissue Dry.Wt(g).
Rhizobium Strains						
1)Australia	90a*	0.552bcd	69ab	0.478bcd	24bc	0.207d
2)Canada	18bc	0.331cd	34bc	0.268cd	68ab	0.677bcd
3)Batina	18bc	0.210d	50abc	0.574bcd	53abc	0.911ab
4)Sharquia	52abc	0.691bcd	72ab	0.741bcd	46abc	0.674bcd
5)Nitrogen Control	0.0c	0.626bcd	0.0c	0.663bcd	0.0c	1.419a
6)Uninoc. Control	0.0c	0.794bc	0.0c	0.426bcd	0.0c	0.211d

*Numbers followed by the same letters within columns are not significantly different at the 0.05 level of significance by the Duncan Multiple Range Test

Table 2: Chemical and Physical properties of the soils used in the study

EC(DS/m)	2.6
pH	7.3
CaCO3 (%)	28
Soil Texture	Silt Loam
N(%)	0.03
Available P (ppm)	36.0
Soluble K (ppm)	25.3
Exchangeable K (meq/l)	4.6
Soluble Ca (meq/l)	12.6
Soluble Mg (meq/l)	7.0
Soluble Na (meq/l)	6.3
Exchangeable Na (meq/kg)	3.7
Organic matter (%)	0.9
SAR	2

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Table 3. The Effect of Sinor*hizobium* strain and inoculation carriers on the Mean* nodulation/plant of two local alfalfa cultivars

CULTIVAR	***Batinah Cultivar***		***Dakhylia Cultivar***	
Salinity level∴	**S1**	**S2**	**S1**	**S2**
Treatment				
Control	0h	0h	0h	0h
Nitrogen	0h	0h	0h	0h
Sinorhizobium strain				
Batinah				
Peat	7bcd	.4defg	3efgh	4defg
Khos	3efgh	5cdef	4defg	3efgh
By-products	4defg	6bcde	8abc	4defg
Sharquia				
Peat	0h	5cdef	0h	2fgh
Khos	7bcd	7bcd	4defg	4defg
By-products	7bcd	5cdef	5cdef	5cdef
Australia				
Peat	9ab	7bcd	2fgh	3efgh
Khos	7bcd	5cdef	4defg	2fgh
By-products	7bcd	6bcde	4defg	4defg
Mixture				
Peat	5cdef	8abc	2fgh	4defg
Khos	0h	10a	0h	4defg
By-products	9ab	8abc	1gh	4defg

LSD=2.542

∴ **S1**=0.8Ds/m, **S2**=4 Ds/m.

* Means having the same letters within the same column are not significantly different at the 5% level of significance by the Duncan Multiple Range Test.

Table 4. The Effect of *Sinorhizobium* strain and inoculation carriers on the Tissue dry weights means*/plant of two local alfalfa cultivars

CULTIVAR	***Batinah Cultivar***		***Dakhylia Cultivar***	
Salinity level∴	**S1**	**S2**	**S1**	**S2**
Treatment				
Control	0.1390	0.1280	0.0760	0.2160
Nitrogen	0.1360	0.1250	0.0990	0.0960
Rhizobium strain				
Batinah				
Peat	0.1240	0.1400	0.0990	0.0940
Khos	0.1180	0.1350	0.0760	0.1300
By-products	0.09100	0.1510	0.0870	0.1050
Sharquia				
Peat	0.1210	0.1540	0.0480	0.0110
Khos	0.1250	0.1260	0.0990	0.1250
By-products	0.1250	0.1370	0.0990	0.0910
Australia				
Peat	0.2010	0.1460	0.1040	0.1660
Khos	0.1400	0.1660	0.0380	0.1830
By-products	0.1240	0.1790	0.1270	0.1150
Mixture				
Peat	0.1580	0.1490	0.1380	0.1290
Khos	0.1630	0.1910	0.0480	0.1190
By-products	0.1610	0.2230	0.1890	0.1120

LSD= 0.05120

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Table 5. The Effect of Sinor*hizobium* strain and inoculation carriers On the percent tissue nitrogen of two local alfalfa cultivars

CULTIVAR	***Batinah Cultivar***		***Dakhylia Cultivar***	
Salinity level∴	**S1**	**S2**	**S1**	**S2**
Treatment				
Control	3.132	2.284	2.740	2.336
Nitrogen	3.154	2.417	2.568	2.687
Rhizobium strain				
Batinah				
Peat	2.873	2.163	2.767	2.510
Khos	2.831	2.563	2.465	2.483
By-products	3.004	2.437	2.646	2.664
Sharquia				
Peat	2.758	2.284	2.980	2.555
Khos	2.902	2.410	2.680	2.444
By-products	2.778	2.375	2.933	2.725
Australia				
Peat	2.406	2.165	2.345	2.411
Khos	2.667	2.563	2.450	2.455
By-products	2.323	2.437	2.378	3.174
Mixture				
Peat	2.468	2.375	2.336	2.150
Khos	2.558	2.417	2.218	2.036
By-products	2.364	2.410	2.411	2.577

LSD= 0.4803

Table 6. Summary results of the inoculum carriers used in the study

Sinorhizobium strain				
Batinah				
Peat	7bcd	4defg	3efgh	4defg
Sharquia				
Peat	0h	5cdef	0h	2fgh
Australia				
Peat	9ab	7bcd	2fgh	3efgh
Mixture				
Peat	5cdef	8abc	2fgh	4defg
Khos (**Batinah**)	3efgh	5cdef	4defg	3efgh
Khos (**Sharquia**)	7bcd	7bcd	4defg	4defg
Khos (**Australia**)	7bcd	5cdef	4defg	2fgh
Khos (**Mixture**)	0h	10a	0h	4defg
By-products(**Batinah**)	4defg	6bcde	8abc	4defg
By-products(**Sharquia**)	7bcd	5cdef	5cdef	5cdef
By-products(**Australia**)	7bcd	6bcde	4defg	4defg
By-products(**Mixture**)	9ab	8abc	1gh	4defg

* Means having the same letters are not significantly different at the 5% level of significance by the Duncan Multiple Range Test.

∴ **S1**=0.8 Ds/m, **S2**=4 Ds/m.

* Means within and between columns having the same letters are not significantly different at the 5% level of significance by the Duncan Multiple Range Test.

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