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EVALUATION OF SELECTED *Rhizobium* INOCULANTS UNDER SUDAN CONDITIONS

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ABSTRACT

Two field experiments were conducted to test the efficiency of selected rhizobia in charcoal and filter-mud as suitable carriers. *Rhizobium* isolates procured from naturally initiated nodules were compared with imported strains for nodulation in two forage plants, *Medicago sativa* and *Clitoria ternateaa* at two different sites in the Sudan. The local isolates proved to be as efficient as the imported strains. In the field experiment, there was a marked positive effect in response to inoculation with the carrier based inoculants. Fresh and dry weight, total nitrogen content and number of nodules per plant were significantly higher in modulated plants as compared with the control (non-inoculated and non-inoculated plants which received urea. The range of increase was between 16 and 94%.

Keywords: *Rhizobium* inoculants, *Medicago sativa*, *Clitoria ternatea*.

INTRODUCTION

As a result of intensive cropping, cultivable lands suffered depletion of nutrients, and fertilizer application became very essential. Countries all over the world especially the underdeveloped are currently spending a lot of money on fertilizers. The situation made it necessary to look for alternative means to minimize these costs. One alternative is to rely on leguminous crops for fixing the atmospheric nitrogen through the *Rhizobiu* nodules association. In Sudan, nodulation is usually limited by the factors affecting the legume – *Rhizobium* symbiosis. One of these factors may be the absence of the right strain of the rhizobia. Hence, in the past few decades considerable emphasis was given to improve this symbiotic process, and the production of *Rhizonium* inoculants prospered widely.

The production and utilization of *Rhizobium* inoculants in the Sudan has not been tried before, except for the limited work on *Phaseolus vulgaris* (Dawood, 1987).

MATERIALS AND METHODS

The techniques used for seed inoculation with carrier based inoculants were as described previously (ElNour, 1987). Two imported *Rhizobium* strains (TAL 380 and TAL 1372) and one local isolate (SUD 20) were tested with *Medicago sativa* L. while two local isolates (SUD 150 and SUD 160) and one imported strain (TAL 1282) were tested with *Clitoria ternatea* L. The imported strains were obtained from the NIFTAL project, University of Hawaii, U.S.A.

The experimental sites for this study were selected in such way that they represent the region in which the legumes are/or will be grown. On this basis two farmer's fields were selected: i- Soba farm at Khartoum province

and ii- Genetti farm at Northern province (350 miles north of Khartoum). Split plot design with 4 replicates was used for both experiments. The plot size was 7.5 m x 2.5 m. Forty plots were prepared for each crop at the two sites. The treatments were, control (uninoculated), uninoculated plus urea and modulated with the isolates/strains under the test. Urea was applied twice at the same rate of 26 kgN/ha at planting time and after the first cut. Plant samples were taken 30 and 60 days from sowing in the case of *Medicago sativa* and 45 and 90 days in the case of *Clitoria ternata*. Randomly selected plants from each replication were carefully dug out.

Roots were thoroughly washed, and the nodules from each root system were collected and counted. Plant tops were separated. Fresh and dry weights (oven dried at 65^{o}C) were determined. The yield of the first and second cut was also taken. The total nitrogen content was determined in the dried tissues by the conventional micro kjeldahl method as described by Pirie (1955). The results were statistically analysed using the Duncan's new multiple range test (Steel and Torrie, 1980).

The difference in yield between the two sites could be attributed to climate differences or/and to the physico-chemical properties at the two locations (Table 1). Properties such as pH, salinity and concentration of certain ions are crucial for good nodulation.

Table 1: Physico – chemical properties of Genetti and soba soils.

Property	Soil series			
	Soba			Genetti
pH	7.8			7.1
EC, m mbos/cm	1.5			0.35
Saturation %	51.5			72.6
Texture	Sand 52%	Silt 7.58	Clay 40.58	Clay
N%	0.03			0.1
P_2O_5 (ppm)	0.09			0.28
Anion m. e/L C^{--}_3	1.0			2.0
HCO^{-}_3	5.5			9.0
Cl^{-}	2.7			1.7
SO^{--}_4	10.0			3.56
Cation m. e/L ca^{++}	3.0			6.0
Mg^{++}	4.0			12.0
Na^{+}	14.3			2.0
K^{+}	0.12			0.51

*** Soil analyses was carried out according to the procedure listed in the USDA by Dr. H.M.A./Magid Soil, Department, Faculty of Agriculture, University of Khartoum.**

RESULTS AND DISCUSSION

Data presented in Tables 2, 3, 4 and 5 show that inoculation has resulted in a significant increase in dry matter accumulation, total nitrogen content and nodules number per plant. The degree of increase differed with the type of isolate/strain used and the field location. In case of *Clitoria ternatea* at Genetti site, inoculation with TAL 1282 resulted in 78% increase

in dry weight when incorporated in charcoal and 79% increase when the rhizobia were carried in filter-mud (Table 2). Inoculation with SUD 150 increased the yield by about 60% over the control for both carriers. This percentage increase dropped to 16 and 17% when the plants were inoculated with SUD 160 carried in charcoal and filter-mud respectively. However, non-inoculated plants treated with urea resulted in an increase in yield of about 25% compared with the control plants. At the Soba site, the plants responded positively to inoculation with TAL 1282, SUD 150 and SUD 160 based on both carriers. The response was similar to that of Genetti, but slightly higher values were observed (Table 3).

Table 2: Response of *Clitoria ternatea* to inoculation with charcoal and Filter-mud-based inoculants in the Genetti Farm Variance analysis was done.

	Shoot					Nodules	
Treatment per plot	**Fresh wt. g/plant**	**Dry wt. g/plant**	**Fresh wt. ton/ha**	**Dry wt. ton/ha**	**% N^-**	**Number of nodules per plant**	**Nodule Fresh wt. g/plant**
TAL1282	90.00	44.17a	25.20	11.20a	2.57a	19.00	0.23a
SUD 150	75.00	41.00b	21.80	10.10b	2.38b	17.00	0.12b
SUD 160	60.50	35.15c	18.98	7.30d	2.31c	14.00	0.18c
Uninoculated + N	61.00	33.17d	20.10	7.90c	2.40b	0.00	0.00
Control	48.40	28.15e	14.90	6.30e	1.00d	2.00	0.001d
TAL 282	92.10	44.00a	25.30	11.30a	2.50ab	20.00	0.14a
SUD 150	77.00	41.15b	22.00	10.20b	2.46ab	19.00	0.22b
SUD 160	61.70	37.07c	19.00	7.40d	2.30c	16.00	0.19c
Uninoculated + N	61.10	33.10d	20.20	7.90c	2.40b	0.00	0.00
Control	48.50	28.30e	15.20	6.30e	1.00d	2.00	0.001d
LSD	-	0.15**	-	0.10**	0.14**	-	0.01**

In a column means followed by the same letter are not significantly different at P = 0.05 as given by Duncan's New Multiple Rang Test.

**** Highly significant difference.**

Inoculation of *Medicago sativa* with TAL 380, TAL 1372 and SUD 120 at both sites resulted in a significant increase in dry matter accumulation, nodule number, total nitrogen and dry weight per plant.

At the Soba farm, when the plants were inoculated with TAL 380, the increases in dry yield were 65% in the charcoal inoculant and 96% in the filter-mud inoculant. The imported strain TAL 1372 produced a comparable increase. However, inoculation with the local isolate, SUD 120, increased the dry yield by 93 and 94% when the rhizobia were carried in charcoal and filter-mud respectively (Table 4). Similar results were observed at Genetti site when *Medicago sativa* was inoculated with the above mentioned isolates/strains (Table 5). The plants treated with urea only, responded positively and showed an increase in dry weight of 32% and 1% at Soba and Genetti sites respectively (Tables 4 and 5). The results obtained in this study agree well with those obtained by Hickey *et al*. (1974) and Dawood (1987). It

was observed that the urea caused total inhibition of nodule production at both sites. The inhibitory effect of combined nitrogen on nodule initiation was also observed by Dart and Mercer (1965), Dart and Wildon (1970), Khalifa (1987) and Van Heerden *et al*. (2007).

Table 3: Response of *Clitoria ternatea* to inoculation with charcoal and Filter-mud-based inoculants in the Soba Farm the Variance analysis was done.

	Shoot					Nodules	
Treatment/ plot	Fresh wt. g/plant	Dry wt. g/plant	Fresh wt. ton/ha	Dry wt. ton/ha	% Total N^-	Number of nodules/ plant	Nodule Fresh wt. g/plant
TAL 1282	95.00	48.15a	26.30	11.80a	3.15ab	20.00	0.27a
SUD 150	77.00	39.70b	22.80	10.70b	3.00c	18.00	0.21bc
SUD 160	62.20	32.20d	20.20	7.90c	2.70	15.00	0.20c
Uninoculated + N	63.20	33.70c	21.90	8.20d	2.30	0.00	0.00
Control	49.50	29.80e	16.20	6.90e	1.00d	2.00	0.001d
TAL 1282	96.00	48.10a	26.50	11.90a	3.20a	23.00	0.28a
SUD 150	77.00	39.80b	22.70	10.80b	3.10	20.00	0.22b
SUD 160	64.00	32.10d	20.30	7.70c	2.80	17.00	0.21bc
Uninoculated + N	63.30	33.80c	21.70	8.20d	0.00	0.00	0.00
Control	49.30	29.90e	17.10	6.80	2.00	2.00	0.001d
LSD	-	0.14**	-	0.19**	0.09**	-	0.01**

In a column means followed by the same letter are not significantly different at P = 0.05 as given by Duncan's New Multiple Rang Test.

**** Highly significant difference.**

Table 4: Response of *Medicago sativa* to inoculation with charcoal and Filter-mud-based inoculants in the Soba Farm the Variance analysis was done.

	Shoot					Nodules	
Treatment/ plot	Fresh wt. g/plant	Dry wt. g/plant	Fresh wt. ton/ha	Dry wt. ton/ha	% Total N^-	Number of nodules/ plant	Nodule Fresh wt. g/plant
TAL 380	18.51	8.27b	4.51	1.719c	2.25c	9.00	0.09c
TAL 1372	19.80	8.30b	5.76	1.737bc	2.50b	10.00	0.10bc
SUD 120	22.43	9.60a	6.43	2.01a	2.65ab	11.00	0.11ab
Uninoculated + N	16.90	7.50c	4.19	1.37d	2.00d	0.00	0.00
Control	13.20	2.25d	3.70	1.04e	1.00e	0.00	0.00
TAL 380	18.60	8.30b	4.60	1.72c	2.20c	11.00	0.10bc
SUD 1372	19.90	8.40b	5.78	1.74b	2.55b	13.00	0.11ab
SUD 120	22.60	9.70a	6.45	2.02a	2.70a	14.00	0.12a
Uninoculated + N	16.50	7.40c	4.18	1.37d	2.00d	0.00	0.00
Control	13.20	5.20d	3.70	1.04e	1.00e	0.00	0.00
LSD	-	0.10**	-	0.01**	0.08**	-	0.008**

In a column means followed by the same letter are not significantly different at P = 0.05 as given by Duncan's New Multiple Rang Test.

**** Highly significant difference.**

Table 5: Response of *Medicago sativa* to inoculation with charcoal and Filter-mud-based inoculants in the Genetti Farm the Variance analysis was done.

	Shoot					Nodules	
Treatment/ plot	**Fresh wt. g/plant**	**Dry wt. g/ plant**	**Fresh wt. ton/ha**	**Dry wt. ton/ha**	**% Total N^-**	**Number of nodules/ plant**	**Nodule Fresh wt. g/plant**
TAL 380	22.30	10.20b	14.00	5.05c	2.60b	17.00	0.21c
TAL 1372	24.40	11.80b	14.20	5.46b	2.70a	19.00	0.21bc
SUD 120	26.00	12.70a	14.80	6.25a	2.90	22.00	0.24a
Uninoculated + N	20.90	9.80d	13.20	4.29c	2.20c	1.00	0.00
Control	18.20	8.15e	11.00	3.80e	1.10d	2.00	0.001d
TAL 380	22.50	8.30c	14.20	5.10c	2.60b	19.00	0.21d
SUD 1372	24.60	11.90b	14.50	5.50b	2.70a	22.00	0.22b
SUD 120	26.00	12.80a	14.90	6.30a	2.80	24.00	0.25a
Uninoculated + N	21.00	9.70d	13.30	4.30d	2.20c	1.00	0.00
Control	18.20	8.15e	11.00	3.80e	1.10d	2.00	0.001d
LSD	-	0.10**	-	0.06**	0.07**	-	0.01**

In a column means followed by the same letter are not significantly different at P = 0.05 as given by Duncan's New Multiple Rang Test.

**** Highly significant difference.**

Conclusion

The two experimental crops responded positively to inoculation with the carrier based inoculation. The degree of response varied according to the isolate/strain used and the site of experimentation. Thus, the response of *Clitoria ternatea* and *Medicago sativa* at the Genetti farm was better compared with that at the Soba farm, whereas at both sites the isolate/strain of *Rhizobium* used had a significant effect on dry matter yield expressed in ton/ha.

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تقييم ملقحات مختارة من الرايزوبيوم تحت الظروف السودانية.

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أجريت تجربتان حقليتان لاختبار كفاءة سلالات الرايزوبيوم فى بيئات مناسبة من الفحم النشط والطمى الراشح. تم عزل الرايزوبيوم من عقد جذرية طبيعية حيث قورنت بسلالات مستوردة لها القدرة على تكوين العقد فى نوعين من نباتات العلف هما البرسيم الحجازى وبسلة الفراشة وذلك فى موقعين مختلفين فى السودان.

لقد أوضحت السلالات المحلية كفاءة مناظرة للسلالات المستوردة، ففى التجارب الحقلية كان هناك تأثير ايجابى واضح كاستجابة للتلقيح بالبيئات الحاملة للملحقات.

كما أوضحت النتائج أن الوزن الطازج والجاف للمجموع الخضرى أو العقد الجذرية، وكذلك محتوى النيتروجين الكلى وعدد العقد الجذرية لكل نبات كان معنويا أعلى فى النباتات الملقحة مقارنة بالنباتات الغير ملقحة بالرايزوبيوم وكذلك النباتات التى عوملت باليوريا دون تلقيح بالرايزوبيوم حيث تراوحت الزيادة بين ١٦ – ٩٤%.