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INFLUENCE OF *RHIZOBIUM* INOCULATION AND SEED PELLETING ON NODULATION OF GREEN MANURE LEGUMES IN AN ACIDIC COCONUT SOIL*

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ABSTRACT

The effect of rhizobial inoculation and seed pelleting was studied in an acidic soil on nodulation, N_2 fixation and growth of green manure legumes which were found to be suitable for cultivation in coconut gardens. Three *Rhizobium* strains (32 HI, EGI, PAU Cowpea) were used as inoculants for *Calopogonium mucunoides* and an IARI strain (L1) and a local isolate (L2) for *Leucaena leucocephala*. The effectiveness of pelleting materials such as rock phosphate, Ca CO_3, charcoal and dolomite was compared along with different inoculants. Rhizobium inoculation resulted in increased growth and nodulation in both legumes and the extent of response varied when different cultures were used. The response in nodulation behaviour, dry matter yield and nitrogen concentration in tissues was significantly more only when pelleting was done on *Rhizobium* inoculated seeds. The difference in response due to the use of different pelleting materials was not significant. The present study indicated the need for pelleting along with rhizobial inoculation to get substantial improvement in nodulation and nitrogen fixation of green manure legumes in acidic coconut soils.

INTRODUCTION

The advantages of growing *Calopogonium mucunoides* as a green manure crop in coconut basins and the suitability of including *Leucaena leucocephala* in coconut-based cropping systems have already been reported (Bavappa et al, 1986; Thomas and Shantaram, 1984). Cultivation of leguminous cover crops is also recommended for other plantation crops such as oil palm, rubber etc. The legume – *Rhizobium* association is one of the most efficient systems of biological nitrogen fixation. Nodulation of *Calopogonium* and *Leucaena* was reported to be poor in acidic coconut soils when compared to other promising green manure/cover legumes (Thomas and Shantaram, 1984). *Rhizobium* inoculation is vital to obtain maximum benefits from leguminous crops when natural nodulation is not effective.

The soils in coconut growing tract of Kerala are acidic in nature. The survival of introduced rhizobia and nodulation were reported to be severely affected under acidic soil conditions (Vincent, 1965; Holding and Lowe, 1971). Rhizobial population and nodulation could be enhanced by liming of acidic soils (Jones, 1966) but it is very expensive and impractical. The impregnation of rhizobia in lime coatings on legume seeds was reported to alleviate the detrimental effects of soil acidity on rhizobial survival, nodule formation and N_2 fixation (Shipton and Parker, 1967). The present study compares the effectiveness of different rhizobial

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cultures alone and with pelleting using different materials on nodulation and growth of *C. mucunoides* and *L. leucocephala* in an acidic coconut soil.

MATERIALS AND METHODS

A creeping cover legume, *Calopogonium mucunoides* Desv. and a tree legume, *Leucaena leucocephala* (Lam) de wit. were used as test plants in this study. Three *Rhizobium* strains of cowpea miscellany group, *Rhizobium* sp. 32HI, EGI (IARI) and PAU cowpea formed inoculants in case of *C. mucunoides*. A *Leucaena Rhizobium* culture (LI) from IARI, New Delhi and a local strain of Rhizobium from *Leucaena* (L2) were tested on *L. leucocephala*. The local culture of *Rhizobium* was isolated from naturally nodulated *Leucaena* grown in CPCRI Kasaragod farm. It was purified and characterised as *Rhizobium* by standard procedures (Vincent, 1970). The effectiveness of the four pelleting materials *viz*., lime, rock phosphate, charcoal and dolomite was compared alongwith the five rhizobial cultures. Rock phosphate and dolomite were powdered and sieved before use. *Leucaena* seeds were given hot water treatment prior to inoculation with rhizobial cultures.

The rhizobial cells grown in yeast extract mannitol media (Vincent, 1970) were suspended in sterile distilled water. The number of rhizobial cells in the inoculum were 4.1 x 10^9, 2.1 x 10^9, 4.5 x 10^9, 4.6 x 10^9 and 2.0 x 10^9 per ml for the cultures 32 HI, EGI, PAU cowpea, L1 and L2, respectively. Drug resistant strains of rhizobia were used to know whether the nodulation by introduced rhizobia is increased by seed pelleting. Resistance to 100 μg of streptomycin and 100 units of penicillin was developed in *Rhizobium* strains 32 H1 and L1 before inoculation to the crops.

Seeds of the two legumes were surface-sterilised and aliquots of rhizobial suspension were inoculated directly on the seeds using gum arabic as adhesive. Pelleting was done as per the methods described by Roughley (1970). Controls were maintained without pelleting and inoculation treatment. The seeds were sown in acidic (pH 5.2) sandy loam soils in pots. The treatment details for *C. mucunoides* and *L. leucocephala* are given in Tables I and II, respectively. The plants were thinned to one per pot after germination.

The plants were harvested at 60 days growth and roots were examined for nodules. The nodular contents of plants inoculated with rhizobia having antibiotic markers were plated on YEM media supplemented with the antibiotics and occurrence of rhizobia with markers (str^+P^+) was recorded. Dry weight of shoot and nodules were determined after drying at 70°C for 48 h. Shoot nitrogen was determined from oven dried and powdered plant samples by microkjeldahl analysis.

RESULTS AND DISCUSSION

Laboratory testing of rhizobial cultures *viz*., *Rhizobium* sp. 32HI, EGI, PAU cowpea and L1, used in this study showed optimum growth in yeast extract mannitol broth at a pH of 7.0 and there was considerable reduction in growth at the acidic pH range of 5.0–5.5 (Anonymous, 1985). Such low pH values are generally observed in coconut growing soils of Kerala. In inoculation trials with green manure legumes, these rhizobia are to be protected from acidic soil conditions in the period between sowing of inoculated seeds and infection of roots by rhizobia. The effect of seed pelleting has been tested in this study using alkaline or neutral materials.

Nodulation behaviour, shoot dry weight and shoot nitrogen accumulation were the

Table I. *Total nitrogen, shoot dry weight and nodulation in* C. mucunoides *as influenced by* Rhizobium *inoculation and seed pelleting*

Rhizobium Treatment	Pelleting Treatment	Nitrogen yield mg plant^{-1}	Shoot dry wt. g plant^{-1}	Nodule dry wt. mg plant^{-1}	Nodule No. plant^{-1}
Uninoculated	Unpelleted	15.61	0.61	28.67	28.00
Rhizobium sp. EGI	Unpelleted	21.72	0.81	40.67	45.33
,,	Calcium carbonate	36.40	1.08	56.00	54.33
,,	Rock phosphate	25.16	0.90	63.70	62.00
,,	Charcoal	39.22	1.18	61.67	58.00
,,	Dolomite	24.76	0.88	44.00	30.67
Mean		29.45	0.97	51.67	48.58
Rhizobium sp. PAU	Unpelleted	22.56	0.76	45.00	37.00
,,	Calcium carbonate	27.08	0.87	56.00	46.00
,,	Rock phosphate	27.61	0.86	56.67	42.00
,,	Charcoal	26.28	0.84	53.33	43.33
,,	Dolomite	32.29	0.97	55.67	53.33
Mean		27.16	0.86	53.13	44.33
Rhizobium sp. 32H1	Unpelleted	16.46	0.63	29.33	28.67
,,	Calcium carbonate	20.11	0.65	32.00	33.67
,,	Rock phosphate	20.23	0.65	36.00	27.33
,,	Charcoal	24.83	0.84	40.67	38.38
,,	Dolomite	27.14	0.86	43.33	41.33
Mean		21.75	0.73	36.27	33.87
LSD (P = 0.05) for comparison of control *VS* treatments		8.16	0.19	14.89	NS
LSD (P = 0.05) for comparison of rhizobial cultures		5.07	0.12	9.29	NS

parameters used to evaluate symbiotic effectiveness of rhizobia. Of the three rhizobial cultures inoculated to *C. mucunoides* significant response to inoculation was obtained in shoot dry weight only with the culture EGI when inoculated without pelleting treatments (Table I). When pelleting was done with the rhizobial inoculation, significant increases were observed in nitrogen yield, shoot dry weight and nodule weight with all the three rhizobial cultures compared to uninoculated control. The extent of increase in growth and nodulation also varied significantly when different cultures were inoculated. Cultures EGI and PAU cowpea were superior to 32HI in increasing growth and nodulation characteristics. Shoot dry weight and nitrogen (Fig. 1) differed significantly

Table II. *Total nitrogen, shoot dry weight and nodulation in* L. leucocephala *as influenced by* Rhizobium *inoculation and seed pelleting*

Rhizobium Treatment	Pelleting treatment	Nitrogen shoot mg plant^{-1}	Shoot dry wt. g plant^{-1}	Nodule dry wt. mg plant^{-1}	Nodule No. plant^{-1}
Uninoculated	Unpelleted	18.70	0.67	20.50	18.25
Rhizobium sp. IARI (LI)	Unpelleted	23.79	0.74	25.50	19.75
,,	Calcium carbonate	25.10	0.75	32.75	23.75
,,	Rock phosphate	31.82	0.81	33.00	24.00
,,	Charcoal	25.76	0.77	26.50	22.50
,,	Dolomite	32.16	0.86	28.25	25.50
Mean		27.73	0.78	29.20	23.10
Rhizobium sp. Local (L-2)	Unpelleted	25.90	0.83	24.50	20.75
,,	Calcium carbonate	30.19	0.88	36.00	23.75
,,	Rock phosphate	33.66	0.97	45.75	30.00
,,	Charcoal	28.85	0.82	35.75	34.75
,,	Dolomite	38.99	0.99	46.25	30.25
Mean		31.52	0.89	37.65	27.50
LSD (P=0.05) for comparison of control *VS* treatments		9.44	NS	11.42	6.73
LSD (P=0.05) for comparison of rhizobial cultures		NS	NS	7.30	4.04

when inoculation + pelleting treatments were compared with unpelleted inoculation treatments. The growth and nodulation responses were not significantly different in pelleting treatments using different materials. However, maximum responses was observed when charcoal was used as pelleting material.

The data on the response of *L. leucocephala* to rhizobial inoculation and seed pelleting are presented in Table II. Significant response to rhizobial inoculation was obtained in nitrogen yield, nodule dry weight and nodule number when pelleting was done with inoculation. The two rhizobial cultures also differed significantly in causing nodulation in the host. As far as the nitrogen yield was concerned, inoculation + pelleting treatments were superior to unpelleted inoculation treatments (Fig. 1). In *Leucaena* also the difference in response due to the use of different pelleting materials was not significant.

Plating of the nodular contents of plants inoculated with rhizobia having antibiotic resistant markers revealed higher colonisation by introduced rhizobia in nodules of plants which received inoculation+ pelleting

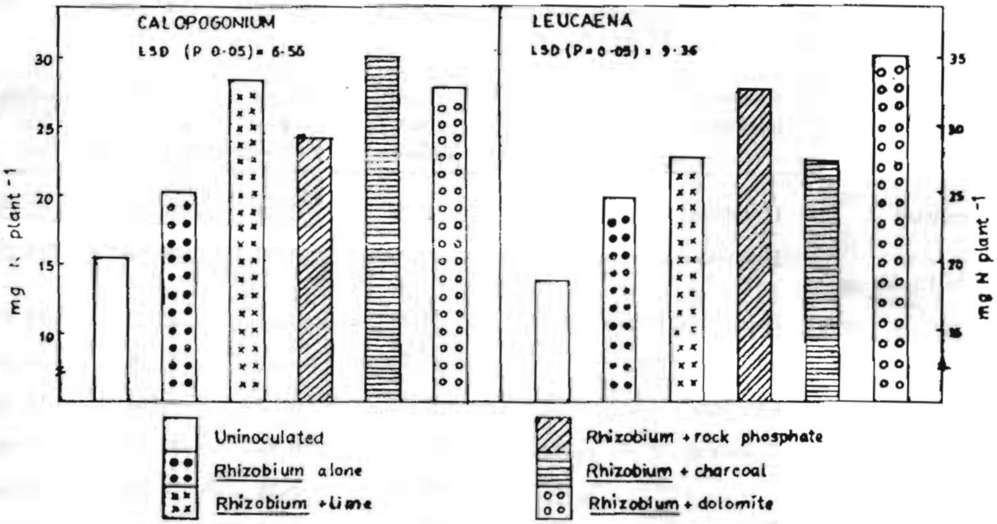

Fig. 1. Effect of *Rhizobium* inoculation and pelleting on nitrogen yield in Calopogonium and *Leucaena*

treatments when compared to inoculation alone. Such higher colonisation rates were observed in nodules of both *Calopogonium* and *Leucaena* when inoculated with drug resistant strains of 32 H1 and L1 cultures, respectively. This indicate that increase in the symbiotic efficiency is due to better survival and subsequent infection of roots by introduced rhizobia under pelleting treatments.

Infectivity and effectiveness of the rhizobial cultures varied significantly on both the legumes. This may be due to the factors such as strainal variation in tolerance to acidic soils and compatibility of the rhizobia with a particular host. Strain differences in tolerance to acidic soils have been reported in *R. meliloti* and *R. trifolii* (Ssali, 1981). Variation in the response of different *Leucaena* cultivars was also reported in an earlier study (Thomas et al 1985).

All the rhizobial cultures tested on *C. mucunoides* and *L. leucocephala* elicited significant responses by increasing shoot weight and/or increasing the nitrogen content over uninoculated control when pelleting was done along with inoculation. Only one rhizobial culture (EGI) could induce significant response in the absence of pelleting. This shows the need for pelleting to protect rhizobia from acidic conditions in the tested soil. Studies conducted in Nigeria (Kang, Hangju and Ayanaba, 1977) and West Africa (Danso, 1988) have demonstrated that lime pelleting of inoculated soybean seeds grown in acid soil increased rhizobial numbers in soil, nodulation and growth of soybean. The pelleting material around the inoculum provides a microenvironment, favourable for survival and growth of rhizobia till the roots develop and rhizobia get a chance to infect the roots. Once infection has taken place the rhizobia are protected from acidic conditions inside the roots. The present study has shown that seed inoculation alone may not give substantial improvement in nodulation and growth of green manure/cover legumes unless pelleting treatment is done in the acidic coconut soils.

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