IDENTIFICATION OF A CARRIER FOR SOYBEAN INOCULANT PRODUCTION IN SRI LANKA*

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ABSTRACT

Three local materials, peat, filter mud and coconut shell powder were evaluated as potential carriers for soybean inoculation in Sri Lanka. On the basis of physical and chemical analysis and growth studies filter mud was identified as the most suitable and a field trial showed that there was no significant difference ($p=0.05$) between the effectiveness of the *R. japonicum* strains on filter mud in comparison with the standard imported peat.

INTRODUCTION

Soybean (*Glycine max*) is not indigenous to Sri Lanka. The extensive cultivation of this species was begun only after the establishment of the International Soybean Program (INTSOY) in 1973. By 1980 over 1,100 ha of soybean were in seasonal cultivation. One condition for successful soybean production is the presence of and effective nodulation by *Rhizobium japonicum* strains, and where soybean has not been cultivated before it is necessary to inoculate the seed with effective *R. japonicum* strains.

Inoculants normally consist of a mixture of an appropriate rhizobia strain or strains and a carrier material. A wide range of carriers has been used as a base for *Rhizobium* inoculants (Burton, 1981) but peat has remained the preferred material because of the protection it offers to cultures against high storage temperatures and its ability to maintain hydration of the cultures. Peat also usually contains certain essential

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nutrients which not only help survival of the cultures but also allow further growth and multiplication during storage (Pugashetti *et al*, 1971). Imported inoculum for large scale cultivation of soybeans is expensive and for that reason it was important to investigate the possibilities of developing a local inoculant. The choice of inoculant carrier material depends largely on its suitability as a good carrier medium for rhizobia, and the criteria for suitability have been summarised by Burton (1978) as non-toxicity to *Rhizobium* species, good absorption qualities, easy preparation and sterilization, good adhesion to seeds and ready availability at moderate costs. On the grounds of availability three potential local carriers, peat, filter mud (a waste product from the sugarcane industry) and coconut shell powder were identified. This paper reports their evaluation as potential carriers for soybean inoculation in Sri Lanka.

MATERIALS AND METHODS

1. Carrier materials

Standard peat was obtained from the Nitrogen Fixation for Tropical Legumes Centre (NifTAL), Hawaii, United States of America and used as a standard in all experiments. Local peat, obtained from Horton Plains Sri Lanka, was prepared by drying, grinding in a hammer mill and passing through a 200 mesh sieve. Coconut shell powder was obtained from a local coconut mill and prepared by passing through a 200 mesh sieve. Filter mud, a deposit obtained during the filtration and clarification of crushed sugarcane, was similarly prepared. Forty nine samples of carrier material were packed in high density (0.32 mm) polythene bags (10×15 cm) and sterilised by autoclaving for 1.5 hours at 121°C, 1.06 kg/cm.

2. Strains

1. Cultures of *Rhizobium japonicum* which had the strain numbers TAL 379, TAL 377 and TAL 102 (obtained from NifTAL, Hawaii) were used for these studies.

2. Media. The standard media used was yeast mannitol agar (YMA) and yeast mannitol broth (YMB, Vincent, 1970) .20 ml starter cultures in YMB were added to 2.5 l of YMB in a simple fermenter and allowed to grow at 28°C for 10 days. Sufficient culture was then added to the carrier to bring it to a predetermined moisture content.

3. **Enumeration of rhizobia.** The drop method of Miles and Misra (Vincent 1970) was used to count the viable number of rhizobia per g of sterile carrier.

4. **Evaluation of effectiveness of strains.** Soybean seeds were first coated with gum arabic by mixing 50 g of plant seed with 1.5 ml of gum arabic solution in a polythene bag. When all the seeds were wet, 5 g. of carrier material were added and the contents of the bag remixed until the seeds were well coated. The seeds were then allowed to dry at room temperature for 20 minutes. For pot trials 5 inoculated seeds were planted in a 150 ml pot containing sterilized sand and thinned to three plants per pot after a week. Plant nutrient solution was added daily to the pots and after 45 days plants were harvested and dried at 78°C for 48 hours. In the field trials when mixed strain inoculants were evaluated, an experimental area of 10m × 10m where soybean had not been grown before was used. Plots 5m × 0.8m were prepared with furrows 20 cm wide and 15 cm deep on either side to prevent plot to plot contamination. A basal fertilizer treatment of 28 kg P / ha and 30 kg K / ha was applied to all plots and 70 kg N / ha was applied in split doses as basal and at 3rd and 6th week to the plots that received the nitrogen treatment. Five treatments (no added nitrogen or inoculant, added nitrogen, filter mud inoculant, local peat inoculant and standard peat inoculant) were arranged in a randomised complete block design with four replicates. Plots were irrigated every fourth day for four weeks. Ten plants per plot were sampled at 50% flowering and nodule colour, abundance and distribution recorded. The total plant dry matter at 50% flowering was used to measure the effectiveness of the inoculants.

RESULTS

1. Physical and chemical properties of the carriers

The data presented in Table 1 summarizes the salient properties of the carriers under evaluation. The standard peat, local peat and coconut shell powder were all acidic and required addition of calcium to adjust their reaction to the desirable range pH 6.9 — 7.1 (Date and Roughley, 1977). Moisture holding capacity is an important physical property of carriers and influences the degree of adjustment required to bring the inoculant to the desired range of moisture content (35% to 55% moisture). The local peat had a lower moisture holding capacity when compared with the other carriers and indeed its final moisture content was below the minimum value recommended for carriers. Vincent (1970) has identified the necessary requirements of inorganic ions and trace elements in carrier material and comparison of these with the average composition of the carrier materials (Table 1) indicated the potential suitability of the filter mud as a carrier. Both the local peat and the coconut shell powder would need supplementation with additional nutrients if they were to be used as carriers.

2. Short term growth studies

The objectives of the short term growth studies were to determine a suitable temperature for the incubation of the carrier with *R. japonicum*, the incubation time required for *R. japonicum* to reach its maximum population in the inoculants and the interaction of carrier, strain and temperature on the change in viable number of *R. japonicum* per g of inoculant. Viable counts were taken at three day intervals for a month. No differences were detected between different strains and therefore the results are summarized for one strain, TAL 379, in Fig. 1. For filter mud and the local peat, 30 days growth at temperatures of 4°, 28° and 38 °C resulted in final populations of greater than 10^7 / g. which is in excess of the minimum recommendation of 10^6 / g suggested by Roughly (1970). For filter mud, local peat and peat, an incubation temperature of 28°C gave the highest counts and maximum or near - maximum populations were achieved with an incubation period of 12 — 16 days. However for the coconut shell powder rhizobia populations decreased very rapidly at the two highest temperatures but much more slowly at 4°C at which temperature viable populations of 10^5 / g could still be detected after a month.

3. Long term growth studies

The objective of these studies was to determine the maximum storage period and most suitable storage temperatures since under normal conditions inoculants generally have to be stored up to at least six months. The results for strain TAL 379 (no differences were detected between different strains) are summarized in Fig. 2. For both local peat and filter mud, decrease in rhizobia populations was observed at the higher storage temperatures (28 °C and 38°C) while storage at 4°C resulted in little change. However the final populations at all temperatures were above the minimum viability number for an inoculum (10^6 / g) and compared favourably with survival in the standard peat. However coconut shell powder did not support the survival of populations of *R. japonicum* since even at 4°C the population had declined to less than 10^7 / g by two months.

4. Effectiveness of the *R. japonicum* inoculants using local carriers

A good quality inoculum should have sufficient rhizobia per g of carrier material to result in good field nodulation and the inoculant strains should have the ability to form nodules and fix nitrogen effectively. The objective of this study was to examine the effectiveness of the strains on the local carriers under two environmental conditions: (*i*) single strain inoculants in pots containing sterile sand and (*ii*) multi-strain inoculants tested in the field.

(i) Single strain inoculants. The results of the pot experiment are summarized in Table 2. No significant differences in plant dry weight could be detected between different strains stored on different carriers under the three storage temperatures which indicated that the local carriers of local peat and filter mud could be used for inoculant production without the *R. japonicum* losing their effectiveness.

(ii) Multi-strain inoculant. Table 3 summarizes the result of the field trial. A marked difference was observed between the inoculated treatments and the uninoculated control. The plants which received inorganic nitrogen (70 kg N / ha) formed no nodules. No. nodules were observed in the control plants and the top dry weights were significantly lower ($p=0.05$) when compared to the other treatments. There was no significant difference between the effectiveness of the *R. japonicum* strains on the local carriers as compared to that on the NifTAL peat.

DISCUSSION

The evaluation of possible carriers is an important step in the inoculant production programme in Sri Lanka. With readily available and cheap alternatives to imported peat, these studies have demonstrated that filter mud would prove to be a highly suitable alternative to peat. Although the physical and chemical studies of the coconut shell powder and local peat had suggested that these had potential, the handling problems of peat combined with its low water holding capacity and the inability of the coconut powder to support and maintain rhizobia growth, finally ruled these out as possibilities. Further studies have shown (Thirukumaran, 1983) that the value of the filter mud can be further enhanced by the mixing of three parts filter mud to one part local peat. This has the effect of improving the survival inoculum at low moisture contents (below 25%).

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Table 1. Physical and chemical properties of the carrier material

	$CaCo_3$ added to bring to neutrality	Moisture holding capacity % w/w	N	Inorganic constituents % P	K	Ca	Mg
Standard peat	20	65	1.63	0.02	0.07	2.5	0.49
Coconut Shell Powder	3.1	60	0.29	0.008	0.11	0.002	0.002
Filter Mud	0	208	1.39	1.65	0.11	3.94	0.81
Local Peat	33	34	0.62	0.06	0.20	0.02	0.45
Vincent (1970) recommendation			0.831	0.403	0.967	0.018	0.024

Table 2. Mean top dry weight of the soybean plant inoculated with different carrier inoculants stored at 4°C, 28°C and 38°C for six months

Carriers	*Strains*	*Mean top dry weight*[a] *g / plant* 4°C	28°C	38°C
Standard Peat	TÅL 379	1.68 (0.31)[b]	1.96 (0.12)	1.83 (0.07)
	TAL 377	1.60 (0.22)	1.99 (0.03)	1.96 (0.08)
	TAL 102	1.94 (0.11)	1.93 (0.11)	1.78 (0.20)
Local Peat	TAL 379	1.63 (0.18)	2.12 (0.15)	1.61 (0.19)
	TAL 377	1.89 (0.66)	1.60 (0.40)	1.84 (0.55)
	TAL 102	2.14 (0.80)	1.63 (0.44)	1.92 (0 19)
Filter Mud	TAL 379	1.54 (0.22)	1.81 (0.19)	1.79 (0.47)
	TAL 377	1.36 (0.31)	2.11 (0.30)	1.91 (0.70)
	TAL 102	2.15 (0.77)	1.45 (0.30)	1.62 (0.29)

a. mean of nine plants, three plants per plot

b figures are the standard deviation

Table 3. The effect of mixed strain inoculants in the field on nodulation and top weight

Mixed strain inoculants	*Nodules*	*Mean top dry weight (g)*
Filter mud	Well nodulated	3.54**
Final peat	Well nodulated	3.55**
Peat	Well nodulated	3.97**
Urea application (70 kg N/ha)	No nodules	3.95**
Control (no inoculation and no nitrogen)	No nodules	1.20

** Significantly higher ($p = 0.05$) compared to control

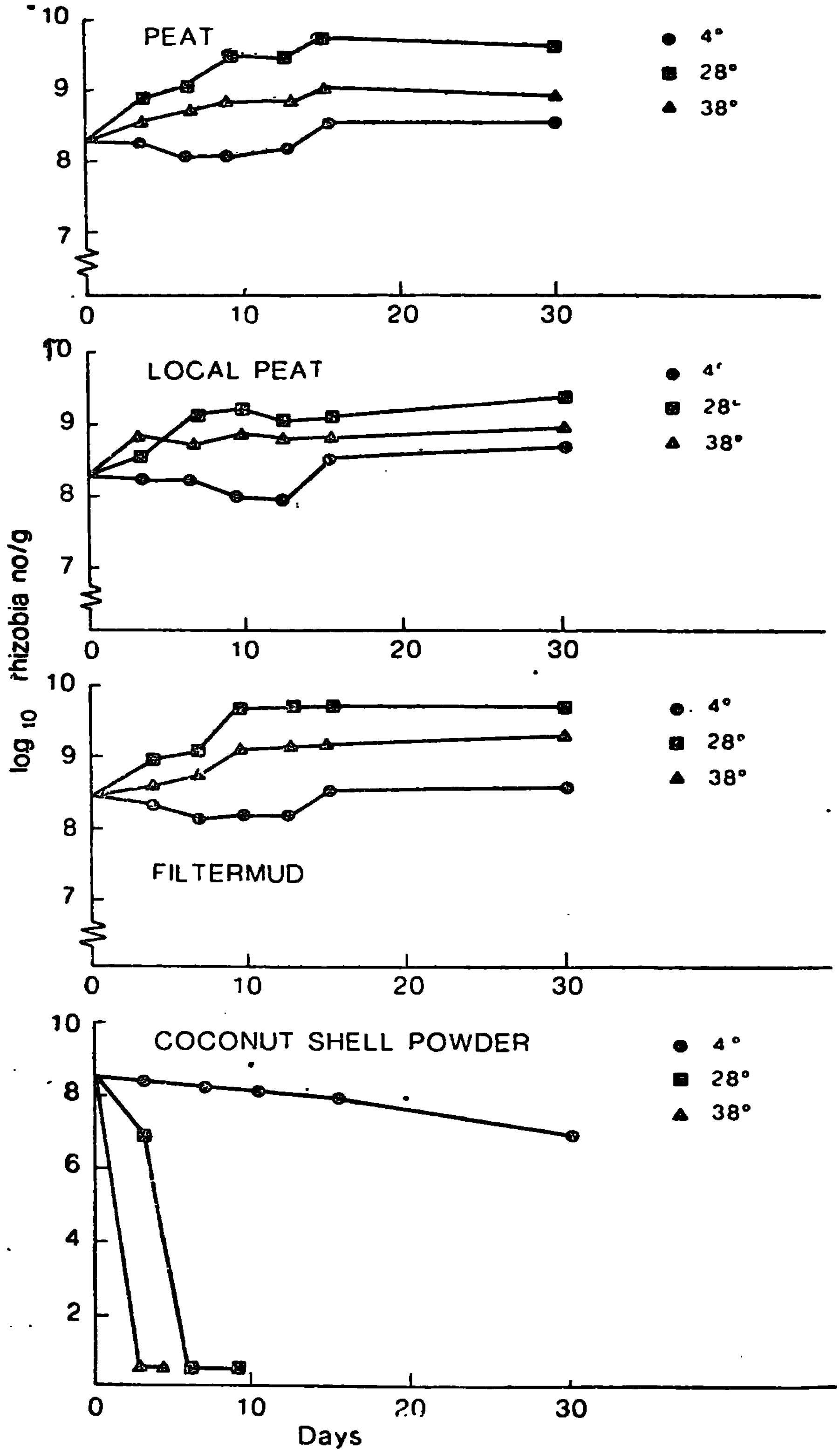

Figure 1. Survival of *R. japonicum* strain TAL 379 on autoclaved carrier material

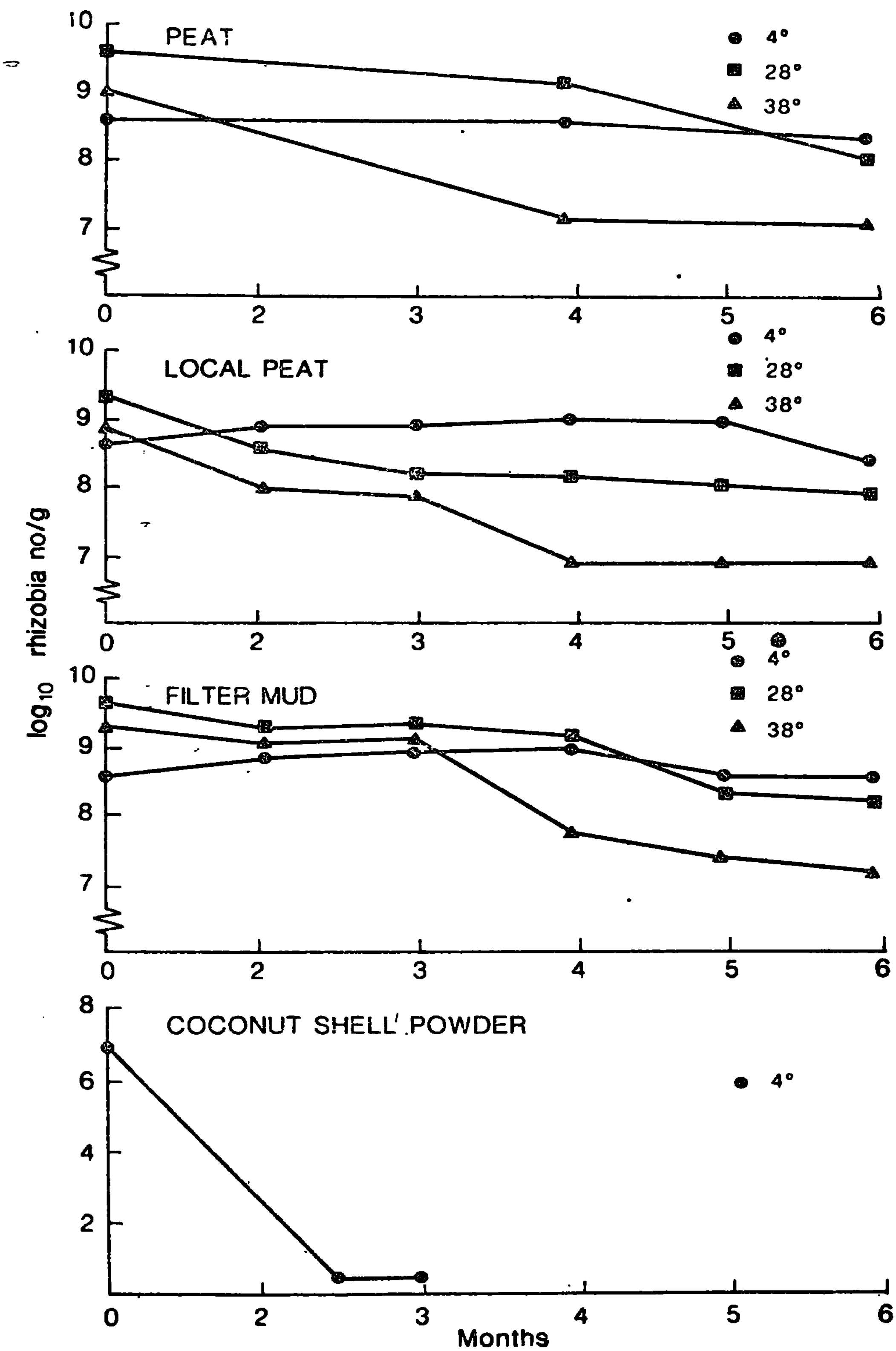

Figure 2. **Survival of *R. japonicum* strain TAL 379 in long term storage**