A Coir-Dust Soil Compost for Rhizobium

K. P. JOHN

A method of preparing Rhizobium *compost using coir dust, soil and calcium carbonate is explained in detail. The compost has been in commercial use since 1956 when it was first described. Tests are outlined in which the compost is compared with the previously used pure cultures on agar slopes: the compost differs little from agar slopes in respect of numbers of viable* Rhizobium *and effectiveness in inoculating* Centrosema pubescens.

The Pathological Division of the Rubber Research Institute of Malaya in 1937 started isolating and supplying *Rhizobium* cultures for inoculation of legume cover plant seeds in the form of pure cultures on agar slopes in test tubes (BEELEY, 1938). This practice was continued till the outbreak of war in 1941, when all work was disrupted, but was resumed at the end of the war in 1945. Later, as replanting gathered momentum, aided by the government-sponsored rubber-replanting schemes, there was a greatly increased demand for *Rhizobium* cultures to inoculate the legume seed planted as a soil cover. The task of preparing and packing the tubes became so large that it was necessary to look for a simpler and cheaper method of distribution. Although the use of peat and/or soil as a medium for *Rhizobium* compost preparation was known, no details were available (NEWBOULD, 1951; VAN SCHREVEN *et al.*, 1954; BONNIER, 1955). Work on a *Rhizobium* compost using coir dust* and soil as a base started in 1955 and a suitable method was developed by 1956 (RUBBER RESEARCH INSTITUTE OF MALAYA, 1956; 1959).

METHOD OF PREPARATION

Various trials were made using different proportions by weight of coir dust, soil and calcium carbonate, to determine which formula gave the maximum number of *Rhizobia* per gram of compost after inoculation and storage at room temperature. The mixture finally selected contained equal weights of finely sifted soil and sifted coir dust, with 5% of calcium carbonate. The ingredients are mixed dry and moistened by adding fractionally more than an equal weight of water (usually 1:1.1). The mixture is autoclaved at 20 lb pressure for two hours and, when cooled, inoculated with the appropriate *Rhizobium* suspended in sterile water; one tube of culture is used to inoculate five pounds of compost, which is then incubated at room temperature for seven days, the contents being shaken for a few minutes each day. After incubation the compost is tested for viability or fungus contamination by streaking a small quantity on yeast extract agar and observing the growth of the organisms that emerge, before packing in prepared polythene bags, about 10 gm to each, and sealing. A packet is treated as equivalent to one tube of the culture previously distributed, although when fresh it contains nearly six times the number of bacteria.

* The light powdery substance embedded among the fibres in coconut husk—a waste product of the coir industry.

RESULTS OF TESTS

Growth of Rhizobium in Compost

The numbers of viable cells per gram wet weight of inoculated compost counted at four-weekly intervals and presented in *Table 1* show that the medium is very suitable for *Rhizobium* culture and that the maximum number of cells is obtained about one week after preparation. The number gradually fell to half in about 16 weeks, but remained at about that level at 20 weeks—the duration of the tests.

COMMUNICATION 398

TABLE 1. MEAN NUMBERS OF VIABLE RHIZOBIUM CELLS PER GRAM OF COIR DUST COMPOST COMPARED WITH A STANDARD AGAR SLOPE CULTURE

Type of culture	Weeks after preparation					
	1	4	8	12	16	20
Compost	3×10^9	2×10^9	2×10^9	1.8×10^9	1.2×10^9	1.3×10^9
Agar	5×10^9	—	—	—	—	—

Nodulation Efficiency of the Compost

Nodulation tests on *Centrosema pubescens* Benth., using one tube of one-week-old agar slope or one gram of a 17- or 23-week old compost culture to inoculate 20 or 30 pounds of seed, were carried out in pots containing sterilised washed sand. The results presented in *Table 2* show that the nodule-forming efficiency of the compost was about the same as that of the pure culture; that one gram of compost was sufficient to inoculate 20 to 30 pounds of seed and that the effectiveness of the compost is not lost on storage at room temperature (26°—30°C) for 23 weeks.

Some of the compost stored in the dark at room temperature in a 500 ml conical flask with a tight cotton wool plug was found to be capable of producing nodules on the test plant *C. pubescens* after two years.

DISCUSSION

Coir dust absorbs so much water that it will remain moist for several months when sealed in polythene; and at the same time it retains good aeration, having no tendency to form clumps, so that the numbers of bacteria are not greatly reduced (*Table 1*). The bacterial counts are in general much higher than those considered satisfactory for peat cultures (McLeod, 1963) and compare favourably with the growth on a nutrient agar slope. A gradual reduction in the numbers of bacteria in soil- or peat-based inoculants kept at room temperature or in a refrigerator has also been observed by Bonnier (1955) and Sannaram (1960) though in some cases the numbers increased after one or two months before beginning to decline.

The nodule-forming efficiency of one gram of the compost is more or less the same as that

TABLE 2. NODULATION OF CENTROSEMA PUBESCENS INOCULATED WITH AGAR SLOPE OR COMPOST CULTURE

Type of culture	Age of culture, weeks	Quantity of seed, pounds	Mean no. of nodules per plant, 4 weeks after inoculation
Agar slope	1	20	13.1
		30	12.6
Compost	17	20	12.4
		30	10.5
Compost	23	20	10.2

of an agar-slope culture sufficient for 20 pounds of seed, although as an added safeguard a packet for that amount of seed contains ten grams of compost. The viability and nodule-forming efficiency of the compost compare well with peat cultures used in Australia (ROUGHLEY, 1961), but these have not been followed up for more than two years; SEN and SEN (1956) and JENSEN (1961) found that different strains of *Rhizobium* survived for 10—45 years in a soil medium stored at room temperature.

Coir-dust *Rhizobium* compost is ideal for distribution by post in ordinary envelopes. At the time of application, each packet is suspended in half a pint of water and used for inoculating 20 pounds of scarified seed which absorbs most of the suspension. The inoculated seed is spread out for surface drying before sowing which is best done on the same or on the following day.

ACKNOWLEDGEMENT

The author is indebted to Dr A. Newsam, Head of Pathological Division, for assistance in preparing the manuscript for publication.

Pathological Division
Rubber Research Institute of Malaya
Kuala Lumpur *December 1965*

REFERENCES

BEELY, F. (1938) Nodule bacteria and leguminous cover plants. *J. Rubb. Res. Inst. Malaya* **8**, 149.

BONNIER, C. (1955) The survival of *Rhizobium* in sterile soils. *Bull. Inst. agron. Stns. Rech. Gembloux* **23**, 359.

JENSEN, H. L. (1961) Survival of *Rhizobium meliloti* in soil culture. *Nature, Lond.* **192**, 682.

MCLEOD, R. W. (1963) Quality of finished cultures. *Rhizobium Newsletter. Rep. University Dept. Agric. Lab. Service 1961-62* **8**, 5.

NEWBOULD, F. H. S. (1951) Studies on humus type legume inoculants. I. Growth and survival in storage. *Scient. Agric.* **31**, 463.

ROUGHLEY, R. J. (1961) Inoculation of legumes with nitrogen fixing bacteria. *Agric. Gaz. N.S.W.* **72**, 40.

RUBBER RESEARCH INSTITUTE OF MALAYA (1956) *Rhizobium* compost cultures. *Pltrs' Bull. Rubb. Res. Inst. Malaya* No. 26, 97.

RUBBER RESEARCH INSTITUTE OF MALAYA (1959) *Rep. Rubb. Res. Inst. Malaya 1956*, 44.

SANNARAM, A. (1960) Survival of *Rhizobia* in peat based legume inoculants. *Curr. Sci.* **29**, 97.

SEN, A. AND SEN, A. N. (1956) Survival of *Rhizobium japonicum* in stored air dry soils. *J. Indian Soc. Soil Sci.* **4**, 215.

VAN SCHREVEN, D. A., OTZEN, D. AND LINDENBERGH, D. J. (1954) On the production of legume inoculants in a mixture of peat and soil. *Leeuwenhoek ned. Tijdschr.* **20**, 33.