BRIEF REPORT

CPO246

a Microbiol. 27. 281–283 (1982)

A Method for Laboratory-Scale Mass Cultivation of *Metarhizium anisopliae*

S. MOHAN and G.B. PILLAI

...al Plantation Crops Research Institute, Regional Station, ...kunnuram P.O., Kayangulam 690 533, Kerala, India

Received July 16, 1981

ABSTRACT. *Metarhizium anisopliae* was mass cultured on tapioca chips and rice bran, supplemented with ...e fish meal extract or urea, in specially designed large aluminium vessels.

The entomogenous fungus *Metarhizium anisopliae* (Metcg.) Sorokin is a well ...umented pathogen of many insect pests (Roberts and Yendol 1971; Steinhaus ...73). The commercial utilization of the fungus as a pesticide necessitates mass ...oduction. Several workers have employed various substrates and different culture ...ssels for mass cultivation of *M. anisopliae* for field use (Rorer 1910; Gough 1911; ...rer 1913*a*, *b*; Vouk and Klas 1931; Dresner 1949). An inexpensive and simpler ...thod for the mass cultivation of *M. anisopliae*, pathogenic to the grubs of coconut ...noceros beetle, *Oryctes rhinoceros* (L.), is described.

Large aluminium basins 50 cm in diameter and 20 cm high with a well formed ...m, served as culture vessels. Small chopped pieces of fresh tapioca tuber (*Manihot ...lissima*) approximately 1 cm^3, were mixed with rice bran (8 : 1, W/W) and sup...mented with inexpensive nitrogen sources, since growth of *M. anisopliae* on tapi... without nitrogen supplement was very poor (8×10^7 spores per g blanched ...strate). Commercially available urea or boiled extract of waste fish meal powder ...g and 7.4 g per 100 g of tapioca-bran mixture, respectively) were used as ...gen supplements at a 0.5 % nitrogen level. Rice bran helps to prevent the ...king of tapioca pieces and forming a pasty mass due to gelling of starch when ...ooked, conserves moisture and also serves as a nitrogen source. The substrate ...spread evenly to cover the entire bottom of the vessel. Each vessel could accom...date 4–4.5 kg of the substrate. Alternatively, blanched cereals such as wheat, ...ley, and rice could be used as substrates, requiring no nitrogen supplement.

The mouth of the vessel (Fig. 1) was covered with a thick, dark-coloured cotton ...cloth of close mesh. The stem of a glass funnel was inserted through a small hole ...ched in the centre of the cloth cover, with the mouth of the funnel remaining ...de the vessel. A second sheet of cloth with a central hole, to let the stem of the ...nel, was laid over the first and both the sheets were tied individually around the ...el. Strips of adhesive tape were used to hold the stem of the funnel in position and

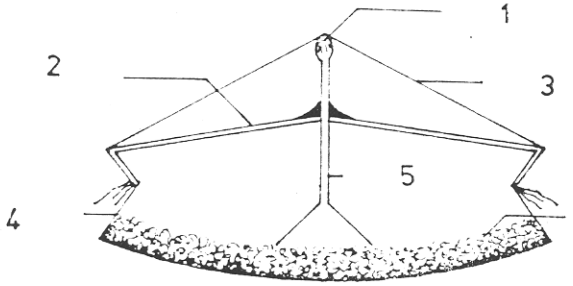

FIG. 1. Cross section of cul... 1 Inoculation port, 2 ... (double sheet), 3 po... 4 aluminium culture ... funnel, 6 substrate.

to seal the gaps around the stem. The opening of the funnel stem served as the inoculation port, which was cotton-plugged and covered with aluminium foil. The culture vessel was autoclaved twice (110 kPa, 121 °C, 25 min) on successive days. A polythene sheet was tied over the culture vessel to prevent dust settling on the cloth during transport and loss of moisture during incubation.

Inoculation was done using an all-glass wash-bottle containing the spore suspension of *M. anisopliae*, harvested from potato dextrose agar slants in 0,01 % of a neutral detergent (*e.g.*, Teepol, by Shell Petroleum, distributed by Glaxo Laboratories, India). The spore load was adjusted to be approximately 10^5 spores per mL of inoculum. A jet of about 50 mL spore suspension was blown into the stem of the funnel and maximum coverage of the substrate was ensured by manipulating the stem. The inoculated vessels were stacked in the laboratory at 27—30 °C and relative humidity of 80—85 %, for three weeks. The containers were gently agitated on alternate days by swaying and manipulating the vessel. Abundant sporulation, completely covering the surface of the substrate was obtained after three weeks. The polythene sheet was removed after two weeks, as excessive moisture in the substrate did not support sporulation. In the trials conducted so far, contamination has not been a problem but small amounts of substrate showing contamination growth could be picked up and discarded. The yield of *M. anisopliae* spores obtained by this method at a 0.5 % nitrogen supplement level, using urea and waste fish meal extract was 370 and 450 spores per µg of substrate, respectively. The spore yield was determined by picking up at random 1 g of substrate containing spores from ten different locations in the culture vessel and pooling the samples in 100 mL of 0.8 % saline containing 0.01 % Teepol as dispersing agent. The flasks were agitated in a rotary shaker for 20 min glass beads. The spore count per mL was determined using a hemocytometer.

Dried, powdered cattle dung was mixed with the tapioca bits containing fungus (1 : 1 W/W) using a plastic spatula (adequate precautions by the use of gown, face mask and gloves are recommended). The carrier material preserved moisture and the fungal spores can be a stored at 4 °C for a week before use. Cattle dung pits or farmyard organic refuse heaps in the farmers' homesteads are inoculated with the fungus preparation at approximately 5×10^{11} spores per m^3 of the heap by thorough mixing. These decomposing organic heaps form the chief breeding sites of the rhinoceros beetle, *O. rhinoceros*, in South India. The larval stages of the pest are predominantly found in these sites and are thus exposed to lethal infection by *M. anisopliae*. The fungal propagules establish an infection either by being ingested alone with the organic matter by the grub or by penetrating the cuticle causing mortality in 15—20 days.

REFERENCES

BRIEGER E.: Culture and use of entomogenous fungi for the control of insect pests. *Boyce Thompson Inst. Plant Res.,Inc.* **15**, 319 (1949).

BURGH L.H.: Results obtained in the study of the frog hopper during the wet season of 1910. *Trinidad Dept. Agric.Circ.* **8**, 46 (1911).

ROBERTS D.W., YENDOL W.G.: Use of fungi for microbial control of insects, p. 125 in H.D. Burges, N.W. Hussey (Eds.): *Microbial Control of Insects and Mites.* Academic Press, London—New York 1971.

BORER J.B.: The green muscardine of frog hoppers. *Proc.Agric.Soc.Trinidad & Tobago* **10**, 467 (1910).

BORER J.B.: The use of the green muscardine in the control of some sugarcane pests. *Phytopath.* **3**, 88 (1913*a*).

BORER J.B.: The green muscardine fungus and its use in cane fields. *Board Agric., Trinidad & Tobago, Circ.* **8**, 15 (1913*b*).

STEINHAUS E.A.: Microbial diseases of insects, p. 525 in P. DeBach (Ed.): *Biological Control of Insect Pests and Weeds.* Chapman and Hall, London 1973.

TOCK V., KLAS Z.: Conditions influencing the growth of the insecticidal fungus *Metarrhizium anisopliae* (Metch.) *Sor.Internat.Corn Borer Invest., Sci.Rept.* **4**, 24 (1931).