J. Biol. Control, 11: 97-100, 1997

Medium for mass production of *Beauveria bassiana* (Balsamo) Vuillemin

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ABSTRACT: A medium was developed with rice hull, saw dust and rice bran at a ratio of 75:25:100, respectively, to mass culture *Beauveria bassiana* (Balsamo) Vuillemin, a potential biocontrol agent against the rice hispa, *Dicladispa armigera* (Olivier) (Coleoptera: Chrysomelidae). It produced 39.33 x 10^7 conidia/ml of water 24 days after inoculation. The mortality of the adult could be achieved up to 78.6 per cent at spray concentration of 10^6 spores/ml of water.

KEY WORDS : *Beauveria bassiana*, *Dicladispa armigera*, rice hull, rice bran, saw dust

Various media have been used for production of *Beauveria bassiana* (Balsamo) Vuillemin in different parts of the world (Smith and Grula, 1981; Filho *et al.*, 1985; Mazumder *et al.*, 1995). The major drawback of such mass cultured *B. bassiana* is loss of virulence over time (Bell, 1985; Sandhu *et al.*, 1993). The present need is to increase the infective propagules to compensate for the loss of virulence. Keeping this in view, the present study was under taken to augment spore production by using an improved medium.

The experiment was conducted in the laboratory of Mycology Research Section, Assam Agricultural University, Jorhat, during January and February, 1996. Three wastes products *viz.*, rice hull (RH), rice bran (RB) and saw dust (SD) were taken individually and in different proportions (RH, SD and RB = 50:50:100; 100:50:50; 75:25:100; 100:25:75; 75:75:50 and 50:75:75). Fifty grams of each of the combinations + 2% Dextrose mixed with 70 ml of double distilled water was taken in 100 ml (Erlenmeyer) conical flask and

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was sterilised by autoclaving at 121°C and 1.05 kg/cm^2 pressure. Performance of these media were compared with potato dextrose agar (PDA) and potato agar for production of *B. bassiana.*

The entomopathogen *B. bassiana* was isolated from a dead rice hispa which was inoculated seven days earlier and maintained on PDA. Each medium was inoculated with a fungal disc of 6 mm diam. from a 10 day old culture, and was inoculated at 25 ± 1°C. The time required to cover the substrate completely in each of the flasks was recorded. Twenty grams of medium with homogenous fungal growth from each flasks were suspended thoroughly in 70 ml water using a rotary mixer (Remi Motors, Remi Udyog, Mumbai) for 20 minutes. The homogenate was allowed to pass through a muslin followed by a Whatman No 1 filter paper. Conidia were counted from each filtrate with the help of a haemocytometer and mean count of 25 such samples was estimated.

Inoculum obtained from different treatments at the concentration of 10^6 conidia/ml of water was used to test their pathogenecity against rice hispa. Twenty laboratory reared adults 48 h after eclosion (starved for 6 h) were released into 30 day old rice (Culture 1) seedlings grown in plastic pots and then caged with paired lantern chimney.

Six and a half ml of inoculum mixed with Tween 80 @ 0.023g/litre was sprayed over leaf surface of one treatment which was replicated four times. After 4 days, seedlings were replaced with healthy ones.

The experiments were conducted in a completely randomized design. The data were subjected to analysis of variance.

The combination 75:25:100 of RH: SD: RB gave maximum support for the growth of the fungus and took 24 days to produce 39.33 x 10^7 conidia/ml of water (Table 1). This was followed by the combination 50:75:75 and 100:25:75 with an average yield of 12.57 x 10^7 and 11.09 x 10^7 conidia/ml of water, respectively. The reason for such a high conidial population in the formulation may be due to the availability of sufficient carbon source from the substrates rice bran and rice hull. The proportionate addition of saw dust,

Table 1. Evaluation of different combinations using three industrial wastes for mass production of *B. bassiana*

Medium/Combinations	Conidia (x 10^7/ml) Mean ± SD
RH:SD:RB (50:50:100)	2.09 ± 0.02
RH:SD:RB (100:50:50)	6.74 ± 1.15
RH:SD:RB (75:25:100)	39.33 ± 1.67
RH:SD:RB (100:25:75)	11.09 ± 1.36
RH:SD:RB (75:75:50)	3.23 ± 0.15
RH:SD:RB (50:75:75)	12.57 ± 1.14
RH	3.50 ± 0.67
SD	0.00
RB	1.70 ± 0.51
PDA	5.00 ± 0.09
Potato Agar	4.30 ± 0.26
CD (P=0.05)	1.50
CV (%)	10.80

contributed to minimize the compactness of the medium during sterilization resulting in better utilization of space and aeration within the medium which was responsible for better growth of the fungus with complete coverage within 24 days, while in others it required 26-52 days (Fig. 1). Although rice bran is a rich source of nutrients along with certain essential oils (Mazumder *et al.*, 1995), the growth of the fungus was found to be highly restricted (1.7×10^7 conidia/ml). The reason may be that the medium became a compact mass after sterilisation which did not allow *B. bassiana* to ramify easily within the medium. But when saw dust and rice hull were added the compactness of the medium was gone and a very high growth of the fungus (39.33×10^7 conidia/ml) was obtained. In saw dust the fungal growth was totally restricted due to the poor nutritional status.

During pathogenecity test, when the conidial suspension obtained from the formulation was inoculated on rice hispa @ 10^6 spores/ml of water, the mortality obtained was 78.6 per cent as against 88.0 per cent from PDA medium and 91.83 per cent from rice hull supplemented with 2 per cent dextrose. Our study corroborates with Maniania (1993) reported dry rice grain based inoculum had highest fungal infection of *Chilo partellus* (Swinhoe) in Kenya. The results indicated suitability of rice hull, saw dust and rice bran at a ratio of 75:25:100 + 2% Dextrose for mass production of *B. bassiana.*

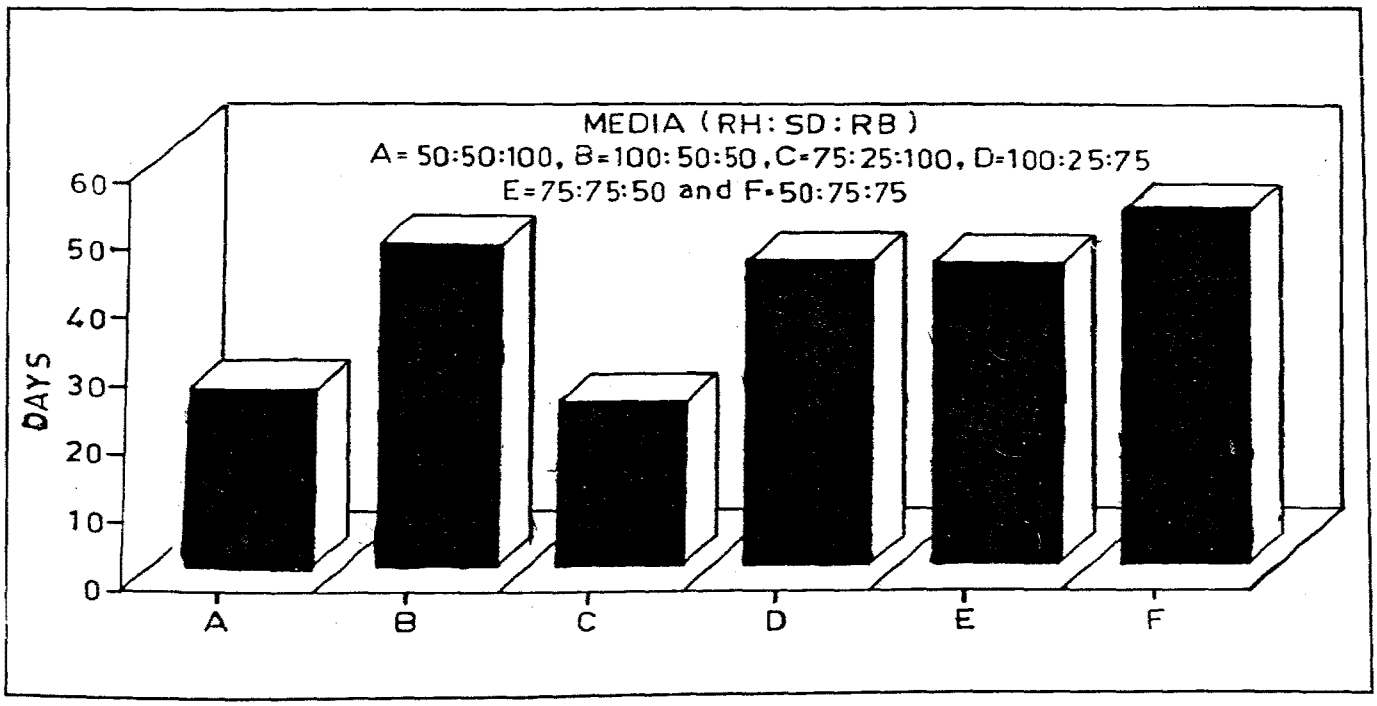

Fig. 1 : Time required (days) for complete coverage of different media by *B. bassiana*

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