Mass multiplication of entomopathogenic fungus, *Paecilomyces lilacinus* (Thom) Samson with solid substrates

U. Amala*, T. Jiji and A. Naseema

ABSTRACT

Various solid substrates like rice bran, wheat bran, gingelly oil cake, coir pith, neem cake were evaluated for the mass multiplication of the fungus, *Paecilomyces lilacinus* (ITCC 6064). Among the substrates, rice bran recorded the maximum spore count of $4.32x10^8$ spores/ml followed by wheat bran ($3.19x10^8$ spores/ml), gingelly oil cake ($2.63x10^8$ spores/ml), coir pith ($2.44x10^8$spores/ml) and neem cake ($2.16x10^8$ spores/ml)on 28th day after inoculation. There was a gradual decline in spore count from fourth week after inoculation. The fungus mass multiplied in rice bran can be used with an effective spore concentration for a period of one month in the management of insects. Therefore, rice bran serves to be the suitable substrate for the mass multiplication of the entomopathogenic fungus, *P. lilacinus*.

Keywords: Entomopathogenic fungi, *Paecilomyces lilacinus,* rice bran

INTRODUCTION

The use of microbial control agents including entomopathogenic fungi, an alternative to chemical control as a component of integrated pest management (IPM) strategies is being widely explored for the management of wide range of insect pests. *Paecilomyces* sp. is a soil fungus with a good potential for biological control of nematodes and also cause widespread epizootics in fruit flies (Jiji *et al.,* 2006), stink bugs (Rambadan *et al.,* 2011), reduviid bugs (Marti *et al.,*2006), green house white flies (Gokce *et al.,* 2005; Wraight *et al.,* 2000) and mite pests (Fielder and Sosnowska, 2007).The ready availability of the mycoinsecticides unlike chemical insecticides is a challenging factor in testing the Pathogenecity of the fungal pathogens against target insect hosts. For the evaluation of the entomopathogenic fungus under field conditions, mass multiplication of the fungus on suitable substrate (Jagadeesh Babu *et al.,* 2008) was necessary. Lack of reliable substrates was found to be another major constraint in the mass production and utilization of the mycoinsecticides. Hence an attempt was made to determine the most suitable and locally available solid substrate for the mass multiplication of the fungus.

MATERIALS AND METHODS

In the present study, the entomopathogenic fungus, *Paecilomyces lilacinus* (ITCC 6064) was collected from the infected cadavers of melon fruit fly, *Bactrocera cucurbitae in* the Instructional Farm, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala. The objective of the study was to select a suitable medium for the mass multiplication of the fungus. To evaluate the suitable medium for the growth of the fungus, five locally available solid substrates like rice bran, wheat bran, gingelly oil cake, coir pith and neem cake were used for the mass multiplication of the fungus. The experiment was conducted in Completely Randomized Design (CRD) with four replications.

Preparation of substrates

Sixty gram each of the solid substrate added with 50 ml of sterile distilled water was taken in 250 ml conical flask. The substrate was sterilized in an autoclave at 121.1°C for 15-20 minutes at 1.02 kg/ cm^2 pressure. After sterilization the substrate was artificially inoculated with five mm fungal disc from well sporulating seven-day old cultures maintained in Potato Dextrose Agar slants. Each treatment was replicated four times. After inoculation, the flask was incubated at room

Table1. Effect of solid substrates on the sporulation of *Paecilomyces lilacinus*

Solid substrates	Spore count x 10^8 spores / ml (days after inoculation)							
	7th	14th	21st	28th	35th	42nd	49th	56th
Rice bran	1.56	2.68	3.71	4.32	3.97	2.47	1.79	1.13
Wheat bran	1.30	2.33	2.87	3.19	3.73	2.29	1.25	0.89
Gingelly cake	0.90	1.51	2.15	2.63	2.86	1.88	0.85	0.56
Coir pith	0.72	1.23	1.58	2.44	2.54	1.64	0.72	0.57
Neem cake	0.72	1.10	1.46	2.16	1.26	0.39	0.00	0.00
CD(0.05)	0.08	0.07	0.08	0.08	0.09	0.11	0.08	0.67

temperature (28 ± 4°C). The conical flasks were shaken daily for the uniform growth of the fungus.

Recording the spore count

The conidia was taken from each of the substrate and suspended in sterile distilled water. The suspension was filtered through double layered muslin cloth and the spore count was recorded microscopically at weekly intervals for a period of two months using a Naubauer's Haemocytometer. The data was subjected to Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

Among the different solid media tested, rice bran recorded the maximum spore count ($4.32x10^8$spores/ml) which differed significantly from the spore count of wheat bran ($3.19x10^8$spores/ml) on the 28th day after inoculation (Table 1).The high spore count recorded in rice bran may be due to the higher nutrient status of the media that supported maximum growth and sporulation of the fungus. Similar observations were recorded by Ibrahim and Low (1993) who suggested rice bran as the suitable medium for the mass culture of *Beauveria bassiana*. The results were in conformity with the observations recorded by Filho *et al*., 1989 who evaluated rice bran as a medium for the mass multiplication of deuteromycetes fungi.

The spore count of gingelly oil cake ($2.63x10^8$spores/ml) was on par with spore count on coir pith on the 28th day after inoculation. Neem cake recorded a spore count of $2.16x10^8$spores/ml on the 28th day. There was a gradual decline in spore count of *P. lilacinus* grown on all the media twenty eight days after inoculation. The study indicates that the fungus mass cultured on these media cannot be stored for more than four weeks at room temperature with an effective spore concentration.

From the observations it was concluded that rice bran was the suitable media for the mass multiplication of the fungus. The fungus grown on rice bran could be effectively used for a period of one month for the management of insects.

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