Madras Agric. J., 95 (7-12) : 415-417 July-December 2008
https://doi.org/10.29321/MAJ.10.100604

Mass production and commercial formulation of *Paecilomyces lilacinus*

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Abstract: The opportunistic nematode egg parasitic fungus, *Paecilomyces lilacinus* was mass produced in different liquid media for evaluation of spore production and formulation. Potato dextrose broth (PDB), Richards's medium; 10% Mdlasses and Semi selective medium was used as culture medium. The Semi selective medium served as control. Highest mycelial weight was found in Semi selective medium (19.82g), followed by 10% Molasses medium (16.85g). The least mycelial weight was found in PDB (10.5g). Highest spore load was found in Semi selective medium (32.8 X10^7 cfu/g) followed by 10% molasses medium (28.8 X10^7 cfu/g). The spore production was least in PDB (10.3 X10^7 cfu/g). The mass produced fungus was formulated with different carrier materials like fly ash, vermiculite, rice hull ash and talc and the viability of the spores were tested at frequent intervals. The viability of the fungus lasted for 120 days in talc and fly ash. Rice hull ash and vermiculite had a viability period of 90 and 75 days respectively.

Key words: Paecilomyces lilacinus , mass production, commercial formulation

Introduction

The annual growth rate of synthetic pesticides is 1-2% as against 10-15% in the case of microbial pesticides (Singh, 1998). The synthetic chemical pesticides resulted in environment pollution leads to the search for antagonists for pest management by the researchers world wide. Nematodes are one of the important limiting factors in crop production (Sasser and Freckman, 1987).The nematophagus fungus *Paecilomyces lilacinus* has been identified as an effective biocontrol agent of *Meloidogyne* spp. and *Globodera* spp. (Jatala *et al.,* 1979). Due to high cost of chemical nematicides and their risk to human beings and environment, use of biocontrol agents has become renewed interest (Jatala, 1985). Many authors reported the multiplication of *Paecilomyces* (llyanitidinow, 1992; Meyer *et al.,* 1997; Vyas *et at.,* 1995) but they involve high cost in multiplication of the fungus. Hence, the present study was conducted to study the various liquid media for multiplication of the fungus *P. lilacinus.*

Materials and Methods

Mass multiplication

One hundred ml of the different liquid media *viz.* PDB (Rangaswami, 1972), 10% Molasses, Richard's medium (Rangaswami, 1972) and Semi selective medium (Mitchell *et al.,* 1987) were taken in a 250 ml conical flask. They were autoclaved at 15 psi for 20 min. Each flask was inoculated aseptically with 8mm disc of the *P. lilacinus,* maintained on Potato dextrose agar (PDA) as a pure culture. The flasks were incubated at room temperature for 30 days. The fungus inoculated in semi selective medium served as control.

Table 1. Mycelial weight and spore load of fungus multiplied in different media

Media	Mycelial weight in g*	Spore load X10^7 cfu/ml of medium*
PDB	10.5[a]	10.3[d]
Molasses 10%	16.85"	28.8[b]
Richard's medium	13.55[C]	23.2[C]
Semi selective medium	19.82[3]	32.8[a]
CD p=(0.05)	2.75	3.89

* Mean of five replications

Means followed by a common letter are not significantly from each other (p=0.05) according to DMRT

All the treatments were replicated five times. Fungal biomass was recorded in each treatment. Spore load was enumerated by serial dilution and plating method on Rose Bengal Agar (Martin, 1950).

Formulation

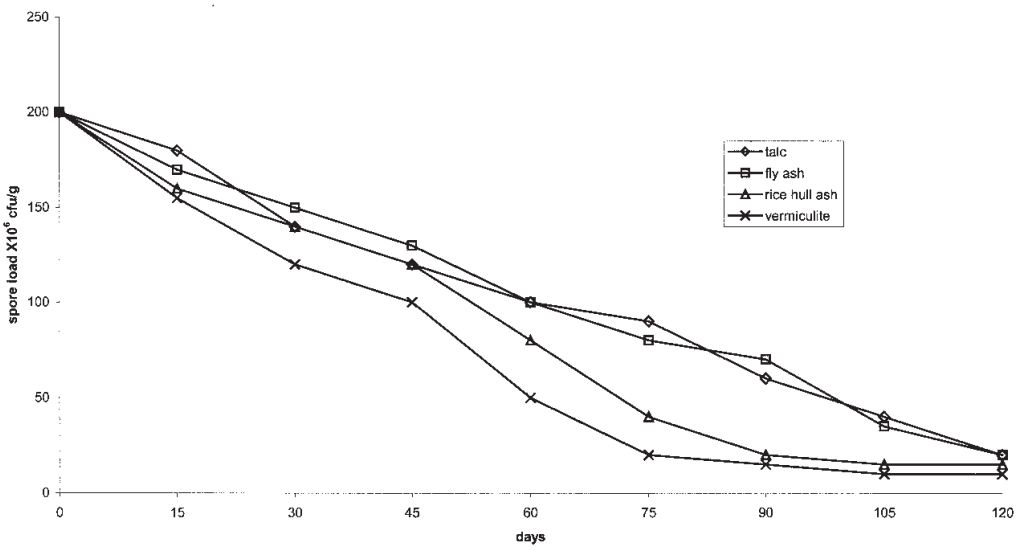
The culture flasks in the above treatments were used for formulation with different carrier materials *viz.*, talc, fly ash, rice hull ash and vermiculite. The mycelial mat along with the broth was homogenized and mixed with carrier material in the ratios 1:2 (Richard 1981). Carboxyl methyl cellulose was added @5g/kg of the product. The acidity of the medium was neutralized by adding 20g of chalk /kg of product. Then the product was shade dried to reduce the moisture content to 12% and packed in opaque polythene bags and stored at room temperature for further studies. The spore load at the time of packing was 20X10 cfu/g in the product. The formulated product was tested for the viability by serial dilution and plating at 15 days interval for 120 days.

Results and Discussion

The fungus was found multiplied well in all the media. In semi selective medium the growth was rapid. The fungus took about 28-30 days at 28±1°C to cover the entire flask in all the media. Highest fungal biomass (19.82g) and spore load (32.8 X10^7 spores/ g) was observed in semi selective medium (Table 1). Molasses 10% medium yielded moderately high biomass and spore production (16.85g and 28.8 X10^7 spores/g respectively). The least biomass and spore production were recorded in PDB. Mass production of *P. lilacinus* on molasses has certain advantages over other media. It is cheaply available and there is no quality loss in the fungus. Calderen *et al.* (1995) also reported similar findings. Although semi selective medium gave significantly high population, considering the cost of production, 10% molasses medium was a cheaper substrate for mass multiplication.

Among the various formulations tested, talc and fly ash were to be the best. The mass production in various media has no significant effect on storage. In talc and fly ash, the spore load drops from the initial spore load to 2X10^7 cfu/g in 120 days. In vermiculite and rice hull ash the viability drops down in 90 and 75 days respectively, (Fig. 1). As the time proceeds the shelf life of the product was gradually deteriorated.

Fig.1. Shelf life of various formulations and spore load/g of medium



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