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Mass Production and Economics of Entomopathogenic Fungus, *Beauveria bassiana, Metarhizium anisopliae* and *Verticillium lecanii* on agricultural and industrial waste

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Abstract: *Beauveria bassiana* (Balsamo) Vuellemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Vertillium lecanii* are entomopathogenic fungi (EPF) that occur naturally in the environment and have a potential as biological control agents against many insect pests. A laboratory study was conducted to mass produce entomopathogenic fungus, *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii* on nine different agricultural and industrial waste substrates. The maximum yield (278.75 X 10^6spores/ml) of *B. bassiana*, 171.75 X 10^6 spores/ml of *M. anisopliae* and 185 X 10^6 spores/ml of *V. lecanii* was obtained in Farm Yard Manure (FYM) followed by SDB (246.25, 157.25 and 180.0 X 10^6 spores/ml, respectively. The lowest number of spores of *B. bassiana*, *M. anisopliae* and *V. lecanii* was obtained in sugarcane bugasse (65.25, 34.25 and 39.0 X 10^6 spores/ml), respectively. The economics of *B. bassiana*, *M. anisopliae* and *V. lecanii* production was evaluated based on the final yield. Among *in vitro* produced media, the production of spores, FYM was the best low cost substrate (Rs. 0.275, 0.440 and 0.410) for 1X 10^6 spore production followed by Pressmud + FYM (1:1) + 1.0g dextrose (Rs. 0.39) for *B. bassiana*, while SDB (Rs. 0.68) for *M. anisopliae* and Crushed jowar grain + 1.0g dextrose (Rs.0.52) for *V. lecanii*. The highest cost of spore production was recorded in sugarcane bagasse (Rs. 1.14, 2.18 and 1.92) followed by pressmud for *B. bassiana*, *M. anisopliae V. lecanii*.
Keywords: *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii*, mass production, economics.

INTRODUCTION

The global consensus to reduce inputs of chemical pesticide which are perceived as being hazardous by some consumers has provided opportunities for the development of novel, benign, sustainable crop protection strategies. A great many chemical pesticides have been or are being phased out (e.g. organochlorine insecticides, methyl bromide) either because of potential human health risks, environmental pollution, effects on non-target organisms or the development of pest resistance.

Biological control agents such as entomopathogenic fungi (EPF) can be used as a component of integrated pest management (IPM) of many insect pests. Under natural conditions, these pathogens are a frequent and often cause natural mortalities of insect populations. Several fungal species including *Metarhizium anisopliae*, *Verticillium lecani* and *Beauveria bassiana* are being used as biocontrol agent for a number of crop, live stock and human nuisance pests [1].

Moreover, entomopathogens are cheap to mass produce, easy to store and effective over a wide range of temperatures and humidity levels. It also provides a rapid kill at economical doses and recently, the fungus has commercially been widely developed as a microbial agent for pest management [2] and encouraging results were obtained against whiteflies, aphids, thrips and mealybugs in greenhouses and nurseries [3]. *B. bassiana*, *M. anisopliae* and *V. lecani* are a facultative pathogen and can be mass-produced on various substrates. The choice of a suitable and economic medium, which supports rapid growth without loss of virulence for number of generations, is one of the basic requirements in the mass production of fungi for microbial control of insect pests. The production can be achieved using different methodologies, which can be classified in to low input and industrial technologies. However, most production of fungal spores worldwide is carried out using simple technologies that demand low inputs[4]. Mass production of entomopathogenic fungi and testing of germination are important steps in successful utilization of EPFs as biocontrol agents.

Some attempts to mass produce *B. bassiana*, *M. anisopliae* and *V. lecani* on low cost materials have been carried out by Mazumdar *et al.* [5] and Babu *et al.* [6]. They evaluated solid and liquid substrates,

including sugar industry by-products and observed that addition of dextrose was indispensable for accelerated growth on some of them. The type of growing medium, affects conidial production of entomopathogenic fungi [7]. Hence this study was design to mass culture the entomopahogenous fungi on locally available agricultural and industrial waste for a cheap and suitable substrates for the low cost production of entomopathogens of virulent spores i.e. liquid and solid media. Despite the many attempts to screen commercially available, low-cost ingredients of industrialized biological pesticides, the research on nutritional requirements of fungal agents were overlooked and a systematic investigation of fungal nutrition utilization is much needed to improved mass production and accelerates commercialization[8]. The outcome of this research will help in mass production technology of entomopathogens in a suitable and economic medium for its dissemination in field.

MATERIALS AND METHODS

The present study was carried out in Bio-control Laboratory, S.V.P. University of Agriculture & Technology, Meerut (India) in 2010. The experiment was conducted in a laboratory to test the effect of different agricultural and industrial waste, solid and liquid substrates on mass production and economics of entomopathogenic fungi (EPFs).

Nucleus culture

The nucleus culture of *B. bassiana, M. anisopliae* and *V. lecani* was isolated from the infected lepidopteran larva and soil-isolated fungi from different location and orchards of Meerut district. It was thereafter maintained on Sabouraud Dextrose Agar (SDA) medium as per the procedure of Prasad *et al*. [9]. Briefly, SDA was prepared and sterilized at 121^{o}C (15 lbs) for 20 minutes. Then cooled and poured in pre-sterilized Petri plates and *B. bassiana* from stock culture was inoculated aseptically. The Petri plates were incubated at 25 ± 1^{o}C in BOD incubator for two weeks to harvest the inoculums culture.

Substrates and *in vitro* production

Nine mass production substrates were evaluated for the conidial production of *B. bassiana, M. anisopliae* and *V. lecanii* under controlled conditions of 25 ± 1^{o}C in BOD incubators for 3 weeks. The substrates were (i) FYM, (ii) Sabouraud dextrose broth (SDB), (iii) Sugar industry Press mud, (iv) Sugarcane bagasse, (v) FYM liquid + 1.0g Dextrose, (vi) Pressmud liquid + 1.0g Dextrose, (vii) FYM liquid + Pressmud liquid (1:1) + 1.0g Dextrose , (viii) *Corcyra* rearing waste (Maize) and (ix) Jawar grain + 1.0 g Dextrose. The last six medium were supplemented with dextrose. There were nine treatments in three replications.

A quantity of 130 g dehydrated SDB was suspended in 2000 ml distilled water, heated to dissolve the medium (pH 5.6) and sterilized by autoclaving at 15 lbs pressure (121^{o}C) for 15 minutes. The lukewarm liquid media was poured in conical flask. Then *B. bassiana, M. anisopliae* and *V. lecanii* were inoculated aseptically and then conical flasks were incubated in BOD.

The *Corcyra* rearing waste (crushed maize grain) and sorghum grain were soaked over night, cleaned with fresh water and 100 g of each was put in separate Conical flasks (250-ml capacity) supplemented with dextrose (1.0 g), plugged with non-absorbent cotton and autoclaved. Upon cooling of media, *B. bassiana, M. anisopliae* and *V. lecanii* were inoculated aseptically and incubated in BOD incubator. The crushed jowar grain was collected from the Bio-control Laboratory as a waste material from *Corcyra* Rearing Unit. The crushed jowar grain (weight 50 g) was put in flask and 15 ml distilled water with 1 g dextrose was added. The sugarcane bagasse and Pressmud was procured from Modipuram market, Meerut. The sugarcane bagasse was cut into small pieces and half-filled in flask with 15 ml distilled water.

Spore counting

A drop of conidial suspension of *B. bassiana, M. anisopliae* and *V. lecanii* (obtained from the growing media by filtering through muslin cloth) was placed on the hemocytometer. The cover glass was put over the grid carefully so that no air bubble entered between cover glass and slide. The conidia of entomopathogenic fungi were counted under Olympus BX41 phase contrast microscope at higher resolution in the middle square (V) of hemocytometer which contained 25 groups of 16 small squares, each group 0.2 mm square.

Statistical analysis

The conidial production of entomopathogernic fungi from different substrates were subjected to analysis of variance (ANOVA) using SPSS 10.0 for Windows software (SPSS, 1999) [10]. The means were separated using LSD and differences between treatments were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The optimum nutrient medium is necessary for the adequate growth of microorganism in general and fungi in particular (Altomare e*t al*., 1999). In the present study, one chemical media (and SDB), and six waste materials (i.e., FYM, Pressmud, sugarcane bagasse, broken maize grain (*Corcyra* rearing waste) and broken jowar were evaluated *in vitro*.

Mass production of *B. bassiana*

The production was more on media containing FYM, SDB and FYM liquid + Pressmud liquid (1:1) + 1g Dextrose as compared to the rest of the media. The least conidial growth was observed in crushed jowar grain and sugarcane bagasse (Table 1).

The results showed that among the media tested, FYM (278.75 X 10^6spores/ml), SDB (246.25 X 10^6 spores/ml) and FYM liquid + Pressmud liquid (1:1) + 1g Dextrose (200.25 X 10^6 spores/ml) were the most suitable media with higher growth of mycelium and production of large number of spores of *B. bassiana*. While the sugarcane bugasse (65.25 X 10^6 spores/ml) produces least number of spores followed by FYM liquid + 1g Dextrose (97.50 X 10^6 spores/ml). Pandey and Kanaujia [11] obtained 6.4 × 10^7 conidia/ml in SDA medium followed by finger millet (5.4 × 10^7 conidia/ml) whereas Purwar and Sachan [12] recorded SDA with yeast extract and sorghum-based media producing higher biomass and conidial counts. Many earlier researcher successfully used SDA medium for the mass culture of fungi. However, surprisingly in the present study the spore count was less in SDB medium as compared to FYM. Hallsworth and Magan [13] offered SDA medium for the successful culture of *B. bassiana* and *M. anisopliae*.

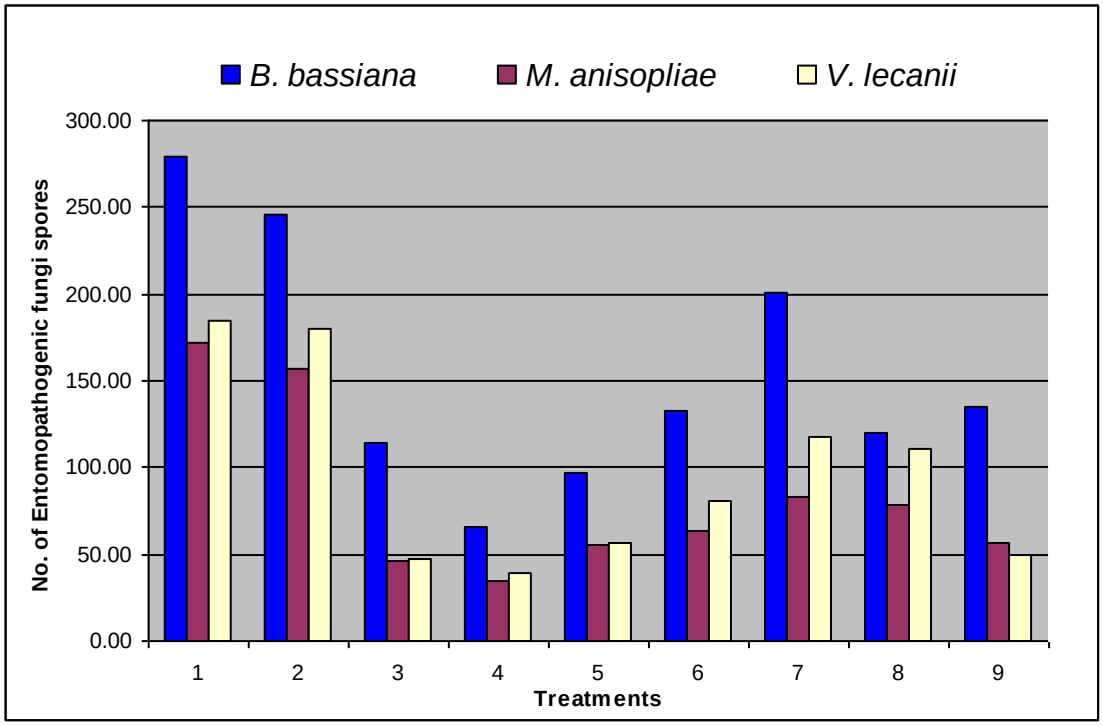

Fig. 1. Mass production of Entomopahogenic fungi on agricultural and industrial waste

Table 1. Mass production of *B. bassiana, M. anisopliae* and *V. lecanii* on different substrates.

S. No.	Media	Spore count (X 10^6)		
		B. bassiana	***M. anisopliae***	***V. lecanii***
1	Farm Yard Manure (FYM)	278.75	171.75	185.00
2	Sabouraud dextrose broth (SDB)	246.25	157.25	180.00
3	Pressmud (Sugar mill)	114.00	45.75	47.50
4	Sugarcane bagasse	65.25	34.25	39.00
5	FYM liquid + 1.0g Dextrose	97.50	55.50	56.50
6	Pressmud liquid + 1.0g Dextrose	132.50	63.50	80.50
7	FYM liquid + Pressmud liquid (1:1) +1.0g dextrose	200.25	83.25	117.50
8	Corcyra rearing waste (Maize)	119.75	78.75	110.75
9	Jowar grain + 1.0 g Dextrose	135.25	57.00	49.50
CD at 5%		**35.850**	**18.832**	**31.585**
SE (m)		**11.977**	**5.289**	**10.549**

Figures are parenthesis in no transformation

*Means (of three replicates where each replicate comprised of 15 individual flasks containing each substrate) ± standard error indicated with the same letter are not significantly different according to LSD test at $p < 0.05$.

Table 2. Economics of mass production of *B. bassiana, M. anisopliae* and *V. lecanii* (1×10^6 spores) on different substrates of agricultural and industrial waste.

S. No.	Media	Cost of production of 1 X 10^6spores (Rs.)		
		B. bassiana	*M. anisopliae*	*V. lecanii*
1	Farm Yard Manure (FYM)	0.27	0.44	0.41
2	Sabouraud dextrose broth (SDB)	0.43	0.68	0.59
3	Pressmud (Sugar mill)	0.66	1.66	1.60
4	Sugarcane bagasse	1.14	2.18	1.92
5	FYM liquid + 1.0g Dextrose	0.78	1.37	1.33
6	Pressmud liquid + 1.0g Dextrose	0.57	1.20	0.94
7	FYM liquid + Pressmud liquid (1:1) +1.0g Dextrose	0.39	0.94	0.66
8	*Corcyra* rearing waste (Maize)	0.62	0.96	0.68
9	Jowar grain + 1.0g Dextrose	0.56	1.34	1.55
CD at 5%		**0.054**	**0.035**	**0.035**
SE (m)		**0.021**	**0.012**	**0.012**

Figures are parenthesis in no transformation value

Mass production of *M. anisopliae*

The results showed that among the media tested, FYM (171.75 X 10^6spores/ml), SDB (157.25 X 10^6 spores/ml) and FYM liquid + Pressmud liquid (1:1) + 1g Dextrose (83.25 X 10^6 spores/ml) were the most suitable media with higher growth of mycelium and production of large number of spores of *M. anisopliae*. While the sugarcane bugasse (34.25 X 10^6spores/ml) produces least number of spores followed by Pressmud (45.75 X 10^6 spores/ml). Pandey and Kanaujia [11] obtained 6.4×10^7 condia/ml in SDA medium followed by finger millet (5.4×10^7 conidia/ml) whereas Purwar and Sachan [12] recorded SDA with yeast extract and sorghum-based media producing higher biomass and conidial counts of *B. bassiana*. No any fungal spore in sugarcane bagasse + 1.0% yeast extract and pressmud + 1% yeast extract while 1.70 X 10^4 spores was obtained in pressmud with 10% molasses reported by Bharati *et al*[14]. However, surprisingly in the present study the spore count was less in SDB medium as compared to FYM. Hallsworth and Magan [13] offered SDA medium for the successful culture of *B. bassiana* and *M. anisopliae*. Soundrapandian and Chandra [15] were also found 300X10^4 spores of *M. anisopliae* in SDA.

Mass production of *V. lecanii*

The results showed that among the media tested, FYM (185.00 X 10^6spores/ml), SDB (180.00 X 10^6 spores/ml) and FYM liquid + Pressmud liquid (1:1) + 1g Dextrose (117.50 X 10^6 spores/ml) were the most suitable media with higher growth of mycelium and production of large number of spores of *V. lecanii*. While the sugarcane bugasse (39.00 X 10^6spores/ml) produces least number of spores followed by Pressmud (47.50 X 10^6 spores/ml). The reports of sorghum grains were found to be ideal for mass production of *V. lecanii* was documented by Sahayraj and Namasivayam [16]. Pandey and Kanaujia [11] obtained 6.4×10^7 conidia/ml in SDA medium followed by finger millet (5.4×10^7 conidia/ml) whereas Purwar and Sachan [12] recorded SDA with yeast extract and sorghum-based media producing higher biomass and conidial counts of *B. bassiana*. The above findings are closely agreed with Bharati *et al*.[14] who recorded no fungal spores of *M. anisopliae* in sugarcane bagasse + 1.0% yeast extract and pressmud + 1% yeast extract and also found spore yields were considerably low in agro-wastes as compared to food grains.

Economics of mass production of *B. bassiana, M. anisopliae* and *V. lecanii*

Based on cost incurred for the production of spores FYM was the best low cost substrate (Rs 0.275, 0.440 and 0.410.) for 1X 10^6 spore production followed by Pressmud + FYM (1:1) + 1.0g Dextrose (Rs. 0.39)for *B. bassiana*, while SDB (Rs 0.68.) for *M. anisopliae* and Crushed jowar grain + 1.0g dextrose (Rs 0.52.) for *V. lecanii* (Table 2). The highest cost of spore production was recorded in sugarcane bagasse (Rs. 1.14, 2.18 and 1.92) for *B. bassiana, M. anisopliae V. lecanii* followed by FYM liquid + 1.0g Dextrose (Rs 0.78.) for *B. bassiana* and pressmud (Rs. 1.66 and 1.60) for *M. anisopliae* and *V. lecanii.*

The methodology evaluated in this study allows for the production of high quality *B. bassiana, M. anisopliae* and *V. lecanii* fungal spores, but the quantity produced are only suitable for small-scale laboratory and field trails.

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