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MASS PRODUCTION OF ENTOMOPATHOGENIC FUNGI USING AGRICULTURAL PRODUCTS

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ABSTRACT
The success of microbial control of insect pests depends not only on the isolation, characterisation and pathogenicity, but also on the successful mass production of the microbial agents in the laboratory. Large-scale availability of the pathogen is a primary requirement in the biocontrol programme. *Beauveria bassiana* is an entomopathogenic fungus which is used against a number of insect pests. To develop an efficient method for the utilization of this fungus as a bio control agent, various grains and liquid media such as Potato Dextrose Broth and Sabouraud's Dextrose Broth were screened. Pea amended media produced maximum biomass of the test fungus while SDB produced significantly higher spore production of the fungi. Highest conidial count (9.06×10^7 conidia mL^{-1}) was observed on cowpea media followed by soybean.

INTRODUCTION

Biopesticides based on bacteria, viruses, entomopathogenic fungi and nematodes are often having considerable scope as plant protection agents against several insects (Noris *et al.,* 2002). Use of entomopathogenic fungi as biological control agents for insect species has increased the global attention during the last few decades. The myco-insecticide based on *Beauveria bassiana* (Balsamo) Vaillemin (Babu *et al.,* 2001; Sharma, 2004), *Paecilomyces fumosoroseus* (Wize) Brown and Smith (Alter and Vandenberg, 2000; Avery *et al.,* 2004) and *Verticillium lecanii* (Zimm.) Viegas (Butt *et al.,* 2001) have been used to control various insect pests. Production of adequate quantities of good quality inoculum is an essential component of the biocontrol programme. The production of entomopathogen may be taken up by two ways either a relatively small quantity of the inoculum for laboratory experimentation and field-testing during the development of mycopesticide and or development of a basic production system for large-scale production by following the labour intensive and economically viable methods for relatively small size markets. China (Feng *et al.,* 1994) and America (Alves and Pereira, 1989) supplied fungal biocontrol agents in sufficient quantities for niche markets in their immediate area. Development of simple and reliable production system follows the basic multiplication procedures of submerged liquid fermentation for the production of blastospores, which are short lived, and hydrophilic (Romback, 1989) or solid state fermentation (Rousson *et al.,* 1983) for the production of aerial conidia. However, the most viable mass production technologies include making use of a diphasic strategy in which the fungal inoculum is produced in liquid culture, which is further utilized for inoculating the solid substrate(s) for conidia production (Burges and Hussey, 1981). Therefore, the present study was undertaken to evaluate grains of rice, wheat, ragi, sorghum, pearl millet and maize and liquid media such as Potato Dextrose Broth and Sabouraud's Dextrose Broth for the mass production of *B. bassiana*.

MATERIALS AND METHODS

Entomopathogenic fungal culture: *B. bassiana* strains were isolated from the diseased caterpillar of *Spodoptera litura* and Mango mealy bug collected from the soybean and mango orchard fields of Pantnagar Uttarakhand, India. The diseased larva is of white colour and showed slight reddish mycelial growth of *B. bassiana*. The larvae were collected in screw cap vials (18 x 4 mm), were surface sterilized with 0.1% mercuric chloride for few seconds and then thoroughly washed with sterilized double distilled water and kept on Whatman filter paper No. 1to remove excess water. These larvae were then cut into small pieces with the help of sterile blade and the bits were aseptically transferred on Sabouraud's Dextrose Agar enriched with 1% yeast extract (SDYA) slants with the help of sterile inoculation needle. The slants were kept at 25 ± 1°C. Diseased larvae were also kept on moist filter paper in Petri dish for mycelial growth and sporulation. The fungi were identified based on the morphological character as per Humber (1997). All the cultures were maintained on SMYA and PDA slants.

Whole grain media: Fifteen whole grains viz, wheat, maize, paddy, sorghum, rice, groundnut, jhangora, chick pea, lentil,

pea, black gram, rajma, soybean, green gram, cow pea and pearl millet were used for estimating the sporution of *B. bassiana*, at 28ºC. 100g of each grain was washed and soaked in water overnight except rice which were soaked for 2 - 3h prior of starting the experiments. The excess water was drained by decanting and shade drying it for half an hour to further remove the excess moisture. The grains were packed separately in 500 mL conical flask, with cotton plug and auto calved at 15 psi for 20 min. After cooling, 1 mL of the spore suspension of fungal pathogen was inoculated into each flask under laminar air flow chamber. They were incubated in BOD incubator at 28ºC for 15 days. Three replications were maintained for each grain. To avoid clumping, after 7 days of inoculation, the flasks were shaken vigorously to separate the grain and to break the mycelial mat. After 15 days of incubation, 10g homogenous grain sample drawn from each replicate of uniformly sporulating flasks was transferred to 100 mL sterilized distilled water containing Tween 80 (0.05%) solution in 250 mL conical flasks. The flasks were shaken in mechanical shaker for 10 min. The suspension was filtered through double layered muslin cloth. Counting of spore's were made after the serial dilution of the suspension using double ruled Neubauer haemocytometer for determining the number of conidia in 1 g of the cereal grains.

Liquid media: Potato Dextrose Broth and Sabouraud's Dextrose Broth, were evaluated for the growth and sporulation of *Beauveria bassiana* fungi. 100 mL of each medium was poured in 250 mL capacity conical flasks and autoclaved at 15 psi pressure for 30 min. Five flasks of each medium was inoculated with 1 mL of spore suspension of fungi separately and incubated at 28ºC for 15 days. The spore suspension was subjected to spore counting and it was carried out as described in the previous section.

Statistical analysis: ANOVA was used to analyse the significance of temperature and media on sporulation of fungal pathogens using 'STATISTICA' computer package.

RESULTS AND DISCUSSION

Beauveria bassiana is an entomopathogenic fungus. The present experiments were conducted to find out morphological variation, growth patterns and suitable media for its mass production. Table 1 revealed that dry weight of Pantnagar isolate of *B. bassiana* varied from 0.0 g to 0.765g on different media. Growth of this isolate on liquid media indicated maximum dry matter production in pea broth followed by chickpea. Liquid media, SDB produced significantly higher spore production of test fungi. Biomass production of 0.69 and 0.63 g was recorded in *B. bassiana* on SDB. Potato Dextrose Broth (PDB) also supported spore production of the fungus (Table 1). Dangar *et al.* (1999) also observed similar findings in *Metarizhium anisepliae*. They found that abundance of glucose and minerals in the coconut water may enhance the growth and spore production of fungi. Rice and wheat washed water also supported the growth and sporulation of test fungi. Patel *et al.* (1990) reported that the purified rice wash water gave the best spore count in *M. anisepliae*. No significant difference in dry matter production of the fungus was observed among solid media having wheat, paddy, sorghum, groundnut, lentil and black gram, separately.

Table 1: Biomass production (g) and sporulation (x10^7) of *Beauveria bassiana* on various substrates

S. No.	Media	Dry weight of fungus (g)	Conidial count (10^7 mL^{-1})
1	Wheat	0.378 (0.936)	2.66 (1.76)
2	Maize	0.238 (0.859)	2.33 (1.67)
3	Paddy husk	0.383 (0.939)	3.33 (1.95)
4	Sorghum	0.506 (1.003)	5.00 (2.34)
5	Rice	0.555 (1.027)	7.60 (2.84)
6	Groundnut	0.488 (0.994)	4.40 (2.21)
7	Jhangora	0.000 (0.7071)	0.00 (0.70)
8	Chickpea	0.701 (1.096)	6.40 (2.62)
9	Lentil	0.629 (1.062)	7.60 (2.84)
10	Pea	0.765 (1.124)	8.00 (2.91)
11	Black gram	0.616 (1.056)	6.80 (2.70)
12	Rajma	0.195 (0.833)	2.33 (1.67)
13	Soybean	0.671 (1.082)	8.00 (2.91)
14	Green gram	0.500 (0.999)	5.40 (2.42)
15	Cowpea	0.652 (1.073)	9.06 (3.09)
Liquid media			
16	SDB	0.696 (1.090)	6.53 (2.65)
17	PDB	0.635 (1.060)	5.60 (2.47)
	CD at 5%	0.028 (0.014)	0.928 (0.211)

* Parentheses values are square root transformed $\sqrt{x+0.5}$

However, significant difference was observed in mycelia growth of fungus on SDB and PDB liquid media. Generally, higher dry matter production was observed on broth of pulses as compared to broths of cereals and oilseed. However, we widely used jhangora grains for the mass production of *Trichoderma* spp. as it is very cheap and easily available. In flasks having jhangora broth, due to the growth of undesireable fungi like *Penicillium*, *Aspergillus, Mucor* etc. no growth of *B.bassiana* was observed which indicate its non suitability for mass production of this fungus. Pilot studies using Jhangora grains will help to confirm these findings.

Highest conidial count (9.06×10^7 conidia mL^{-1}) was observed on cowpea media followed by soybean. Significant differences in conidial count were observed on maize, paddy, sorghum, rice and chickpea. Whereas, no significant difference was observed on the fungal growth among rice, lentil, pea and soybean added media. All the pulses along with wheat are known to have appreciable amounts of protein. Higher nitrogen has been shown to be necessary for mycelial growth of fungi (Riba and Glande, 1980; IM *et al*., 1988) which may be a reason for maximum production of biomass and conidia on Chickpea, pea, soybean etc. Most of these pulses and grains are part of human diet and their cultivation charges make them less economical for the mass multiplication of *B.bassiana*. Cheap culture medium is required in order to increase the cost-benefit ratio. Hence, several sources were tested for mass multiplication. Low cost sources of nutrients like millets such as sorghum, jhangora, and pearl millet were assessed for their utility in terms of conidial yield of the test fungus. Some of these grains are inexpensive, easily available and act as best nutritive media for the mass multiplication of many micro and macro organisms. Previously, Ibrahim and Low (1993) and Sharma *et al*. (2002) found rice as a suitable media for the mass culture of *B. bassiana*. This cereal was also used for the mass production of other deuteromycete fungi. Gopalakrishnan *et al*. (1999) reported that sorghum was the ideal cereal for the mass production of *Paecilomyces farinosus,*

it also supported satisfactory growth and sporulation of *B.bassiana* in our experiments also. In the case of *Verticillium lecanii,* sorghum was found to be the ideal cereal for mass production, which is in confirmation with the findings of Lakshmi *et al*. (2001). The ability of an entomopathogenic fungus to grow on different pests depends on the nutrients present on the insect body which is directly based on the plant they feed (Ambethagar *et al.,* 1998). The present study also supported this fact in which among several naturally available substrates tested for mass multiplication of *B. bassiana* SDB and Pea seeds are most suitable for its growth and development. Moreover, *B.bassiana* has wide host range and it was isolated from pests of banana (Kaaya *et al.,* 1991), rice (Puzari *et al.,* 1997), cashew (Ambethagar, 1991, 1996, and 1997), beans (Landa, 1984), crucifers (Butt *et al.,* 1994), chickpea and pigeonpea (Gopalkrishnan and Narayanan, 1988) around the world. Previous workers have also successfully used agro-wastes such as crushed maize cobs, wheat bran, rice bran, bagasse and press mud singly and along with supplementation for mass multiplication of *Metarhizium* and *Verticillium* (Vimaladevi and Prasad, 1996). The use of different agricultural wastes is economical and also helps in their efficient utilization. For a successful integrated pest management programme, the agents like the entomopathogenic fungi should be amenable to easy and cheap mass multiplication. From this study it was clear that the test fungus is able to grow on a variety of cheap and easily available grains. These grains can be used for the mass multiplication of the fungus and it may increase its efficiency as a biocontrol agent which is also economic and easily available. Refined experiments using other easily available nutrient sources like corn steep liquor, rice and wheat straw, corn stalks and other agricultural wastes will probably provide more information on the utility of different agro wastes for production of entomopathogenic fungi.

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