



Asian Journal of Medical and Pharmaceutical Sciences

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Research Article

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A Comparative Study on Different Media Substrates for Mass Production of Entomopathogenic Fungi *Beauveria bassiana* Meerut (U.P.) Isolate

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ABSTRACT

The production of locally isolated (Meerut Isolate) Entomopathogenic fungi in suitable media for large scale application has not yet been studied, therefore, the present study was undertaken to evaluate grains, pulses, oilseeds and liquid media such as Potato Dextrose Broth and Sabouraud's Dextrose Broth for the mass production of *B. bassiana*. Studies revealed that dry weight of Meerut (UP) isolate varied from 0.623 g to 0.811 g on different media. Growth on broth media indicated maximum dry matter production in chickpea (0.811 g) followed by SDB (0.802 g) > pea (0.796) > soybean (0.751 g) > cowpea (0.715 g) > PDB (0.711) > urd (0.663 g) > lentil (0.623 g) and groundnut (0.582 g). Significant differences in conidial count were observed on maize, paddy, sorghum, rice and chickpea. Whereas, no significant difference was observed among rice, lentil, pea and soybean. And highest conidial count (8.97×10^7 conidia ml⁻¹) was observed on cowpea broth followed by soybean (8.00×10^7) and pea (8.00×10^7) > Rice (7.7×10^7) > lentil (7.6×10^7) = Groundnut (7.6×10^7) > SDB (7.3×10^7) > urd (6.8×10^7) and chickpea broth (6.2×10^7).

Keywords: Entomopathogenic Fungi, *B. bassiana*, Mass Production

ARTICLE INFO

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Article History: Received 08 February 2016, Accepted 28 March 2016, Available Online 19 June 2016

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PAPER-QR CODE

Citation: Punia Gudia, et al. A Comparative Study on Different Media Substrates for Mass Production of Entomopathogenic Fungi *Beauveria bassiana* Meerut (U.P.) Isolate. *A. J. Med. Pharm. Sci.*, 2016, 4(1): 17-21.

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1. Introduction

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) Vaillem (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 good quantities of good quality inoculums is an important component of the biocontrol programs. The production of entomopathogen may be taken up by two ways: either a relatively small quantity of the inoculums for laboratory experimentation and field-testing during the development of mycopesticide or development of a basic production system for large-scale production which follows labor intensive and economically viable methods for relatively small size markets. The knowledge of nutrition requirements is the main need in the cultivation of microorganisms using any cultural technique. The nutrients like carbohydrates, proteins, lipids, nucleic acids are made up of microelements like carbon, hydrogen, nitrogen, sulphur, phosphorus and these are involved in mechanisms like host pathogen interaction and self defense mechanisms. Carbon is the major component and the molecules of carbon also contribute to oxygen and hydrogen. The effect of the nutrient sources on the growth and development of microorganisms is studied. The fungus which is used and the media components used are responsible for the mycelia growth and spore yield. Although the saprophytic fungi utilize a range of nutrient sources but for the mass production and commercialization, simple and cheap media needed (Raimbault, 1998). For the full growth of microorganisms, the macro elements like carbon, hydrogen, oxygen, sulphur, phosphorus and nitrogen are required which are the components of carbohydrates, nucleic acids and proteins. The growth characteristics in addition to growth substances are useful in the tolerance level selection studies. The preparation being used for the growth, storage and transport of microorganism can be used in solid or liquid forms (Jenkins, 1995). The media should have all the nutritional requirements for the growth of microorganisms. According to Roberts and Yendol (1971), wheat, corn and potato products are the basic substrates for the mass production of *B. bassiana* spores. Pandit and Som (1988) recommended potato dextrose agar for the maintenance of *B. bassiana* cultures and soybean chunks for its mass (Production) multiplication. The different grain media that were found suitable for the mass multiplication of fungi include cooked rice and carrot broth for *B. basiana* (Rammohana Rao, 1989). Jowar was found to be the best medium (Patel, 1990) and finger millet, pea and jowar were found good for growth of *B. bassiana* (Dayakar, 2011). Sharma et al. (2002) reported cowpea grain was found to be superior for better sporulation of *Beauveria* spp. than other substances viz. bajra, barley, gram, maize, rice, sorghum and wheat. Pandey and Kanaujia (2005) conducted experiments on mass multiplication of *B. bassiana* on grain based media viz., barley, finger millet, maize, sorghum, soybean and wheat. Highest spore production (5.39×10^7

conidia/ml) and spore viability (86.6%) was observed in conidia produced from finger millet. However, the production of locally isolated Entomopathogenic fungi in suitable media for large scale application has not yet been studied 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*.

2. Experimental

Isolation of Fungus from Soil

Generally, isolation of *B. bassiana* from soil requires a selective medium. DOC2 medium that contained no dextrose, containing 3g Bactopeptone, 0.2g CuCl₂, 2mg crystal violet, 15g Agar and 1000 ml distilled water (Shimazu and Sato 1996) was used for isolating fungus from soil samples (pH 10). The medium was autoclaved at 120°C for 20 minutes and poured into 9 cm Petri plates. Soil sample (1g) from a sugarcane field, Sardar Vallabhkhair Patel University Of Agriculture And Technology, Meerut (UP) was suspended in 200 ml of sterile distilled water containing 0.03 % Tween @80 as surfactant. Suspensions were applied at concentration of 0.2 ml/plate using Spread Plate Method. Plates were incubated at 25°C in complete darkness. Obtained colonies were transferred to Sabouraud's Dextrose Agar Plates supplemented with 1% Yeast extract (SDYA) for primary morphological identification. Regular sub culturing of fungal colonies on SDYA plates maintained fungal inoculums for further experiments. For mass multiplication of isolate of *B. bassiana*, eight pulses (Bengal gram, black gram, greengram, cowpea, rajma, lentil, pea and soybean), four cereals (rice (husked + unhusked), sorghum, wheat and maize) one oilseed (groundnut) and one small millet jhangora seeds were used. The potentiality of these media for mass production of these two isolates were compared with potato dextrose broth and Sabouraud's dextrose broth for evaluating the growth and sporulation of entomogenous fungi following the standard microbiologist methods (Booth, 1971; Sundrababu, 1980).

Preparation of Media

A 100 gram sample of each raw material was taken separately washed, soaked in water for 30 minutes. After straining through double muslin cloth, the extract was distributed in 250 ml conical flasks at the rate of 100 ml per flask. Another set of PDB (Potato Dextrose Broth) and SDB (Sabouraud's Dextrose Broth) was also prepared simultaneously. The medium contained in the flasks were sterilized at 15 psi 20 minutes in an autoclave.

Biomass Production of *B. bassiana* On Different Media

Five mm discs of the 15 days old *B. bassiana* culture grown on Sabouraud's Dextrose Agar Plates supplemented with 1% Yeast extract (SDYA) were carved out using a flame-sterilized cork borer and inoculated into each of the conical flasks containing the respective test media broth for testing the biomass production of *B. bassiana*. Antibiotic (streptomycin) was also mixed @ 100 mg/flask before

inoculation to inhibit the bacterial contamination. Thereafter, these flasks were incubated in BOD incubator at $25 \pm 1^\circ\text{C}$ and 85 ± 5 per cent relative humidity. The experiments were terminated 20 days after inoculation. The biomass of fungus was measured by filtering the mycelial mats on a dried pre-weighed flask. Four replications were maintained for each medium. Conidial count and conidial germination of *B. bassiana* grown on different media were evaluated.

Spore Harvesting and Drying

The spores were harvested at 3 and 12 days after the inoculation for liquid and solid media respectively, to evaluate spore yield. To harvest the spores as powder it was necessary to dry the fungus to reduce moisture content and allow the spores to separate from the substrata. The spores harvested following this procedure can be preserved for a long time without loss of germinative power or pathogenicity (Bateman, 1995). To dry the cultures the conical flasks were opened in a room with a temperature of $20 \pm 5^\circ\text{C}$ and an average relative humidity of $50 \pm 5\%$ and allowed to air dry. Harvesting was done both mechanically and manually for 20 minutes. The mechanical harvest involved the use of a shaker Ro-Tap that uses horizontal circular motion and vertical taping motion to stratify and screen the particles. The manual harvest consisted of back and forth movements of the sieve. The spore powder that was collected after sieving was weighed and kept in separate sterile vials for further assessments, such as moisture content and quality assessment.

Culture Moisture Content Assessment

In order to monitor air drying of the cultures, sample was weighed after incubation durations. At the same time, from independent samples that were kept under the experimental conditions, subsamples were taken daily to assess the humidity content using oven drying where the sub samples were dried for 24 hours at 105°C (Rao et al. 2006). The aim of this procedure was to determine the moisture content of the fungus culture.

3. Results and Discussion

Suitability of Different Growth Media for Mass Multiplication of *B. bassiana*: In recent years crop protection based on intensive use of biocontrol agent have occupied an important place in insect pest management programme. Among the bioagent entomopathogen fungus is important as it can be produced economically and has the desired effect in controlling insect pests. Investigation on fungal entomopathogen (Ferron, 1978; McCoy et al., 1988; Samson et al., 1988; Feroon et al., 1991; Glare and Milner, 1991; Tanada and Kaya, 1993) revealed that insect mycoses occurred in nature in epidemic form in different periods of time. This results in lowering of insect pest populations below economic injury level. Therefore, mass production of effective entomopathogenic fungi becomes a pre-requisite for successful implementation of biological control in integrated pest management. So selection of suitable media for entomogenous fungi is essential not only for obtaining maximum growth and sporulation but also for production of infective propagules which ultimately decides the success of mycoinsecticides. Suitability of seventeen media

including grains and synthetic media in the growth and sporulation of *B. bassiana* was determined in the present studies. Among these PDA and SDB were considered as check. The dry weight of Meerut (UP) isolate varied from 0.623 g to 0.811 g on different media. No significant difference in dry matter production was observed in between wheat and paddy, sorghum and groundnut, lentil and urd. However, significant difference was observed among wheat, maize, sorghum, chickpea, lentil, pea, rajma and soybean broth media. Generally, higher dry matter production was observed on broth of pulses as compared to broths of cereals and oilseed. Table below revealed that dry weight of Meerut (UP) isolate varied from 0.623 g to 0.811 g on different media. Growth on broth media indicated maximum dry matter production in chickpea (0.811 g) followed by SDB (0.802 g) > pea (0.796) > soybean (0.751 g) > cowpea (0.715 g) > PDB (0.711) > urd (0.663 g) > lentil (0.623 g) and groundnut (0.582 g). Significant differences in conidial count were observed on maize, paddy, sorghum, rice and chickpea. Whereas, no significant difference was observed among rice, lentil, pea and soybean. And highest conidial count (8.97×10^7 conidia ml^{-1}) was observed on cowpea broth followed by soybean (8.00×10^7) and pea (8.00×10^7) > Rice (7.7×10^7) > lentil (7.6×10^7) = Groundnut (7.6×10^7) > SDB (7.3×10^7) > urd (6.8×10^7) and chickpea broth (6.2×10^7). Crospovidone and CCS perform their disintegration by wicking through capillary action and fibrous structure respectively with minimum gelling. Tablets of each batch were evaluated for *in vitro* disintegration time. The results showed that the disintegration time of prepared tablets were in the range of 3.6 to 6 seconds. These trials indicated that amongst the disintegrants used, Crospovidone and CCS were better disintegrants to formulate fast dissolving tablets of Flunarizine than Sodium starch glycolate. Tablets were evaluated for *in vitro* dissolution studies in acid buffer (pH-1.2) and the results were shown in the Table 6 and Fig.1-3. Among the various formulations tablets of batch F8 prepared CCS and crospovidone showed 98.17% release of drug within 30 min.

Table 1: Dry Weight count and Conidial count (conidia/ml) of *Beauveria bassiana* Meerut (UP) Isolate

Culture Media	Dry Weight(g)	Conidial Count (Conidia/ml)
Chickpea	0.811	6.2×10^7
SDB	0.802	7.3×10^7
Pea	0.796	8.00×10^7
Soybean	0.751	8.00×10^7
Cowpea	0.715	8.97×10^7
Urd	0.663	6.8×10^7
PDB	0.711	6.9×10^7
Lentil	0.623	7.6×10^7
Rice	0.659	7.7×10^7
Groundnut	0.582	7.6×10^7

Significant differences in conidial count was observed among PDB, SDB, cowpea, soybean, chickpea, lentil and pea, which ranged from 6.2×10^7 to 8.97×10^7 conidia ml^{-1} .

Similar findings were also given by Sharma et al., (2002). They reported Cowpea grains were found to be superior for better sporulation of *Beauveria spp.* than other substrates viz., Bajra, Barley, Gram, Maize, Rice, Sorghum and Wheat. So on the basis of above findings; we can say that following grains were found suitable for mass production of *B. bassiana* as cowpea, pea, soybean, chickpea, urd and lentil. Pulses are rich in protein so they enhance the dry matter and spore production as compared to cereals. Therefore, good results were observed in dry matter and conidial count on pulses broth as compared to cereals broth.

Discussion

Mass multiplication of entomopathogenic fungi is an important area in developing biocontrol strategies for management of pest population. Therefore, growth and sporulation of two isolates of *B. bassiana* were investigated on media prepared with eight pulses, four cereals, one oil seed and one small millet. In the present study, several naturally available substrates of both solid and liquid media were tested for mass multiplication of *B. bassiana*. The success of microbial control of insect pests depends not only on the isolation, characterization 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 bio-control program. For a successful integrated pest management program, the agents like the entomopathogenic fungi should be amenable to easy and cheap mass multiplication. Environmental efficiency and storage stability and have made conidia the propagule of choice for most commercial formulations (Wraight et al., 2001). Therefore mass multiplication of effective entomopathogenic fungi becomes a pre-requisite for successful implementation of biological control in insect pest management. It is apparent that earlier workers had tried to use different carbohydrate and protein rich commodities and even agricultural as well as industrial by products for mass production of this fungus. potato paste, bean pulp and paste of rolled oats for obtaining good growth of the fungus The results (Table) showed that there were significant differences among the culture Media tested, *B. bassiana* spore production on both media. Results were compared with potato dextrose broth and Sabouraud's dextrose broths were used for estimating dry matter of the fungus. On pulses broth media both the isolates showed good growth and sporulation as compared to cereals broth. In case of Meerut (UP) isolate highest dry matter was produced by chickpea which was followed by SDB > pea > soybean > cowpea > urd > PDB > lentil > Rice and groundnut. Highest conidial count was observed on cowpea which was followed by pea soybean in Meerut (UP) isolate.

The present study was carried out for three weeks at room temperature confirming earlier work (Maniania,1993) who reported that ambient temperature for conidia production in *B. bassiana* and *M. anisopliae* was 20-25 °C. Our results partly agree with observations of Nelson et al. (1996) that demonstrated maximum yield was achieved when fungi were grown on rice for three weeks at 23°C. There is a need to determine yield to confirm whether the substrate with the highest quantity of spores has a maximum yield of spores

per gram of substrate. The quantities of spores achieved here would probably have been different if the different isolates would have been incubated for different periods of time since different scientists have reported different optimal incubation periods for different fungal isolated. For example optimal incubation time for some *B.bassiana* strains has been previously reported as two weeks at 27 ± 1⁰ C (Dorta et al., 1990).

4. Acknowledgement

I would like to sincerely thank Dr. C.S. Prasad (Registrar, (SVPUAT) Sardar Vallabhbhai Patel University Of Agriculture And Technology, Meerut (UP, India) and entire staff of BIOCONTROL LAB (SVPUAT) to let me carry out my research work on *B.bassiana* with this efficacy.

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