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Research Article

Evaluation of economically viable substrates for mass production of *Beauveria bassiana* (Balsamo) Vuillemin

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ABSTRACT: Five substrates, *viz*., sorghum, rice bran, rice husk, pressmud and bagasse were evaluated to identify the most suitable substrate for large scale multiplication of six strains of *Beauveria bassiana*. The results of the study showed sorghum as the most suitable substrate as it yielded highest conidial count for all the strains and the conidial viability was also highest in the conidia harvested from sorghum. The strain Bb-5A was found to be the most vigorous among all the strains as it recorded highest conidial count per gram of the substrate on all the substrates used.

KEY WORDS: Mass Multiplication, *Beauveria bassiana,* strains, substrates

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INTRODUCTION

Crop protection based on biological control of crop pests with microbial pathogens like virus, bacteria, fungi and nematodes has been recognized as a valuable tool in pest management due to their eco-safety, target specificity, no development of resistance, reduced number of applications, higher yields, quality improvement, higher acceptability and value of produce and suitability for exports. Among all the microbials, entomopathogenic fungi, which are promising alternatives with advantages such as entry by contact, replication in target insect, safety to non-target organisms, ease and economical *in vitro* mass culture. Mass multiplication is one of the important criteria for commercialization of an identified potent strain of the fungi. Hence, this study was formulated to identify a suitable substrate for *Beauveria bassiana* and a potent strain for mass multiplication.

MATERIALS AND METHODS

The present research was conducted in the Department of Entomology and AICRP on Biological control of crop pests and weeds, College of Agriculture, Rajendranagar, Hyderabad during 2004-07.

Isolates

Six isolates of *Beauveria bassiana* [Bb-13, Bb-11 and Bb-5A from NBAII, one commercial isolate coded as Bb-N and two local isolates from two different localities *B. bassiana* Local-1 (Bb-L-1) and *B. bassiana* Local-2 (Bb-L-2)] were obtained. The commercial isolate and isolates from NBAII were host passaged through *Spodoptera litura* and the fungus was recovered from dead cadavers following single spore isolation procedure on Sabouraud's Dextrose Agar medium with yeast extract (SDAY). The two local isolates were isolated from larvae of *S. litura* showing symptoms of *B. bassiana* infection with white muscardine growth over the body surface which were collected from *rabi* groundnut and castor fields. Single spore isolation method was followed for sub-culturing the growing fungus on fresh plates of SDAY medium regularly till pure cultures were obtained. The culture was then host passaged and pure culture was recovered which was used for the experimental purpose.

Substrates

Different economically viable substrates such as sorghum grain, rice bran, rice straw and sugar industry

wastes such as pressmud and bagasse were selected for the purpose of substrate evaluation for mass production of the six strains / isolates. These substrates were inoculated individually with all the six isolates of *B. bassiana* following different methods.

Sorghum

One kilogram of sorghum grain was washed thoroughly under tap water and soaked in distilled water for about half an hour. The soaked grain was cooked until the grains became soft and formed a paste when pressed between fingers. The cooked grain was air dried in shade for one hour on a filter paper to remove excess moisture.

Fifty grams of the grain was weighed into 250 ml flasks plugged with cotton and autoclaved for 20 min. at 121°C and 15 psi. The sterilized grain was inoculated with 10 ml of conidial suspension containing $5x10^7$ conidia from a vigorously growing 10 days-old culture under aseptic conditions. The inoculated grain was incubated for 25 days at 25±1°C.

Rice bran

Fifty grams of rice bran was taken in 250 ml flasks plugged with cotton and autoclaved at 121°C and 15 psi for 20 min. The sterilized flasks were inoculated with 30 ml of conidial suspension containing $5x10^7$ conidia. Large quantity of water was used for preparation of conidial suspension for inoculating rice bran so as to provide adequate moisture to support fungal growth. The contents of the flask after inoculation were stirred thoroughly using a sterile glass rod to avoid formation of clumps and for uniform mixing of the inoculum. The flasks were incubated at 25±1°C for 25 days.

Press mud

The same procedure followed for rice bran was adopted for press mud, but only 10 ml of conidial suspension ($5x10^7$ conidia) was used for inoculation as the substrate contained enough moisture to support fungal growth.

Rice straw and bagasse

Rice straw cut into small pieces and bagasse when used as substrates did not support growth of *B. bassiana*. These substrates were therefore used separately as physical means of support for the growth of the fungus by soaking them overnight in 10% of molasses and 2.5% of yeast extract mixed in two liters of water separately. The soaked materials were squeezed and air dried on a filter paper for one hour to absorb the excess moisture. Fifty grams of the substrate was weighed into 250 ml flasks and autoclaved. The sterilized substrate was inoculated with $5x10^7$ conidia and incubated for 25 days at 25±1°C.

Growth and biomass production on SDY medium

The growth of different strains of *B. bassiana* on SDY medium apart from different natural substrates was assessed for which 100 ml of SDY broth prepared in 250 ml flasks was sterilized in an autoclave and inoculated with a conidial suspension containing $5x10^7$ conidia. The inoculated flasks were placed in a shaker for 10 days at 25°C. The biomass thus produced was filtered through a double layered muslin cloth and weighed to record the biomass production. Each isolate was replicated five times.

After 25 days of incubation, the growth was measured in terms of spore load per gram by drawing randomly one gram of the substrate from the flask and placing it in known volume of distilled water which was serially diluted to calculate the spore count using improved Neubauer's haemocytometer under a compound microscope at 40x magnification and the number of conidia per ml was calculated and compounded to determine the spore load per gram of the substrate (Aneja, 1996). Twenty samples were drawn from each replication and studied for drawing more meaningful results.

The viability of conidia of different strains obtained after culturing on different substrates was also determined using approximately 500µl of uniformly suspended spore solution placed in the cavities of a cavity slide containing 100µl of SDY medium. The slides were placed in a Petri plate containing a moistened filter paper at its bottom and incubated at 25±1°C and 95% RH. The slides were observed after 24 hours under a microscope and the number of conidia germinated and total numbers of conidia visible in any of the focused region were recorded. The per cent conidial viability (G) was calculated using the formula given below. There were five replications with a sample size of 10 for all the treatments studied.

$$G = \frac{N}{T} \times 100$$

Where G = per cent spore germination
N = number of spores germinated
T = total number of spores observed

Statistical analysis

All the six isolates were tested in the five test substrates and each isolate was replicated three times. The data obtained on conidial concentration per gram of substrate and per cent conidial viability for each isolate on all the five substrates were subjected to Duncan's multiple range tests (DMRT) analysis and results were interpreted at 1 per cent level of significance.

RESULTS AND DISCUSSION

The strains of *Beauveria bassiana* exhibited significant differences in their biomass production when cultured on SDY broth with strain Bb-5A showing the highest biomass production and isolate Bb-L-1 showing the least (Table 1). This difference may be due to the variability in their inherent potential to utilize the available nutrients in the culture medium and proliferate rapidly.

The substrates (sorghum grain, rice bran, rice straw, bagasse and pressmud) exhibited significant differences in their potentiality to yield conidia. Significant differences between the strains for a common substrate were also noticed (Table 1). The highest conidial concentration per gram was yielded by strain Bb-5A on all the substrates and the lowest was yielded by isolate Bb-L-1. This may be due to the variability among the strains as explained earlier. The variability among different substrates in yielding different conidial concentrations per gram can be explained based on the nutritional quantity of the substrates and also by considering the physical form of the substrate, which influences the growth of the entomopathogenic fungi.

Among the five substrates, sorghum grain yielded the highest conidia per gram of substrate which may be due to the presence of rich source of carbon and adequate source of nitrogen, which are essential for higher growth and sporulation. It has been reported that sorghum grain contains 75% of starch and 27 to 30% amylase, which are rich sources of carbon that enhance the growth and sporulation of fungi (Anonymous, 1991).

Im *et al*. (1988) reported that dextrose, a carbon rich source, favours sporulation of *Nomuraea rileyi*. Sorghum grain contains high amount of dextrose (1.8%) (Anonymous, 1991) which might be the reason for its higher spore production potential. Another possible reason could be favourable physical condition of the sorghum grain which becomes soft and fibrous after boiling and gets loosely distributed in the conical flask and gives good scope for easy proliferation of the growing fungus. Muller-Kogler (1967) suggested that loose substrates yield more conidia than solid substrates. Similarly, Roberts & Yendol (1971) reported high production of conidia of *Verticillium lecanii* when broken jack seeds were used because of the availability of enough surface for fungal growth and sporulation.

The findings of the present study are in accordance with the findings of Patel & Kanaujia (1997), Sharma *et al*. (2002) and Pandey & Kanaujia (2005) on *B. bassiana*. Similar findings were reported by Devi (1994), Devi *et al*. (2001) and Kulakarni and Lingappa (2002) where sorghum grain along with 2% yeast extract yielded high spore production in *N. rileyi*. Verma *et al*. (2004) reported high spore production of *Paecilomyces lilacinus* on sorghum grain as substrate.

Although rice bran is a rich source of nutrients along with certain essential oils, it did not support good growth as sorghum grain (Mazumder *et al*., 1995). The reason may be that the medium became a compact mass after sterilization, which did not allow *B. bassiana* to ramify within the medium (Puzari *et al*., 1997). Rice straw and bagasse did not support the growth of the fungus initially. This may be due to the non-availability of carbon source in the substrates (Saikia, 1987). Addition of energy sources exogenously enhances the fungal growth and sporulation as described by Smith and Grula, (1981) and Gopalakrishnan and Mohan (2000). Yeast extract (N_2/vitamin source) has been shown to be necessary for mycelial growth of *N. rileyi* (Riba and Glander, 1980; Im *et al.,* 1988). Hence, 10% molasses and 2.5% yeast

Table 1. Effect of different substrates on biomass and conidial production in different strains of *Beauveria bassiana*

Substrates	Conidial concentration/g (Conidiax10^9) of different strains mass multiplied on different substrates					
	Bb-5A	Bb-N	Bb-13	Bb-11	Bb-L-1	Bb-L-2
Biomass (g) SDY broth	3.95 ± 0.02^a	3.86 ± 0.03^b	3.43 ± 0.04^d	3.56 ± 0.03^c	2.56 ± 0.03^f	2.65 ± 0.02^e
Sorghum grain	4.20 ± 0.04^a	3.87 ± 0.07^a	3.30 ± 0.04^a	3.08 ± 0.04^a	2.82 ± 0.04^a	2.88 ± 0.04^a
Rice bran	3.26 ± 0.06^b	2.49 ± 0.04^c	2.66 ± 0.03^b	2.53 ± 0.03^b	2.33 ± 0.04^b	2.36 ± 0.02^b
Bagasse	2.86 ± 0.02^c	2.52 ±0.03^b	2.44±0.06^c	2.41 ± 0.04^c	2.14 ± 0.04^c	2.27 ±0.03^c
Rice straw	2.25 ± 0.05^d	1.96 ±0.04^d	2.03 ±0.06^d	1.90 ±0.04^d	1.73 ± 0.04^d	1.76 ± 0.03^d
Pressmud	0.11 ± 0.001^e	0.11 ± 0.001^e	0.10 ± 0.001^e	0.10 ± 0.001^e	0.08 ± 0.002^e	0.10 ± 0.002^e
SEM±	0.040	0.041	0.045	0.035	0.034	0.029
CD (0.01)	0.119	0.123	0.134	0.104	0.101	0.086

Figures indicated by the same letters are not significantly different from one another as per Duncan's Multiple Range Test [DMRT]

Table 2. Effect of different substrates on the conidial viability of different strains/isolates of *Beauveria bassiana*

Substrates	Per cent Conidial viability of different strains mass multiplied on different substrates					
	Bb-5A	Bb-N	Bb-13	Bb-11	Bb-L-1	Bb-L-2
Sorghum grain	90.08 ± 0.43[a] (71.63 ± 0.41)	88.34 ± 0.30[a] (70.01 ± 0.27)	89.26 ± 0.37[a] (70.85 ± 0.35)	88.16 ± 0.25[a] (69.85 ± 0.22)	83.68 ± 0.38[a] (66.15 ± 0.29)	84.52 ± 0.37[a] (66.81 ± 0.29)
Rice bran	86.46 ± 0.30[b] (68.39 ± 0.25)	85.30 ± 0.35[b] (67.43 ± 0.28)	86.12 ± 0.28[b] (68.10 ± 0.23)	84.68 ± 0.30[b] (66.93 ± 0.24)	81.94 ± 0.45[b] (64.83 ± 0.33)	82.22 ± 0.29[b] (65.04 ± 0.22)
Bagasse	78.62 ± 0.24[c] (62.43 ± 0.17)	76.16 ± 0.52[c] (60.75 ± 0.35)	76.08 ± 0.33[c] (60.70 ± 0.22)	75.82 ± 0.48[c] (60.52 ± 0.32)	72.30 ± 0.46[c] (58.22 ± 0.30)	73.18 ± 0.487[c] (58.79 ± 0.32)
Rice straw	73.62 ± 0.44[d] (59.07 ± 0.28)	71.28 ± 0.51[d] (57.57 ± 0.32)	73.10 ± 0.33[d] (58.74 ± 0.22)	71.40 ± 0.41[d] (57.65 ± 0.26)	69.48 ± 0.51[d] (56.44 ± 0.31)	70.44 ± 0.48[d] (57.04 ± 0.30)
Pressmud	72.36 ± 0.44[e] (58.26 ± 0.29)	70.02 ± 0.66[d] (56.78 ± 0.41)	69.82 ± 0.69[e] (56.66 ± 0.43)	68.64 ± 0.66[e] (55.93 ± 0.41)	66.44 ± 0.34[e] (54.58 ± 0.21)	66.80 ± 0.43[e] (54.80 ± 0.26)
SEM±	0.380	0.483	0.427	0.447	0.432	0.418
CD (0.01)	1.130	1.436	1.268	1.328	1.284	1.243

Figures in parentheses are angular transformed values; figures indicated by the same letters are not significantly different from one another as per Duncan's Multiple Range Test (DMRT)

extract when added to these substrates triggered the growth and sporulation of *B. bassiana*, but the growth was very poor compared to that on sorghum grain and rice bran. Slightly a better growth of *B. bassiana* on bagasse compared to rice straw may be due to the presence fructose (2%) in the bagasse. The results of the present study are in accordance with the work of Mazumder *et al*. (1995), who reported no growth of *B. bassiana* on bagasse and very poor growth on rice husk. Similar poor growth of *B. bassiana* when cultured on rice hull was reported by Puzari *et al*. (1997). Press mud exhibited least growth of *B. bassiana* among the five test substrates and it may be due to its poor nutritional status and formation of clumps after sterilization which provided little surface area for proliferation of the fungus into the substrate.

The spores produced from different substrates showed differences in spore viability, which were significantly high in all the strains when sorghum grain was used as a substrate, and significantly lowest when press mud was used. Among the strains, Bb-5A produced the highest number of viable spores on all the substrates while Bb-L-1 recorded the least number of viable spores on all substrates (Table 2). The reason could be the strain variations as suggested earlier. The variations in the production of viable spores by different substrates may be directly correlated with the nutritional status of the respective substrates. The substrates which are rich in nutritional composition produced not only high spore concentrations but also higher viable spores (Tables 1 and 2). The positive correlation between spore production and its viability can be clearly understood from the works of Pandey & Kanaujia (2005) who recorded higher spore viability for the substrates which yielded high spore concentrations while evaluating six different grain media for mass production of *B. bassiana*. It was also suggested by them that supplementing of sucrose exogenously resulted in increased viability of spores which clearly explains that spore viability depends on the availability of the nutrients. The present study establishes sorghum as an ideal substrate for mass production of fungi and the strain Bb-5A as the most promising for mass multiplication.

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