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Research Article

Mass production of *Beauveria bassiana* (Bals.)Vuill. for the management of rice hispa, *Dicladispa armigera* (Olivier)

PURNIMA DAS*, L. K. HAZARIKA, D. BORA[1], K. C. PUZARI[2] and PRANAB DUTTA[2]

Department of Entomology, Assam Agricultural University (AAU), Jorhat 785 013, Assam, India

[1] *Department of Life Sciences, Dibrugarh University, Dibrugarh 786 004, Assam, India*

[2] *Department of Plant Pathology, AAU, Jorhat 785 013, Assam, India*

* *Corresponding author E-mail*: purnima.dashazarika@gmail.com

ABSTRACT: Potato broth, rice gruel, coconut water, rice husk, sawdust and rice bran were evaluated for mass culturing of *Beauveria bassiana* (Bals.) Vuill (Strain AAU-09). Amongst the solid media rice husk in addition of 2% dextrose was superior to others in terms of spore production (6.25×10^7 conidia/ml) and pathogenicity (86.67%) to *Dicladispa armigera* (Olivier) adults. Likewise, amongst the liquid media potato broth supplemented with synthetic chitin (2%), dextrose (2%) and peptone (2%) supported maximum spore production.

KEY WORDS: Entomopathogenic fungi, *Beauveria bassiana*, mass production, pathogenicity, spore production

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INTRODUCTION

In a biocontrol programme production of good quality inoculum of any microorganism is prerequisite for receiving excellent result. The production of entomopathogenic fungi had been successfully accomplished in many semisynthetic and synthetic media (Campbell *et.al.,* 1978, Smith and Grula, 1981). While, for harvesting of dried blastospores of the fungus, Ferron (1978) and Alvas and Periera (1989) utilized liquid media such as rice guel, coconut water and potato broth, supplemented with different carbon and nitrogen sources. Aquino *et al.* (1977), Filho *et al*. (1988*)* and Pandit and Som (1988) utilized rice grains and soyaben chunk for mass culturing of *Beauveria bassiana* (Bals.) Vuill. Rice husk, rice barn, and sawdust are agricultural and industrial wastes which cause serious problems in their disposal resulting in environmental pollution as such if these wastes can be managed by utilizing them as media for mass production of entomopathogenic fungi, we may contribute toward economic waste management. Therefore, the present study was undertaken to evaluate these wastes as economically viable substrates for mass production of *B. bassiana* and to evaluate their pathogenicity against a serious major pest of rice, the rice hispa. *Dicladispa armigera* (Oliver).

Similalry, attempt for utilizing liquid media such as potato broth, rice gruel and coconut water has also been made in this study.

MATERIALS AND METHODS

Solid media

Rice husk, rice bran, saw dust were collected from the local mills. One hundred gm of each of the solid media soaked in 25 ml distilled water were taken in an autoclaveable polypropylene bag (24 cm x 14 cm) sterilized by autoclaving at 121°C and 15 lb for 20 minutes, and the process was repeated for two consecutive days and p^H was adjusted to 7 (Mazumder *et al.,* 1994). Each bag was condsidered as a replicate and 3 bags were used for each treatment.

Liquid media

One hundred ml each of rice gruel, coconut water and potato broth were poured in 250 ml conical flask. To each of the flask streptomycin sulphate @ 0.5gm/l was added and all the flasks were autoclaved at 121°C under 15 lb pressure for 20 min. Each flask of containing a medium was inoculated with 1 ml of spore suspension

and incubated at 25±1°C for 15 days and was replicated thrice.

Inoculation of media

Conical flasks containing medium and polypropylene bags as described were inoculated under laminar air flow chamber with 1 ml of pure culture (14-day-old) of conidial suspension of *B. bassiana* as per the method described Puzari and Hazarika (1994). These bags were then incubated at 25°C ± 1°C for 15 days. To avoid clumping after 7 days of inoculation, the bags/flasks were shaken to separate the substrate and to break the mycelial mat.

Conidial Density

After 15 days of incubation, 10 gm homogenous solid sample was drawn from each replicate which were transferred to 100 ml sterilized distilled water containing Tween 80 (0.023%) solution in 250 ml conical flask. The suspension was filtered through double layered muslin cloth and then through filter paper (Whatman No. 1). Spore count was made after the serial dilution of the suspension using double ruled Neubauer haemocytometer for determining the number of conidia per gm or ml of medium

Supplementation of carbon, nitrogen and minerals

Starch, casein, synthetic chitin, yeast extract, dextrose, peptones $MgSO_4$, $CaC0_3$ and manitol were added individually to 250 ml flask containing 100 ml of either potato broth or rice gruel or coconut water at a concentration of 2%. Each treatment was replicated five times. All the supplement were obtained from the Hi Media Laboratories Private Limited.

The liquid media such as potato broth, rice gruel and coconut water were supplemented with sucrose, manitol, starch, dextrose, casein, peptone, chitin, yeast extract, $MgSO_4$, $CaCO_3$ and were added to find out their effect on conidial density and pathogenecity.

Bioassay of *B. bassiana* strains against *D. armigera* adults

Adults of *D. armigera* were bioassayed at room temperature (30±1°C, RH 80-85%) as per the method of Puzari *et al*. (1994). Pathogenecity of the strain, AAU-09 was assayed in the laboratory at a concentration of 1 x 10^7 conidia/ml (Puzari and Hazarika 1991, 1992; Hazarika and Puzari, (1995). Four seedlings of twenty-day-old (*var*. Ranjit) were grown in plastic pots (510 ml capacity) containing soil mixed with manure and fertilizers. Ten laboratory reared pre-starved adults (2-day-old) were released into each pot and caged in paired lantern chimneys one kept atop another. The open end of the top chimney was fitted with a muslin cloth. One day after the release of the test insects, *B. bassiana* at a concentration of 10^7spores /ml in water mixed with Tween 80 @ 0.023% was sprayed @ 20 ml/pot with a glass atomizer. Each treatment was replicated thrice in CRD. Control pots were treated with water mixed with tween 80. Mortality of adults due to infection was recorded after 7 and 10 days of inoculation and data were subjected to ANOVA.

RESULTS AND DISCUSSION

Table 1 shows the mean spore production of *B. bassiana* as grown in three solid media and per cent pathogenicity of these spores against *D. armigera* adults. Though, three substrates favoured the conidial production and infectivity of the fungus, rice husk alone and in combination with 2% dextrose showed highest inoculum density (6.25 x 10^7conidia/ml) and pathogenicity (86.67%) than rice bran and saw dust alone or in combination with 2% dextrose. Nutrient composition of a medium selected for mass production is of key importance for the growth, sporulation and infectivity of entomo-pathogenic fungi (Ferron, 1981). Rice husk contains lignin (20-42%) and cellulose (30-45%), which supported a proliferated growth of this fungus (Mazumder *et al*., 1995, Puzari *et al.* 1997; Sharma *et al*., 2002). Many attempts to utilize rice bran and husk as sole nutrient source had produced satisfactory results in terms of production of good quality inoculum of *B. bassiana* (Sahayaraj *et al.*, 2008; Pham *et al*., 2009). Poor growth of the fungus in saw dust and rice bran individually may be due to absence of sufficient cabrohydrate and protein in order to support sporogenesis. Sawdust contains phenolic compounds which may act as growth inhibitors (Mazumder *et al*., 1995). However, as a result of addition of dextrose and peptone to saw dust and rice bran, productivity of the media improve significantly with concomitant increase in potentiality and ignificant growth. In terms of potentiality, sawdust and rice brane alone were the poorest media, spores harvested out of which caused only 12.00% and 50.00% mortality, respectively.

Coconut water was significantly superior to potato broth and rice gruel in terms of spore counts and pathogenecity. However, when media were supplemented with carbon and nitrogen compounds, peptone in potato broth produced significantly higher number of propagules (5.16 x 10^7 conidia/ml) followed by dextrose (4.32 x 10^7 conidia/ml) and synthetic chitin (4.24 x 10^7 conidia/ml). Similarly, in case of coconut water and rice gruel, there was no significant effect of supplemented nutrients.

Table 1: Mean spore production (x10^7) and pathogenicity (%) of *B. bassiana*

Treatments	Spore Production x 10^7	Mortality (%)
Rice husk	5.25	50.00
Saw dust	0.19	12.00
Rice bran	5.03	50.00
Rice husk + dextrose	6.25	86.67
Saw dust + dextrose	4.31	32.33
Rice bran + dextrose	6.33	83.51
CD ($p = 0.05$)	0.46	2.67

Table 2: Effect of medium on mean spore production of *B. bassiana* (AAU-09) and mean pathogenicity (%)

Treatments	Spore Production x 10^7	Mortality (%)
Coconut water	3.80	53.33
Potato Broth	3.52	40.00
Rice gruel (Rice cooked water)	3.27	36.67
CD ($p = 0.05$)	0.20	1.05

Table 2 shows the mean spore prduction of *B. bassiana* in liquid media and mean percent virulence of the spores' poduced in those media. Out of the three media tested, coconut water served as the best medium in terms of spore production and pathogenicity. Abundance of glucose and mineral present in coconut water may enhance the growth and spore production of *B. bassiana* (Dangar *et al.,* 1991; Sahayaraj *et al.*, 2008). Though, coconut water is proved to be a rich medium for mass production of *B. bassiana* fulfilling all the characteristics necessary for this purpose, availability and cost may prevent it to become commercially viable medium for any biocontrol agent in raw form. It is known that some liquid media, such as rice wash (Sahayaraj *et al.*, 2008), rice gruel (Sudharma and Peethambaran, 2000) produce mycotoxin and quality spores having greater viability and virulence (Akbar *et al*., 2005).

Nutrient supplementation is also an important area of entomopathogenic fungal nutrition; as such experiments conducted in this respect were presented in Table 3. Potato broth supplemented with peptone, a nitrogen source supported maximum sporulation (5.16 x 10^7 conidia/ml) having virulence (66.67%). Addition of peptone in all the liquid media brought about similar changes as observed in this study. Pham *et al*. (2009) observed maximum spore yield (16.5 x 10^7 spores/ml) in corn meal by adding 2% peptone as nitrogen source. Likewise, dextrose, yeast extract and synthetic chitin

Table 3: Effect of supplements on spore production and pathogenecity (%)

Treatments	Spore Production x 10^7	Mortality (%)
Potato broth + 2% casein	3.76	53.33
Potato broth + 2% synthetic chitin	4.24	56.67
Potato broth + 2% yeast extract	4.14	56.67
Potato broth + 2% dextrose	4.32	66.67
Potato broth + 2% peptone	5.16	66.67
Potato broth + 2% starch	4.00	46.33
Potato broth + 2% $MgSO_4$	3.76	43.33
Potato broth + 2% $CaCO_3$	3.81	43.33
Potato broth + 2% manitol	3.84	42.00
Rice gruel + 2% casein	3.52	40.00
Rice gruel + 2% synthetic chitin	3.68	53.33
Rice gruel + 2% yeast extract	3.60	56.67
Rice gruel + 2% dextrose	4.20	53.33
Rice gruel + 2% peptone	4.26	50.60
Rice gruel + 2% starch	3.52	39.90
Rice gruel + 2% $MgSO_4$	3.60	46.67
Rice gruel + 2% $CaCO_3$	3.52	40.00
Rice gruel + 2% manitol	3.60	43.33
Coconut water + 2% casein	3.44	43.33
Coconut water+ 2% synthetic chitin	3.84	50.00
Coconut water + 2% yeast extract	3.52	43.33
Coconut water+ 2% dextrose	4.16	56.67
Coconut water+ 2% peptone	4.20	56.67
Coconut water + 2% starch	3.44	40.00
Coconut water + 2% $MgSO_4$	3.52	46.67
Coconut water + 2% $CaCO_3$	3.68	40.00
Coconut water + 2% manitol	3.68	43.33
CD ($p = 0.05$)	0.26	1.10

also enhanced sporulation. Mazumder *et al.* (1995) reported potato broth supplemented with 2% dextrose showed maximum spore production of *B. bassiana* (4.3 x 10^7 conidia/ml) causing 76.30% mortality to the adults of *D. armigera.* Potato dextrose liquid broth medium and Richard's medium were best media for mycelia growth (Manisegarane and Letchoumanane, 1996). Similarly, bean broth, rice broth and potato broth were shown as good liquid media for *B. bassiana* spore production, and it was interesting to note that 96% of these spores germinated (Batista-Filho *et al*., 1985). Thus, it is clear that liquid media need supplementation with carbon and nitrogen sources to enhance growth and potentiality of *B. bassiana*. From this study it is clear that though *B. bassiana* can grow in a wide variety of agricultural products of both solid as well as liquid state, quantity and quality of nutrients favour diferentially on sporogenesis and mycotoxin production (Latge and Sanglier, 1985), sometimes these being highly specific to carbohydrates

(Campbell *et al*., 1983), amino acids (Campbell *et al*., 1978) and peptones (Barnes *et al.,* 1975). This is an area which requires further studies. Potentiality of conidia grown in different media may vary from one medium to another due to the variability in production of viable conidia and biochemical constituents of conidia. The variation in virulence of pathogen and susceptibility of the host are dependent upon several intrinsic factors in the host pathogen interaction including capacity of the pathogen to produce lethal dose of toxins responsible for causing pathogenesis in the host and also capacity of the host to counteract the same, however, optimization of culture medium through manipulation of nutrients and physical environment can enhance virulence of *B. bassiana* to a great extent (Samsinakova *et al*., 1981).

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