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Mass production of entomopathogenic fungi using agricultural products and by products

K. Sahayaraj and S. Karthick Raja Namasivayam

Crop Protection Research Centre, St.Xavier’s College, Palayamkottai 627002, Tamil Nadu, Department of Biotechnology and Bioinformatics, SRM University Ram, Apuram campus, Chennai 89, India.

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Various agricultural products and by products such as grains, vegetable wastes, seeds, rice husk, saw dust and liquid media such as coconut water, rice and wheat washed water and rice cooked water were evaluated for mass production of three entomopathogenic fungi; *Beauveria bassiana*, (Bals.) Vuil. *Paecilomyces fumosoroseus* (Wize) Brown and Smith and *Verticillium lecanii*. (Zimm) Viegas. Among the grains, wheat supported maximum spore production for *B. bassiana* while sorghum recorded maximum spore production in *P. fumosoroseus* and *V. lecanii*. Similarly carrot, jack seeds and ladies finger also supported good growth and sporulation of all the three tested fungi. Coconut water supported maximum growth and sporulation.

Key words: Entomopathogenic fungi, mass production, agricultural products.

INTRODUCTION

Biopesticides based on bacteria, viruses, entomopathogenic fungi and nematodes are often 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 mycoinsecticide based on *Beauveria bassiana* (Balsamo) Vaillem in (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 a good quality inoculum is an essential component of the biocontrol programme. The production of entomopathogens may be taken up by the following methods based on the quantity of the product desired: 1) relatively small quantities of the inoculum for laboratory experimentation and field-testing during the development of mycopesticide and 2) 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) supply fungal pathogens by this method 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 pro-duced in liquid culture, which is further utilized for inoculating the solid substrate(s) for conidia production (Burges and Hussey, 1981). The present study was undertaken to evaluate grains such as rice, wheat, raghi, sorghum, pearl miket and maize at different tempera-tures, and liquid media such as rice washed water, wheat washed water, coconut water and rice cooked water and naturally available solid media such as carrot, ladies finger, jack seeds, rice husk, and saw dust for the mass production of *B. bassiana*, *P. fumosoroseus* and *V. lecanii*.

MATERIALS AND METHODS

Entomopathogenic fungal culture

*B. bassiana* and *P. fumosoroseus* were isolated from the diseased...
caterpillar of _S. litura_ collected from the groundnut fields in Tamil Nadu, India. The diseased larvae showed white colour for _B. bassiana_ and slight reddish mycelial surface growth for _P. fumosoroseus_. The diseased larvae were collected in screw cap vials (18 x 4 mm) and brought to the laboratory for further studies. The diseased larvae were surface sterilized with 0.1% mercuric chloride for few seconds and then thoroughly washed with sterilized double distilled water. The excess water was removed by keeping the diseased larvae on Whatman filter paper No. 1. The diseased larvae were then cut into small pieces with the help of sterile blade and the bits were aseptically transferred on to the sabourand maltose agar enriched with 1% yeast extract (SMYA) 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). The identified fungi were _B. bassiana_ (Balsoma) Vuillemin and _P. fumosoroseus_ (Wize) Brown et Smith, _V. lecanii_ (Zimm). Viegas was obtained from Microbial Type Culture Collection (MTCC 915) Chandigarh, India. All the cultures were maintained on SMYA and PDA slants.

**Whole grain media**

Six whole grains viz rice, wheat, raghi, sorghum, pearl millet and maize were used for estimating the sporulation of _B. bassiana, P. fumosoroseus_ and _V. lecanii_ at 28°C. 100 g of each grain was washed well and soaked in water overnight except rice and pearl millet which were soaked for 2 - 3 h prior to starting the experiments. The excess water was drained by decanting and shade drying it for half an hour to further remove the excess moisture. Three replications were maintained for each grain. The grains were packed separately in individual 500 ml bottle for _P. fumosoroseus_ and _V. lecanii_ and 500 ml conical flask for _B. bassiana_ separately. They were plugged with cotton wool and auto calved at 15 psi for 1 h. After cooling, 1 ml of the spore suspension of fungal pathogen was inoculated into each bottle, separately. All these procedures were done under laminar air flow chamber. They were incubated in BOD incubator at 26, 28, 30 and 32°C separately for 15 days. To avoid clumping, after 7 days of inoculation, the flasks and bottles were shaken vigorously to separate the grain and to break the mycelial mat. After 15 days of incubation, 10 g homogenous grain sample drawn from each replicate uniformly sperulating bottle/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 spores 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**

Liquid media; rice wash water, wheat wash water, coconut water and rice cooked water were evaluated for the growth and sporulation of three tested fungi. 100 ml of each medium was poured in 250 ml capacity conical flasks and autoclaved at 15 psi pressure for 20 min. Five flasks of each medium was inoculated with 1 ml of spore suspension of each fungi separately and incubated at 28°C for 15 days. The spore suspension was subjected to spore counting and it was carried out as described in the previous section.

**Solid media**

Non-synthetic solid media; carrot, ladies finger, jack seeds, rice husk, and saw dust were tested. 100 g of each solid material was taken in 500 ml conical flasks, inoculated with 1 ml of spore suspension and incubated at 28°C in BOD incubator for 15 days. The spore count was made as mentioned earlier.

**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**

In the present study, several naturally available substrates of both solid and liquid media were tested for mass multiplication of _B. bassiana, P. fumosoroseus_ and _V. lecanii_. 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 bio-control programme. For a successful integrated pest management programme, the agents like the entomopathogenic fungi should be amenable to easy and cheap mass multiplication.

**Grains**

The results (Table 1) showed that among the grains tested, _B. bassiana_ spore production was significantly higher on wheat (11.76 x 10^10). The spore count recorded at the respective temperatures on rice was slightly lower than wheat. But the statistical composition between the wheat and rice were insignificant. In the case of _P. fumosoroseus_, sorghum recorded the highest spore count of 10.37 x 10^11/100 g. Sorghum was found to be ideal for the mass production of _V. lecanii_; it recorded 11.31 x 10^10 spores/100 g. Pearl millet was found to be the next best media for the spore production (10.17 x 10^10 spores/100 g). The lowest spore production was recorded in maize (Table 1) which was statistically insignificant (P > 0.05, P = 0.071).

Grains are cheap, easily available and act as best neutritive media for the mass multiplication of many micro and macro organisms. According to Ibrahim and Low (1993) and Sharma et al. (2002), rice was found to be the 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_. In the case of _V. lecanii_, sorghum was found to be the ideal cereal for mass production, which is in confirmation with the findings of Lakshmi et al. (2001). Lowest spore production was recorded in maize at all temperatures.
Table 1. Spore \((X \times 10^8)\) and biomass production (g) of entomopathogenic fungi on various substrates.

<table>
<thead>
<tr>
<th>Media</th>
<th>Spore count ((X \times 10^8))</th>
<th>Biomass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bb</td>
<td>Pf</td>
</tr>
<tr>
<td>Grains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td>11.24</td>
<td>8.76</td>
</tr>
<tr>
<td>Wheat</td>
<td>11.76</td>
<td>9.71</td>
</tr>
<tr>
<td>Sorghum</td>
<td>10.24</td>
<td>10.37</td>
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<tr>
<td>Pearl millet</td>
<td>9.78</td>
<td>10.26</td>
</tr>
<tr>
<td>Raghi</td>
<td>10.72</td>
<td>10.17</td>
</tr>
<tr>
<td>Maize</td>
<td>9.44</td>
<td>10.11</td>
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<tr>
<td>Non synthetic liquid media</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coconut water</td>
<td>12.10</td>
<td>10.17</td>
</tr>
<tr>
<td>Rice cooked water</td>
<td>10.21</td>
<td>2.12</td>
</tr>
<tr>
<td>Rice wash water</td>
<td>8.76</td>
<td>6.75</td>
</tr>
<tr>
<td>Wheat wash water</td>
<td>8.17</td>
<td>7.31</td>
</tr>
<tr>
<td>Non synthetic solid media</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrot</td>
<td>10.76</td>
<td>9.12</td>
</tr>
<tr>
<td>Jack seeds</td>
<td>9.81</td>
<td>6.12</td>
</tr>
<tr>
<td>Ladies finger</td>
<td>9.17</td>
<td>5.17</td>
</tr>
<tr>
<td>Rice husk</td>
<td>6.31</td>
<td>1.21</td>
</tr>
<tr>
<td>Saw dust</td>
<td>0.17</td>
<td>1.76</td>
</tr>
</tbody>
</table>

Bb = Beauveria bassiana; Pf = Paecilomyces fumosoroseus; VI = Verticillium lecanii.

Liquid media

Among the liquid media, coconut water produced significantly higher spore production in all tested fungi. \((5.27 \times 10^8, 10.17 \times 10^8 \text{ and } 5.27 \times 10^8 \text{ spores/100 ml})\) and 0.51, 0.71 and 0.51 g of biomass production was recorded in B. bassiana, P. fumosoroseus and V. lecanii, respectively. The other media also supported spore production in all tested fungi (Table 1). Dangar et al. (1991) also observed similar findings in M. anisepliae. 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 all the three tested fungi. Patel et al. (1990) reported that the purified rice wash water gave the best spore count in M. anisepliae.

Solid media

Among the seeds, vegetables and solid wastes such as rice husk and saw dust tested, B. bassiana recorded the maximum spore production \((10.76 \times 10^8 \text{ spores/100 g with } 0.73 \text{ g})\) on carrot followed by jack seeds \((P < 0.005, P = 0.006)\). Carrot waste also supported maximum spore as well as biomass production of P. fumosoroseus (Table 1). In V. lecanii, jack seeds produced significantly \((4.11 \times 10^8 \text{ higher spores } (P < 0.05)\) followed by the ladies finger. Carrot also recorded \(2.17 \times 10^8 \text{ spores/100 g and } 0.24 \text{ g of biomass production and } 1.27 \times 10^8 \text{ spores/100 g was recorded in rice husk. However, V. lecanii pro-}

REFERENCES


