EVALUATION OF DIFFERENT SUBSTRATES FOR MASS MULTIPLICATION OF BEAUVERIA BASSIANA (BALSAMO) VUILLEMIN

Gokil Prasad Gangwar*
Department of Plant Pathology,
G.B. Pant Univ. of Agric. & Tech., Pantnagar 263 145, India

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ABSTRACT

Different substrates viz. sorghum, maize, pearl millet, barnyard millet (Echinocloa frumentacea) and cow dung were evaluated for mass multiplication of Beauveria bassiana (Balsamo) Vuillemin. Sorghum grains were found to be best and exhibited maximum population ($6.32 \times 10^9$ cfu) followed by maize (each grain broken into 8-10 pieces) with $5.77 \times 10^9$ cfu and barnyard millet ($5.08 \times 10^9$ cfu), respectively. Addition of 1 per cent sucrose increased the growth and sporulation of $B$. bassiana in sorghum ($6.65 \times 10^9$ cfu) and barnyard millet ($6.24 \times 10^9$ cfu). However, population of $B$. bassiana was significantly reduced in sorghum and barnyard millet grains when amended with 2 per cent sucrose.

Key words: Beauveria bassiana, Cow dung, Grain, Mass multiplication, Sucrose.

INTRODUCTION

The insect/pests cause huge crop losses. Since ancient time Microbial control is the most promising approach to manage insect pest with little disturbance to environment. Entomopathogens offer several advantages i.e. no pollution problems and health hazards, more stable without any development of resistance in insect pest, and cause little or no disturbance in ecological balance. The white muscardine fungus Beauveria bassiana has been recorded to infect almost 500 host insect species belonging to orders Lepidoptera, Hemiptera, homoptera, Coleoptera, Hymenoptera, Orthoptera and Diptera (Moore and Prior, 1996). For successful utilization of entomopathogens in management of insect pest, selection of virulent strain, production of large quantity of spores, pathogenicity against various pest species, selection of suitable media, formulation procedures and condition for storage are important (McCoy et al., 1975; Alyoshima et al., 1976). High doses ($10^{14}-10^{13}$ spores ha$^{-1}$) of entomopathogenic fungi are required for controlling insect pest in the field (Bartlett and Jaronski, 1988). Therefore, mass production of entomopathogenic fungi is an essential component for their utilization in the IPM. Major obstacle in the mass multiplication of $B$. bassiana is its slow growth rate and non-availability of suitable substrate. Therefore, the present study was carried out to evaluate different inexpensive and easily available substrates viz. sorghum, maize, pearl millet, barnyard millet (Echinocloa frumentacea) and cow dung amended with different concentration of sucrose, for mass multiplication of $B$. bassiana.

MATERIALS AND METHODS

Present study was carried out in Bio-control Laboratory, Department of Plant Pathology, G.B. Pant University of Agriculture and Technology, Pantnagar. Beauveria bassiana was multiplied on sorghum, maize, pearl millet, barnyard millet (Echinocloa frumentacea), and cow dung. Sorghum, barnyard millet, maize and pearl millet grains were soaked overnight in sucrose solution at the concentration 0, 0.5, 1.0 and 2.0 per cent strength. Maize grains were broken into 4-5 and 8-10 pieces. Excess water was drained out and soaked grains were weighed 50 g and filled in 250 ml conical flasks. Air dried cow dung was weighed 20 g and filled in 250 ml conical flasks. Moisture was added as 100 per cent v/w by adding sucrose solution at the concentration 0, 0.5, 1.0 and 2.0 per cent. These flasks were plugged with non-absorbent cotton and sterilized by autoclaving at 121.6 °C at 15 lbs psi for

*Corresponding author’s e-mail: gokil_prasad@rediffmail.com
15 minutes. All flasks were inoculated with 5 mm disc taken from periphery of actively growing 12 days old culture of B. bassiana and incubated in BOD incubator at 28±1 °C temperature. Four replications were maintained for each treatment. Growth and sporulation was observed at 20th days after inoculation. Sporulation in was measured by suspending 1 g colonized substrate from each replication. This suspension was serially diluted with adding 9 ml sterilized water to 1 ml conidial suspension. From 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} and 10^{-10} dilutions 1 ml suspension and 20 ml melted PDA was poured into Petri dishes and mixed by rotating dishes. Three replications were taken from each concentration. Sporulation was observed as colony forming units (cfu) per gram colonized substrate.

**RESULTS AND DISCUSSION**

All the substrates tested i.e. sorghum, maize, pearl millet, barnyard millet, and cow dung were supported good growth of B. bassiana. Maximum sporulation of B. bassiana was exhibited by sorghum (6.32 × 10^9 cfu g^{-1}) followed by maize (grain broken in to 8-10 pieces) by 5.77 × 10^9 cfu g^{-1} and barnyard millet (5.08 × 10^9 cfu g^{-1}). Other substrates like pearl millet, maize (grain broken in to 4-5 pieces) and cow dung supported good growth where population recorded by 3.27 × 10^9, 3.09 × 10^9 and 2.11 × 10^9 cfu g^{-1}, respectively. These findings are confirmatory with Sharma et al., (2002) which reported that multiplication of B. bassiana isolates was best on cowpea grains (3.98 × 10^8 conidia g^{-1}) supported maximum sporulation of Bb-3 isolate followed by chickpea grains (3.78 × 10^8 conidia g^{-1}). However, rice grains were best for sporulation of Bb-1 and Bb-2 isolates (9.77 × 10^7 and 7.56 × 10^7 conidia g^{-1}) and sporulation on sorghum, maize and pearl millet ranged between 1.02 × 10^6 to 2.6 × 10^8 conidia g^{-1} for different isolates of B. bassiana. Studies of Rammohana (1989) revealed that cooked rice and carrot broths are suitable for multiplication of B. bassiana. Somasekher et al. (1998) reported that multiplication of B. bassiana and B. brongniartii was significantly high on sorghum grains as compared to the sugarcane byproducts. Nelson et al. (1996) achieved maximum conidial yield of B. bassiana on rice. Patel (1997) reported that pearl millet was the best medium and Dayakar (1999) observed that finger millet, pea and pearl millet were good substrate for growth of B. bassiana. In the present study, population of sporulation on maize was affected by size of broken grain in pieces. Smaller grains were supported better sporulation of B. bassiana than bigger one.

Further, addition of 0.5 per cent sucrose in barnyard millet (5.37 × 10^9 cfu g^{-1}) exhibited significant increase in sporulation as compared to without sucrose (5.08 × 10^9 cfu g^{-1}). Whereas, sporulation in all other substrates amended with 0.5 per cent sucrose was statistically equal to without sucrose. Increase in sucrose concentration from 0.5 per cent to 1.0 per cent showed significant increase in sporulation with sorghum 6.29 × 10^9 to 6.65 × 10^9 cfu g^{-1}, barnyard millet 5.37 × 10^9 to 6.24 × 10^9 cfu g^{-1} and maize (grains broken in to 4-5 pieces) 3.13 × 10^9 cfu to 3.53 × 10^9 cfu g^{-1}, respectively. Patel et al. (1990) also recorded maximum yield of conidia on a medium containing sucrose, yeast extract and basal salt. Similarly, Rambach (1998) reported highest conidial production and hyphal growth of B. bassiana in the medium containing 2 per cent sucrose and 0.5 per cent yeast extract.

Increase in sucrose concentration from 1 per cent to 2 per cent not exhibited significant increase in sporulation on all the substrates. However, a

### TABLE 1: Average colony forming unit (× 10^9) per gram substrate colonized by Beauveria bassiana amended with different sucrose concentration

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Sucrose concentration (%)</th>
<th>0.0</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum</td>
<td></td>
<td>6.32</td>
<td>6.29</td>
<td>6.65</td>
<td>5.91</td>
</tr>
<tr>
<td>Pearl millet</td>
<td></td>
<td>3.27</td>
<td>3.31</td>
<td>3.34</td>
<td>3.22</td>
</tr>
<tr>
<td>Barnyard millet</td>
<td></td>
<td>5.08</td>
<td>5.37</td>
<td>6.24</td>
<td>6.00</td>
</tr>
<tr>
<td>Maize (4-5 pieces)</td>
<td></td>
<td>3.09</td>
<td>3.13</td>
<td>3.53</td>
<td>3.36</td>
</tr>
<tr>
<td>Maize (8-10 pieces)</td>
<td></td>
<td>5.77</td>
<td>5.86</td>
<td>6.03</td>
<td>5.59</td>
</tr>
<tr>
<td>Cow dung</td>
<td></td>
<td>2.11</td>
<td>2.15</td>
<td>2.16</td>
<td>2.10</td>
</tr>
<tr>
<td>CD (P = 0.05)</td>
<td>Substrate (A)</td>
<td>0.23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sucrose concentration (B)</td>
<td>0.18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A × B</td>
<td>0.44</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Mean of four replication
significant reduction in sporulation was observed with sorghum and barnyard millet amended with 2.0 per cent sucrose by $6.65 \times 10^9$ and $6.24 \times 10^9$ cfu g$^{-1}$, respectively as compared to 1.0 per cent sucrose concentration ($5.91 \times 10^9$ and $6.00 \times 10^9$ cfu g$^{-1}$, respectively) and this may be due to increased hyphal growth of B. bassiana at higher sucrose concentration as indicated by Rambach (1998) who reported that higher amount of sucrose enhanced the hyphal growth of B. bassiana. Maximum yield of hyphal bodies of B. bassiana was recorded with higher amount of sucrose and yeast extract in the medium (Patel et al., 1990).

In the present study, maximum sporulation of B. bassiana was exhibited by sorghum amended with 1 per cent sucrose ($6.65 \times 10^9$ cfu g$^{-1}$) and barnyard millet amended with 1 per cent sucrose ($6.24 \times 10^9$ cfu g$^{-1}$). There was no significant effect of addition of sucrose on pearl millet, cow dung and maize when each grain broken in to 8-10 pieces. However, population of sporulation on maize grain was affected by size of grain pieces and smaller grains were supported better sporulation.

REFERENCES


