Occurrence of Entomogenous Fungi, *Beauveria bassiana* and *Fusarium oxysporum* on Teak Leaf Skeletonizer, *Eutectona machaeralis* and Their Pathogenicity

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Abstract: Leaf skeletonizer, *Eutectona machaeralis* (Walker) (Lepidoptera: Pyralidae), is a major pest of teak (*Tectona grandis* L.f.), responsible for severe defoliation in nurseries, plantations and natural forests. During the recent survey (2008-2009) in teak forests of Madhya Pradesh, larvae of this pest collected from Kundam and Bargi, Jabalpur Forest Division, were found to be infested by fungi in nature at the time of epidemic outbreak. The infested fungi were isolated on Potato Dextrose Agar (PDA) medium in laboratory and identified as *Beauveria bassiana* (Bals.-Crv.) Vuill. (Hypocreales: Cordycipitaceae) on larvae collected from Kundam and *Fusarium oxysporum* Schlecht. (Hypocreales: Nectriaceae) on larvae collected from Bargi. The isolated fungi were tested for their pathogenicity against the early last instar larvae of this insect in laboratory. Results revealed that the fungi are pathogenic to induce larval mortality when applied on host plant leaves and larvae together of the insect pest. Among the two pathogens tested, *B. bassiana* was recorded to be more effective in larval killing than *F. oxysporum*. The highest concentration of 1.7 x 10^7 and 1.1 x 10^7 spore/ml of *B. bassiana* and *F. oxysporum* induced maximum of 94 and 52% larval mortality, respectively within five days after inoculation and was found to be significantly (P<0.05) different from other treatments including control.

Key words: *Eutectona machaeralis* · *Beauveria bassiana* · *Fusarium oxysporum* · Infestation pathogenicity

INTRODUCTION

Teak (*Tectona grandis* L.f.) (family Verbenaceae) is a potential tree species of high quality tropical timber [1, 2]. Leaf skeletonizer, *Eutectona machaeralis* (Walker) (syn. *Pyrausta machaeralis* [3], *Hapalia machaeralis* [4]), is the most pernicious pest of teak responsible for epidemic defoliation regularly in nurseries, plantations and natural forests of all teak growing areas [4-10], including Madhya Pradesh [11-13]. Larvae of this insect feed only on the fleshy leaf tissues, leaving all the veins intact, thus it affects adversely the growth and vigour, besides causing certain abnormalities, resulting in both qualitative and quantitative loss in timber production [4,14].

The first record of infestation of larvae of *E. machaeralis* by a fungal pathogen, *Beauveria bassiana* (Bals.-Crv.) Vuill. has been reported from Kunsi village, Shimoga, Karnataka [15]. Singh and Misra [16] have isolated *B. bassiana*, from dead larvae of *E. machaeralis*, collected from teak forests of Melghat, Maharashtra. *B. bassiana*, has been found to be highly infectious to kill the larvae of *E. machaeralis* [17]. Sharma and Joshi [18] have isolated two fungal pathogens, *B. bassiana* and *Fusarium oxysporum* Schldl., from the larvae of leaf skeletonizer collected from teak forests of Kanjai and Jabalpur of Madhya Pradesh respectively. Apart from these, nothing is known about the entomopathogenic fungi, *B. bassiana* and *F. oxysporum* attacking teak leaf skeletonizer [19-22].

The present study has contemplated to describe the occurrence of two entomogenous fungi, *B. bassiana* and *F. oxysporum* infesting the larvae of *E. machaeralis* in teak forests of Madhya Pradesh, India and their pathogenicity against the target insect pest in laboratory.

MATERIAL AND METHODS

To investigate the natural enemies of teak leaf skeletonizer, *E. machaeralis*, periodical surveys were conducted in teak forests of Madhya Pradesh through out the year 2008-2009. During the survey, larvae of this
insect pest were found to be infested by fungi in nature at the time of epidemic outbreak occurred in the month of September, 2009 and October, 2009 in teak forests of Kundam and Bagri, Jabalpur Forest Division (Madhya Pradesh), respectively. These infested larvae were collected and studied. The infested fungus was isolated on Potato Dextrose Agar (PDA) medium at 30°C in B.O.D. The method was same as described earlier for some other entomopathogens [18]. The growth characters of fungus were recorded. For morphological studies, a small piece of mycelium with spores were taken from the seven days old active culture and spread out on the microscopic slide. A drop of lactophenol and cotton blue was added and covered by a glass cover slip. The shape and size of conidiophores and conidial arrangement were noted and identification of entomopathogenic fungus was done with the help of available monographs [23-25].

To confirm the field observations, the isolated native fungi were tested for their pathogenicity against the larvae of this insect pest collected from the heavily infested teak trees planted in the campus of this Institute and were reared in the insectary. Fresh leaves of host plants were provided daily to insects as food. One day old last instar larvae of weight ranged from 0.021-0.030 g (mean 0.026 ± 0.006 g) were separated out and preconditioned by starvation for about one hour.

The desired concentrations of fungi were prepared separately in laboratory by diluting with distilled water. The water suspension was then uniformly sprayed separately on host plant leaves containing preconditioned 10 larvae of E. machaeridis by hand atomizer. The sprayed host plant leaves and larvae were then transferred to clean marked beaker of one liter capacity lined at the bottom with a piece of filter paper and covered with muslin cloth. Similarly, the untreated host plant leaves and larvae served as control. In all, five replications of each concentration were made. The observations on the number of dead and moribund larvae after 1, 2, 3, 4 and 5 days of treatment were recorded. The data on larval mortality recorded after 5 days of treatment were subjected to ANOVA (CRD) after angular transformation to conform to normal distribution [26]. The data of trial were also corrected by using Abbott's formula [27]. The experiments were conducted in laboratory under the prevailing environmental conditions during the month of September 2008 (temperature 31.37°C and RH 56.70%) and October (temperature 30.35°C and RH 55.67%).

RESULTS AND DISCUSSION

The pathogen isolated from the larvae of leaf skeletonizer, E. machaeridis, collected from Kundam teak forest was identified as Beauveria bassiana (Bals.-Criv.) Vuill. (Ascomycetes: Sordariomycetes: Hypocreales: Cordycipitaceae) (Fig. 1), due to the presence of following characters given by Subramanian [23], Brady [24] and Samson et al. [25], which are as follows: mycelium white or slightly coloured with a white fluffy to powdery appearance, conidiophores single, irregularly grouped or in verticillate clusters, inflated at the base, tapering to a slender spore bearing portion which appears zigzag after several spores are produced, conidia (sympodiospores) hyaline, rounded to ovoid, 1-celled, dry and borne singly on small stigmatic. Similarly, fungal pathogen isolated from the larvae collected from Bagri teak forest was identified as Fusarium oxysporum Schlecht. (Ascomycetes: Sordariomycetes: Hypocreales: Nectriaceae) (Fig. 2), due to the presence of following characters given by Subramanian [23], Brady [24] and Samson et al. [25], which are as follows: the mycelium colony on PDA appeared white to light pinkish violet to pale, floccose and about 4.5 cm in diameter in seven days. The chlamydospore remained smooth walled, globose, formed singly or paired, intercalary and terminal or on short lateral branches, conidia two types, microconidia unicellular, ellipsoid, falcate, straight, abundant, variable, measuring 4.5-10.0 x 2.0-3.5 μm in size and

![Fig. 1: Dead larvae of leaf skeletonizer, E. machaeridis, infested by a fungal pathogen, B. bassiana and its isolated culture](image-url)
Fig. 2: An isolated fungal pathogen, *F. oxysporum*, collected from the dead larvae of leaf skeletonizer, *E. machaeris.*

Fig. 3: Data of percentage corrected larval mortality in *E. machaeris* due to treatment of *B. bassiana* in laboratory

Fig. 4: Data of percentage corrected larval mortality in *E. machaeris* due to treatment of *F. oxysporum* in laboratory

from 2-5 and 1.2%.

To confirm the field observations, the isolated fungi, *B. bassiana* and *F. oxysporum* were tested for their pathogenicity against the larvae of this insect in laboratory and the results are summarized in Table 1 and 2, respectively. On the basis of pathogenicity tests made against the target pest, results exhibited larval mortality due to treatment of *B. bassiana* and *F. oxysporum*, when applied on host plant leaves and larvae together, which was significantly (P<0.01) different among their mean values (F=86.663, P<0.01; d.f. 2, 12 for *B. bassiana* and F=35.284, P<0.01; d.f. 2, 12 for *F. oxysporum*). The highest concentration of 1.7 x 10^7 and 1.1 x 10^7 spore/ml of *B. bassiana* and *F. oxysporum* induced maximum of 94 and 52% larval mortality respectively within five days after inoculation and found to be significantly (P<0.05) different from other treatments including control. Further, there was a decline of larval mortality in respect of lowering concentration of *B. bassiana* and *F. oxysporum* as evident from the data based on percentage corrected larval mortality (Fig. 3 and 4). Among the two pathogens tested, *B. bassiana* was recorded to be more effective in inducing larval mortality when compared with *F. oxysporum.*

Table 1: Data (mean) on percentage larval mortality in *E. machaeris* obtained due to treatment of *B. bassiana* on leaf and larvae together in laboratory

<table>
<thead>
<tr>
<th>Treatment concentration (spore/ml)</th>
<th>Indices</th>
<th>Larval mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.7 x 10^7</td>
<td>T1</td>
<td>94.00 (81.00)</td>
</tr>
<tr>
<td>1.6 x 10^7</td>
<td>T2</td>
<td>60.00 (50.87)</td>
</tr>
<tr>
<td>Control *</td>
<td>T3</td>
<td>2.00 (3.69)</td>
</tr>
<tr>
<td>Sem</td>
<td></td>
<td>4.186</td>
</tr>
<tr>
<td>C. D. at 1%</td>
<td></td>
<td>13.005</td>
</tr>
<tr>
<td>C. D. at 5%</td>
<td></td>
<td>13.900</td>
</tr>
</tbody>
</table>

*Without any treatment. Angular transformed values are inside parentheses.

Table 2: Data (mean) on percentage larval mortality in *E. machaeris* obtained due to treatment of *F. oxysporum* on leaf and larvae together in laboratory

<table>
<thead>
<tr>
<th>Treatment concentration (spore/ml)</th>
<th>Indices</th>
<th>Larval mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 x 10^7</td>
<td>T1</td>
<td>52.00 (40.15)</td>
</tr>
<tr>
<td>1.3 x 10^7</td>
<td>T2</td>
<td>39.00 (32.97)</td>
</tr>
<tr>
<td>Control *</td>
<td>T3</td>
<td>4.00 (7.37)</td>
</tr>
<tr>
<td>Sem</td>
<td></td>
<td>3.328</td>
</tr>
<tr>
<td>C. D. at 1%</td>
<td></td>
<td>14.344</td>
</tr>
<tr>
<td>C. D. at 5%</td>
<td></td>
<td>10.220</td>
</tr>
</tbody>
</table>

*Without any treatment. Angular transformed values are inside parentheses.

developed on simple phialids arising laterally on the hyphae, macroconidia generally three septate, gradually or abruptly towards both ends and measured 24.0-52.0 x 3.5-5.0 um in size. Earlier, the occurrence of these two fungal pathogens, *B. bassiana* and *F. oxysporum*, on the larvae of *E. machaeris* has already been reported [15-18].

Field observations revealed that larval infestation of *E. machaeris* due to the fungus, *B. bassiana* and *F. oxysporum* in teak forests of Madhya Pradesh varied...
Earlier, Rajak et al. [17] have reported that B. bassiana, is highly infectious to kill the larvae of E. machaeris and LC₅₀ value for B. bassiana has been worked out to be 2.9 x 10⁶ conidia for 3rd instar larvae and it increases with growth and development of larval instar.

Kill of insects due to fungus is known from very ancient times. Several species of fungi are known to infest insect larvae which ultimately cause their death [28] and often act as natural control agents that limit insect population [29], including forest insects [30]. It has mentioned that fungal conidia can germinate on the insect cuticle and produced specialized structures that allow the fungus to penetrate the cuticle and enter the insect's body [31, 32]. In most instances, as fungal infections progress, infected insects are killed by fungal toxins (mycotoxin), not by chronic effects of parasitism [29]. The same may be the cause of larval mortality in E. machaeris, due to treatment of B. bassiana as well as F. oxysporum.

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REFERENCES


