Bioefficacy of efficient entomopathogenic fungus against branch canker pathogen (*Macrophoma theicola*) in tea plantations of southern India

J MAREESWARAN¹, P NEPOLEAN², R JAYANTHI³, R PREMKUMAR SAMUEL ASIR⁴ and B RADHAKRISHNAN⁵

University of Calicut, Malappuram, Kerala 673 635

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ABSTRACT

Three branch canker pathogens, viz. NBCHE-6, UPA-61 and VPM were isolated from different tea growing districts of south India and four entomopathogenic fungus, viz. *Beauveria bassiana*, *Paecilomyces lilacinus*, *Lecaniiicillium lecannii* and *Paecilomyces fumosoroseus* were procured from the microbial type culture collection and gene bank (MTCC), Chandigarh. *In vitro* studies revealed that *Beauveria bassiana* showed highest antagonistic effect against NBCHE-6 (64.22) followed by *Paecilomyces fumosoroseus* against UPA-61(56.66). *Paecilomyces lilacinus* significantly controlled VPM (54.66), while *Lecaniiicillium lecannii* showed insignificantly control against VPM (47.33). While *Beauveria bassiana* and *Paecilomyces lilacinus* coiled around and shrink branch canker pathogen, *Lecaniiicillium lecannii* breaks into branch canker hyphae and *Paecilomyces fumosoroseus* produces more spore to kill branch canker. In culture filtrate studies, *Paecilomyces fumosoroseus* and *Paecilomyces lilacinus* showed maximum control of VPM (68.44) and UPA-61 (65.59). *Beauveria bassiana* also showed significant control of two isolates VPM and UPA-61 (54.44). *Lecaniiicillium lecannii* showed least control of VPM (30.44). This study concludes that entomopathogens can significantly control branch canker pathogen (*Macrophoma theicola*).

Key words: Antagonist, Entomopathogens, Hyperparasitism, *Macrophoma* sp, Non-volatile culture filtrate, Tea

Tea, being a perennial crop, provides a stable environment for a number of pests and diseases. Tea plantations suffer heavily from the infestation. Pests, pathogens and weeds are important factors limiting the productivity and quality of processed tea. Stem diseases are important as they stagnate crop and sometimes kill the bush. Pruning operation in tea increases the risk of stem diseases since it exposes the wood tissue to parasitic cuts and saprophytic fungi. Branch canker in tea was first noticed in southern India in 1899, but in Sri Lanka the disease was recorded in 1904 (Petch 1923). The pathogen *Macrophoma* sp. is a wound pathogen. The fungal pathogen can easily enter through prune cuts or tissues damaged by sun-scorch. The pruning cuts also provide ideal surface for germination of spores (Otieno 1997). Stem diseases like wood rot (*Hypoxylon serpens*), collar canker (*Phomopsis theae*), branch canker (*Macrophoma theicola*) and thorny stem blight (*Tunstallia aculeata*) are predominant in southern India. Recent researchers reported that, entomopathogen could be used as plant fungal disease. Entomopathogenic fungi such as *Lecaniiicillium* sp. and *Beauveria bassiana* have shown to engage in plant pathogenic fungi interaction (Vega 2008 and Vega et al. 2008). These entomopathogens have been reported to very effectively control plant disease (Goettel et al. 2008 and Ownley et al. 2008). The entomopathogenic fungi are considered integrated control for chewing and sucking insect and pest (Gallego and Gallego 1988). The entomopathogens are (*Paecilomyces fumosoroseus*, *Lecaniiicillium*, *Beauveria bassiana* and *Metarhizium anisopliae*) commercially developed as biopesticides (Goettel et al. 2005). Moreover, these entomopathogens have been confirmed against plant fungal pathogen (Kavkova and Curn 2005). In our attempt, performance of entomopathogens like *Beauveria bassiana*, *Paecilomyces fumosoroseus*, *Lecaniiicillium lecannii* and *Paecilomyces lilacinus* have been evaluated against *Macrophoma* sp. The studies were conducted under *in vitro* level.

MATERIALS AND METHODS

The infected stem portions were collected from different tea growing district of south India, viz. Anamallais,
Coonoor, and Vandiperiyar. The plant parts were then examined under microscope. The fungus was morphologically and culturally characterized identically following “The diseases of tea bush” (Petch 1923). Isolation of genomic DNA amplification (PCR) was performed to 18s rRNA gene of the fungus sequencing analyses were identical to that used by Crous et al. (1999). Total of three strains were isolated namely VPM, UPA-61 and NBCHE-6. These fungal pathogens were confirmed as Macrohoma sp and Macrohoma theicola through molecular technique identifications and submitted to NCBI (NBCHE-6-Accession No. JQ234977, VPM-Accession No. KP004441 and UPA-61-Accession No. KP17922).

The entomopathogen and test pathogen at the opposite edges and were incubated for 7-9 days and interaction between the opposing cultures including hyphal contact or coiling and lysis, which was observed under the microscope. Hyphal interaction gently from the zone of interaction in dual culture plates with the help of a needle and placed in a drop of lactophenol cotton blue on a microscopic slide (Elad et al. 1983).

The effect of culture filtrate of entomopathogen was studied following the method of Dennis and Webster (1971). The entomopathogens were inoculated in 100 ml sterilized Potato dextrose broth in 250 ml conical flasks and incubated at 27 ± 1°C. The liquid culture medium was filtered through Whatman No.1. The filtrate was centrifuged at 10000 rpm for 15 min. The supernatant was filtered using millipore membrane filter paper (0.22µm). The entomopathogen filtrate was added to molten PDA to obtain final concentration of 2% (v/v). The medium was poured into petri plates (20 ml/plate) and plates were inoculated with 6 mm disc of test pathogens. PDA plates inoculated with Macrohoma sp. but amended with sterile distilled water served as control. The plates were incubated at 27 ± 1°C for 6 days. The percentage of inhibitions was calculated by the above formula.

The potential of entomopathogens were screened against Macrohoma sp. branch canker pathogen by dual culture method as described by Rajendiran et al. (2010). The entomopathogens were procured from Microbial Type Culture Collection and Genebank (MTCC), Chandigarh, namely like Beauveria bassiana, Paecilomyces fumosoroseus, Lecanicillium lecanii and Paecilomyces lilacinus. The pathogen and antagonist were inoculated in PDA plates on diametrically opposite points. Since the entomopathogens were slow growing in nature, the antagonists were inoculated only before the pathogen colony grew considerably therefore, after 2 days. Linear growth of the biocontrol agents colonizing either over or meet each other the pathogens growth was measured after 9 days of incubation. For the testing of antagonistic entomopathogens Beauveria bassiana, Paecilomyces fumosoroseus, Lecanicillium lecanii and Paecilomyces lilacinus 6 mm discs of antagonist and Macrohoma sp cut from the edge of 7 days old culture were placed 3 cm apart on potato dextrose agar (PDA) plate. The Petri plates were incubated at 27 ± 1°C and periodical observations on the growth of the antagonist to colonize the pathogen were recorded. The experimental design used was completely randomized with four dishes for each isolate and control plate (without entomopathogen), a sterilized agar disc plate. Antagonistic activity was measured using Bell’s scale method (Bell 1982). The percentage of inhibitions was calculated by the formula, PI=C-T/C×100. PI-percentage of inhibition, C-radial growth of the pathogen in control, T-radial growth of the pathogen in dual culture.

The entomopathogen and test pathogen at the opposite edges and were incubated for 7-9 days and interaction between the opposing cultures including hyphal contact or coiling and lysis, which was observed under the microscope. Hyphal interaction gently from the zone of interaction in dual culture plates with the help of a needle and placed in a drop of lactophenol cotton blue on a microscopic slide (Elad et al. 1983).

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![Fig 1 Hypal interaction between entomopathogen and pathogen (under 10X view)](image1)

![Fig 2 Growth inhibition of Macrohoma sp. by entomopathogen (Paecilomyces fumosoroseus and Lecanicillium lecanii)](image2)

<table>
<thead>
<tr>
<th>Strain code</th>
<th>Location</th>
<th>Identified as</th>
<th>NCBI Gen Bank Accession Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBCHE-6</td>
<td>Coonoor</td>
<td>Macrohoma sp</td>
<td>JQ234977</td>
</tr>
<tr>
<td>VPM</td>
<td>Vandiperiyar</td>
<td>Macrohoma sp</td>
<td>KP004441</td>
</tr>
<tr>
<td>UPA-61</td>
<td>Anamallais</td>
<td>Macrohoma theicola</td>
<td>KP179221</td>
</tr>
</tbody>
</table>
**RESULTS AND DISCUSSION**

In this experiment results revealed that, the different tea growing area infected branch canker specimens were isolated and identified at molecular level. The details of branch canker isolate NBCH-6, VPM and UPA-61 with source of location is given (Table 1). The entomopathogens have been tested for inhibition of branch canker pathogen. The inhibitory effects observed in this study were mainly for antagonistic and competition.

The dual plate method showed maximum growth inhibition of *Beauveria bassiana* against branch canker isolates NBCH-6 (64.22), UPA-61 (62.66) and VPM (61.55). The effect of *Paecilomyces fumosoroseus* against two isolates of VPM and UPA-61 (56.66) and *Paecilomyces lilacinus* against VPM (54.66) was significantly antagonistic potential followed by *Lecanicillium lecannii* observation against VPM (47.33), NBCH-6 (45.33) and UPA-61 (44.22) (Table 2 and Fig 2).

Mycoparasitism of both hyphal interaction vital role in mechanism of antagonistic potential capability. The hyphae of *Beauveria bassiana* and *Paecilomyces lilacinus* coiled around hyphae of *Macrophoma* sp. and shrunk it (Fig 1A). However, same action *Beauveria bassiana* with pathogen of tomato root-rot *Pythium myrotylum* was reported by Klingeman et al. (2008). Kiss (2003) reported that, this *Beauveria bassiana* may control plant pathogens and can act through antibiosis and mycoparasitism. Pathogen hyphae was broken by *Lecanicillium lecannii* spores interaction (Fig 1B). The result conform to the reports of Askary et al. (1997) who reported that, *Lecanicillium lecannii* acts as mycoparasite attaching to powdery mildew mycelia and conidia, producing enzymes such as chitinase, which penetrates to the mildew spore and kills the pathogen. *Paecilomyces fumosoroseus* produced more hyphae and spores interact with pathogen hyphae. Several scientists reported the mycoparasitism interaction as main principle mechanism of biological control (Elad et al. 1983). This study results are in accordance with the reports of Kang et al. (1996), Verharr et al. (1997), Di et al. (1998), Miller et al. 2004 and Kavkova and Curn (2005). This may also be the reason for its antagonistic effect on

**Table 2**  **In vitro screening of entomopathogen against branch canker pathogen (Dual plate technique)**

<table>
<thead>
<tr>
<th>Entomopathogen</th>
<th>Growth inhibition (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Culture (MTCC)</strong></td>
<td><strong>Macrophoma</strong></td>
<td><strong>Macrophoma</strong></td>
</tr>
<tr>
<td></td>
<td>sp (NBCH-6)</td>
<td>sp (VPM)</td>
</tr>
<tr>
<td><em>Beauveria bassiana</em></td>
<td>64.22</td>
<td>61.55</td>
</tr>
<tr>
<td><em>Lecanicillium lecannii</em></td>
<td>45.33</td>
<td>47.33</td>
</tr>
<tr>
<td><em>Paecilomyces lilacinus</em></td>
<td>47.77</td>
<td>54.66</td>
</tr>
<tr>
<td><em>Paecilomyces fumosoroseus</em></td>
<td>54.44</td>
<td>56.66</td>
</tr>
<tr>
<td>CD (P=0.05)</td>
<td>3.1</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Values are means ± SE of four replication of three repeated experiments.

**Table 3**  **In vitro screening of entomopathogen against branch canker pathogen (Non-volatile culture filtrate)**

<table>
<thead>
<tr>
<th>Entomopathogen culture (MTCC)</th>
<th>Growth inhibition (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macrophoma</strong></td>
<td><strong>Macrophoma</strong></td>
<td><strong>Macrophoma</strong></td>
</tr>
<tr>
<td></td>
<td>sp (NBCH-6)</td>
<td>sp (VPM)</td>
</tr>
<tr>
<td><em>Beauveria bassiana</em></td>
<td>53.33</td>
<td>54.44</td>
</tr>
<tr>
<td><em>Lecanicillium lecannii</em></td>
<td>18.55</td>
<td>30.44</td>
</tr>
<tr>
<td><em>Paecilomyces lilacinus</em></td>
<td>57.77</td>
<td>63.1</td>
</tr>
<tr>
<td><em>Paecilomyces fumosoroseus</em></td>
<td>60.04</td>
<td>68.44</td>
</tr>
<tr>
<td>CD (P=0.05)</td>
<td>7.1</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Macrophoma sp.

Culture free extract of entomopathogen namely, *Paecilomyces fumosoroseus* and *Paecilomyces lilacinus* have showed maximum inhibition in growth of the pathogen at 2% concentrations. The maximum inhibition given by *Paecilomyces fumosoroseus* against VPM (68.44) followed by *Paecilomyces lilacinus* against UPA-61 (65.59). Significant inhibition observed by action of *Beauveria bassiana* against both isolates of VPM and UPA-61 (54.44). *Lecanicillium lecannii* was seen to inhibit VPM sparsely (30.44) as compared to other antagonistic treatments (Table 3).

The present studies reveal that, *Beauveria bassiana* and *Paecilomyces fumosoroseus* show higher control growth of *Macrophoma* sp. pathogen. Youssef and Hatem (2012) also reported the control of red palm weevil and Rhizoctonia –root-rot of date-palm with *Beauveria bassiana*. Isaria fumosoroses (formely *Paecilomyces fumosoroseus*) and *Lecanicillium sp* (formaely Verticillium lecannii) are known entomopathogens and have good biopesticidal properties (Goettel et al.2005). Wherever, these kind of entomopathogens against fungal plant pathogens (Benhamou and Brodeur 2000, 2001). In our findings, we conclude that *Paecilomyces lilacinus* showed good antagonistic result. Similar findings were recorded by Perveen et al. (1998) with *Paecilomyces lilacinus* and *Pseudomonas aeruginosa* when used against root rot (*Meloidogyne javanica*) and root knot disease (*Macrophomina phaseolina*) in some vegetable crops. The applications of *Paecilomyces lilacinus* fungus protect plant roots from pathogens, increase plant growth and leaf yield (Manjula and Podile 2001. Wraight et al. 2003 and Muthulakshmi et al. 2010).

Results of our experiment showed low inhibitory effect of *Lecanicillium lecannii*, though, these entomopathogenic fungus have activity against phytopathogenic fungi including powdery mildew (Verhaar et al. 1997, 1998), Spencer and Atkey 1981, Askary et al. 1998). In the present investigation, all the entomopathogens studied, showed antagonistic potential and inhibitory effect against *Macrophoma* sp. pathogen. The evidence for role of competition and parasitism has been convinced and evidence established the importance of antibiotic.
From this study, it is evident that the entomopathogens reduced the growth of all isolates of branch canker pathogen causal organism by *Macrophoma theicola* significantly. Mostly entomopathogen can be used as pest and insect infection disease control. Hence, it may use in integrated approaches for managing plant disease and pest control.

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