Combined effect of *Beauveria bassiana* (Deuteromycetes) with leaf extracts of *Phlogocanthus thyrsiflorus* Nees (Acanthaceae) on Pulse beetle, *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae)

C. Murasing 1, P. Das 2, K. Sathish 3, L.K. Hazarika 4

1Dept. of Entomology, College of Agriculture, Jorhat, Assam Agricultural University, Jorhat, 785013, Assam, India.
2 Dept. of Entomology, College of Agriculture, Jorhat, Assam Agricultural University, Jorhat, 785013, Assam, India.
3 Dept. of Entomology, College of Agriculture, Jorhat, Assam Agricultural University, Jorhat, 785013, Assam, India.
4 Dept. of Entomology, College of Agriculture, Jorhat, Assam Agricultural University, Jorhat, 785013, Assam, India.

**Abstract**

Experiments were conducted to evaluate the compatibility of *Beauveria bassiana* (Balsamo Vuillemin with leaf extracts of *Phlogocanthus thyrsiflorus* and their combined effect on *Callosobruchus chinensis* (L.). In this research leaf extracts of *P. thyrsiflorus* with water, methanol, ethanol and petroleum ether at 5.00% concentration were studied for compatible with the entomopathogen. Compared to other solvent extracts, water extracts of *P. thyrsiflorus* was found to be compatible with *B. bassiana* (1x10³ conidia/ml). The combination of *B. bassiana* and water extract of *P. thyrsiflorus* caused 100% mortality of *C. chinensis* on 9 days after treatment.

**Keyword:** Compatibility, *Beauveria bassiana*, *Phlogocanthus thyrsiflorus*, Combined effect.

1. **Introduction**

*Beauveria bassiana* (Balsamo Vuillemin (Hypocreales: Clavicipitaceae) is a soil-borne ubiquitous entomopathogen having been registered as a biopesticide against 700 crop pests worldwide. *Phlogocanthus thyrsiflorus* is an evergreen shrub up to 2.4m high, branchlets quadrangular. It can kill the vegetation beneath when it grows in moist and shady places suggesting presence of allelopathic properties. Besides, it has medicinal properties. It is used for bronchial troubles. Leaves contain β-sitosterol, lupeol and betulin. Other constituents of the plant are similar to those of *Adhatoda zeylanica* (Ghani, 2003) [5].

2. **Materials and Methods**

The experiments were conducted at the Physiology Laboratory, Department of Entomology, Assam Agricultural University (AAU), Jorhat. The leaves of *P. thyrsiflorus* were collected in and around Jorhat district of Assam, India. The collected leaves were washed and dried in the shade at room temperature, grounded finely and hydro distilled in a Soxhlet apparatus as well as extracted separately with methanol, ethanol and petroleum ether as per method described by Bora et al. (1999) [3]. The solvent were removed under reduced pressure using rotary vacuum evaporator (JSGW) and the residues were further dissolved in respective solvents on weight by volume (W/V) basis making it 100% stock solution and stored in a sealed glass bottle at 4°C refrigerator. Similarly the aqueous extract were prepared grinding leaves in distilled water with weight by volume basis after washing thoroughly with running water which served as 100% stock solution.

For maintaining the culture of adult *C. chinensis*, 1kg green gram seed were put in a 5 lit capacity plastic jar and released five pairs of adult male and female in 1:1 ratio. For proper growth and development of the insect during winter season, the plastic jar containing green gram seed and *C. chinensis* were kept on BOD incubator at temperature 29±2 °C. The Tea Mosquito Bug (TMB) isolate of *Beauveria bassiana* (KR855715) was cultured in Petri dishes of 100 mm diameter in the laboratory at 25± 1 °C and 12:12 hour L: D cycle for 9 days to allow it to grow and sporulate maximum (Puzari et al., 1997) [16]. Each stored stock solution was further diluted into 0% (control) and 5.00%, each of which was mixed aseptically with melted potato dextrose agar (PDA) medium (Himedia) containing streptomycin sulphate @ 0.02% in Petri plates and was allowed to solidify under laminar flow cabinet. Each treatment was replicated thrice. A five mm diameter circular disc of 9 day old *B. bassiana* was augured out and planted on each of the treated plates with the help of a sterilized loop. These plates were incubated in the BOD incubators under similar conditions as described earlier. On 9th day of inoculation, radial growth of *B. bassiana* in terms of diameter was measured (Depieri et al., 2005) [9] and for conidial density estimation, the mycelial mat was scraped off from the surface of each of the plates and dissolved in 10 ml sterile distilled water.
Each suspension was stirred for two minutes at 100 rpm with Vortex stirrer and conidial density was measured from each of the treatments (Puzari et al., 1997)\(^{16}\). Each trial was replicated three times and means were calculated. Germination of conidia was estimated as per method of Ekesi et al. (2001)\(^{8}\) by placing four drops of conidial suspension having 10^7 conidia/ml on a clean sterile microscopic slide loaded with 3 ml smear treated medium and incubating it under conditions stated above with 95-100% relative humidity. A slide smeared with the extract free medium was also prepared in the similar way as control. After 24 hours, numbers of germinated conidia were counted (400x) with an optical microscope (Carl Zeiss). Each treatment was replicated four times with five germination counts. Compatibility of \textit{P. thyrsiflorus} with \textit{B. bassiana} was calculated (Alves et al., 1998)\(^{11}\) and classified the plant extracts based on toxicity as very toxic (0 to 30%), toxic (31 to 45%) moderately toxic (46 to 60%) and compatible (>60%) to \textit{B. bassiana}. The data obtained from the trials was completely randomized and submitted to analysis of variance. The mean values were compared by using the Duncan’s (1955)\(^{7}\) Multiple Range Test (DMRT) using a computer programme, SPSS (Version 20.0). (P≤ 0.01 and 0.05).

The combined effect of \textit{P. thyrsiflorus} and \textit{B. bassiana} were tested against adult \textit{C. chinensis}. For this 10 pairs of adult \textit{C. chinensis} were taken and dipped in fungus suspension for few seconds and air dried before released in to plastic container containing 20g green gram seed. Different concentrations \textit{viz.}, 1x10^7, 1x10^8 and 1x10^9 conidia/ml of water was used in this study and each concentration was replicated 3 times. Control plastic containers were treated with water mixed with Tween- 80 @0.023%. Mortality of adults due to infection was recorded up to 9 days of inoculation and data were subjected to ANOVA (Puzari and Hazarika, 1994)\(^{15}\).

3. Results and Discussion

Table 1 shows the radial growth and germination percentage of \textit{B. bassiana} as affected by different solvent extracts of \textit{P. thyrsiflorus}. While combining the \textit{B. bassiana} with different solvent extract of \textit{P. thyrsiflorus}, water extract showed the highest spore germination of \textit{B. bassiana} (68.70±5.39%) at 5.00% concentration, followed by petroleum ether (32.62±1.38%), methanol (22.67±1.55%) and ethanol extract (14.74±2.51%) after 24 hours of inoculation. At 12 days after inoculation, water extract recorded highest vegetative growth (2.55±1.64cm) at same concentration. The significant reduction in vegetative growth and germination of \textit{B. bassiana} was observed in all treatments. ‘T’ values for compatibility studies of \textit{P. thyrsiflorus} with \textit{B. bassiana} revealed that at 5.00% concentration water extract of \textit{P. thyrsiflorus} leaf was compatible with the mycopathogen and found moderately toxic (55.47%). The present investigation was supported by the findings of Ribeiro et al. (2012)\(^{18}\), who reported the aqueous extracts of neem seeds and leaves catigua at the concentrations of 2.5, 5.0, 7.5, and 10.0% (w/v), were classified as compatible with the entomopathogen, becoming important alternatives to integrate programmes of integrated pest management, especially in organic farming systems. It is also supported by the findings of Islam and Omar (2012)\(^{14}\), who revealed that \textit{B. bassiana} was slightly affected by neem and sub-lethal doses of neem tree extract with \textit{B. bassiana} and improve the effectiveness of the control process and thereby reducing the amount of insecticide used. The present investigation was supported by the findings of Hazarika et al. (1997)\(^{16}\) and Gupta et al. (1999)\(^{10}\), who reported that \textit{B. bassiana} was compatible to neem formulations while fungicides were antagonistic. These findings are also in agreement with the findings of Depieri et al. (2005)\(^{18}\), according to them the Neem seed and leaf extracts were compatible with the entomopathogen and leaf extract at 15% had a small negative impact on vegetative growth and viability of conidia, but it was still compatible with the fungus \textit{B. bassiana}, according to the T model. The compatible formulation with \textit{B. bassiana} (isolate DEBI008) and imidacloprid formulation could be used simultaneously with the entomopathogenic in integrated pest management Alizadeh et al. (2007)\(^{2}\).

Table 1: Radial growth and germination % of the \textit{B. bassiana} (KR855715)treated with different solvent extract of \textit{P. thyrsiflorus}

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Germination % (Mean ± SE)</th>
<th>Radial growth (cm) (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water extract</td>
<td>68.70±5.39</td>
<td>2.55 ± 1.64</td>
</tr>
<tr>
<td>Petroleum ether extract</td>
<td>32.62±1.38</td>
<td>0.00</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>22.67±1.55</td>
<td>0.00</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>14.74±2.51</td>
<td>0.00</td>
</tr>
<tr>
<td>Control</td>
<td>100.0±0.00</td>
<td>4.17±1.61</td>
</tr>
</tbody>
</table>

Table 2 shows the combined effect of \textit{B. bassiana} and the \textit{P. thyrsiflorus} leaf extract against adult of \textit{C. chinensis} and were found that 91.97% adult mortality was recorded at 1x10^7 conidia/ml concentration after 3 days of treatment, which was followed by 1x10^8 (88.33%) and 1x10^9 (86.67%) conidia/ml concentration. At 9th day after treatment 100% adult mortality of \textit{C. chinensis} was found in all the treatments. It shows the synergistic effect of both the biopesticides and caused highest adult mortality of \textit{C. chinensis}.
It was supported by the findings of Mohan et al., (2007)\textsuperscript{[13]}, who reported that the effect of combined treatment with \textit{B. bassiana} and neem in comparison to single treatments with either of them on \textit{Spodoptera litura} Fabricius in laboratory bioassays and found the synergistic effect on insect mortality. The present investigation was also supported by the findings of Purwar and Sachan (2006)\textsuperscript{[14]}, who reported that the combination of insecticides with \textit{B. bassiana} showed 1.26-35.8 fold increase in toxicity of insecticides over sole treatment. It was further supported by the work of Zibae et al. (2013)\textsuperscript{[19]}, who reported that the combination of \textit{B. bassiana} and \textit{Artemisia annua} (0.5%) and \textit{Lavandula stoechas} (0.6%) extracts caused the highest \textit{Hyphantria cunea} mortality. These findings are in agreement with the findings of Islam and Omar (2012)\textsuperscript{[11]}, according to them the efficacy of microbial control agent could be enhanced by applying them in conjunction with reduced rates of insecticides. The interaction between these control agents could be additive, synergistic or antagonistic. Synergistic interactions would enhance the effectiveness of the microbial control agent and reducing the adverse effects of pesticides. The present investigation is further supported by the findings of Puzari et al. (2015)\textsuperscript{[17]}, who reported that chloropyriphos at half of the recommended dose along with \textit{B. bassiana} was found to be the best treatment in controlling the rice hispa along with increased yield of the crop consecutively.

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Conidia/ml} & \textbf{Mean mortality (%)±SE} & \textbf{3rdDAT} & \textbf{6thDAT} & \textbf{9thDAT} \\
\hline
1x10\textsuperscript{7} & 91.67±4.39 & 98.33±1.66 & 100±0.00 \\
& (76.23)\textsuperscript{a} & (85.66)\textsuperscript{a} & (88.72)\textsuperscript{a} \\
1x10\textsuperscript{8} & 88.33±4.39 & 96.67±1.66 & 100±0.00 \\
& (70.66)\textsuperscript{a} & (81.35)\textsuperscript{ab} & (88.72)\textsuperscript{ab} \\
1x10\textsuperscript{9} & 86.67±1.66 & 93.33±1.66 & 100±0.00 \\
& (68.64)\textsuperscript{b} & (75.21)\textsuperscript{b} & (88.72)\textsuperscript{b} \\
\textbf{Control} & 1.67±1.66 & 3.33±1.66 & 5±0.00 \\
& (4.31)\textsuperscript{b} & (8.61)\textsuperscript{c} & (12.92)\textsuperscript{c} \\
\textbf{S. ED (±)} & 3.15 & 2.48 & 0.00 \\
\textbf{CD(P=0.05)} & 7.27 & 5.73 & NS \\
\hline
\end{tabular}
\caption{Combined effect of \textit{Beauveria bassiana} and water extract of \textit{P. thyrsiflorus} leaf on adult mortality of \textit{C. chinensis}}
\end{table}

\* Data presented are the mean of 3 replications each having 20 nos. of insects.
\* Zero and 100% mortality was corrected by using Steel & Torrie formula.
\* Data within the parentheses are angular transformed value, compared by DMRT, (P<0.05)
\* Means followed by same letter are not significantly different.

4. References


******************************************************************************