BEAUVERIA BASSIANA (BALSAMO) VUILLEMIN (STRAIN ITCC - 4668) AS ACARICIDE AGAINST TETRANYCHUS URTICAE KOCH (ACARI: TETRANYCHIDAE)

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ABSTRACT
The bioefficacy of entomopathogenic fungi Beauveria bassiana (strain ITCC-4668) was evaluated against two spotted spider mite Tetranychus urticae Koch under field conditions. Highest reduction in T. urticae population was recorded in B. bassiana (0.3×10⁹ conidia ml⁻¹) treated plots (62.36 %) followed by Ethion 50 EC (0.05%) (61.97 %), B. bassiana at 0.3×10⁸ (56.69 %) and 0.3×10⁷ (55.59%) conidia ml⁻¹. A significant negative correlation was found between the average number of mites and chlorophyll content (r= -0.766). In uninfected leaves higher chlorophyll content was recorded (1.016 µg/ml) as compared to mite infested leaves (0.166 µg/ml). B. bassiana treatment improved the total chlorophyll content (0.346 µg/ml), chlorophyll a (0.079 µg/ml) and chlorophyll b content (0.267µg/ml).

Key words: Beauveria bassiana, Chlorophyll content, Stethorus punctilium, Tetranychus urticae

INTRODUCTION
Tetranychus urticae Koch is a ubiquitous and economically important agricultural pest throughout the world (Farouk and Osman, 2011). It is known to attack about 1200 species of plants (Zhang, 2003), of which more than 150 are economically important (Xie et al., 2006), often causing 50-100 percent yield loss (Chhillar et al., 2007; Kumar et al., 2010). Acaricide resistance is accelerated by its high fecundity, inbreeding, arrhenotokous reproduction, high mutation rate and very short life cycle resulting in many generations per year (Van Leeuwen et al., 2009). As a consequence, T. urticae has attained the dubious reputation to be “the most resistant species” in terms of the total number of pesticides to which it has become resistant (Van Leeuwen et al., 2010). In addition, toxicity to non target organisms, groundwater contamination, depletion of rhizosphere microorganisms and human health hazards have further aggravated the situation.

To overcome these problems, bio-pesticides based on microbes like bacteria, viruses, entomopathogenic fungi and nematodes offer considerable scope as protection agents against several insects and mites (Prakash and Rao, 2006; Lewis, 2006). The virulence of entomopathogenic fungi Beauveria bassiana against T. urticae on tomato under glasshouse conditions (Chandler et al., 2000) and tobacco spider mite Tetranychus evansi (Wekesa et al., 2005) have been reported. Keeping in view the above facts and need for search of effective biointensive control measures, it was decided to evaluate the bioefficacy of B. bassiana against T. urticae on okra.

MATERIALS AND METHODS
Culturing of mite and its predator: Two spotted mite, T. urticae was reared and maintained on potted okra plants in the screen house, Department of Zoology, CCS Haryana Agricultural University, Hisar, India.

Culturing of fungi: Pure mother culture of the entomopathogenic fungus, B. bassiana (strain ITCC-4668), were procured from IARI, New Delhi. The culture of the strain was then raised on Potato Dextrose Agar slants in 250 ml conical flasks and stored under refrigerated conditions until further use. Regular passaging is done for further multiplication and maintenance at 27±2°C, > 90 % RH and 16:8
(Light: Dark) photoperiod. Aqueous conidial suspension was made from conidia harvested from the slants grown in conical flasks after 14 days of inoculation. Tween 80 (0.02%) was used to disperse the conidia. The conidial suspension was filtered through muslin cloth. A suspension of $0.3 \times 10^9$ conidia ml$^{-1}$ concentration was made using haemocytometer counts. The lower conidial concentrations ($0.3 \times 10^7$, $0.3 \times 10^8$) were obtained from the serial dilutions.

**Efficacy of Beauveria bassiana against Tetranychus urticae:** The field experiment was conducted at Research Farm, Department of Entomology, CCS Haryana Agricultural University, Hisar. Seeds of okra (cv. Varsha Upar) were sown in eighteen plots, each plot measuring 3m x 2m in size, following recommended agronomical practices in randomized block design. Three plots per treatment were maintained in the field. In field plant to plant and row to row 30 cm distance was maintained and regular irrigation was provided.

The conidial suspension was sprayed against T. urticae population on okra in different plots. The response of fungal treatments was compared with ethion 50EC at its recommended concentration (0.05%). Water treated and untreated (No spray) plots acted as control. Before spraying, initial count of T. urticae was made from ten randomly selected plants/ plot the day before the spray. Mixed population was counted with the help of hand lens from two leaves each from top, middle and bottom strata. The numbers of mites were expressed in sq. cm. The efficiency of the treatments was evaluated by the number of dead mites and beetles after every 24 h till 7 days of the study period.

**Estimation of chlorophyll content:** Mite infestation leads to chlorotic patches in the leaf. To see the impact of T. urticae infestation on okra leaves, chlorophyll contents of mite infested leaves were measured after seven days of infestation. This was compared with chlorophyll contents of uninfested and B. bassiana treated leaves to control T. urticae infestation. A leaf tissue of okra (0.03g) were blotted dry and dipped in test tubes containing 3 ml of dimethyl sulfoxide (DMSO) for overnight as described by Wellburn (1994). The extracted chlorophyll in DMSO was estimated by recording its absorbance at 663 and 645 nm against DMSO as blank using a spectrophotometer. The chlorophyll content of okra leaves was determined by following formula:

$\text{Chlorophyll a} = \frac{12.3 A_{663} - 0.86 A_{645}}{1000 \times w} \times V$

$\text{Chlorophyll b} = \frac{19.3 A_{645} - 3.6 A_{663}}{1000 \times w} \times V$

where,

$V= \text{volume of DMSO (5 ml)}$;

$a= \text{Path length}$;

$w= \text{weight of tissue taken (0.03 gm)}$

Total Chlorophyll = Chlorophyll a + Chlorophyll b

**Statistical analysis:** The corrected per cent mortality in field population was worked out by using the formula of Henderson and Tilton (1955).

$$\text{Corrected Per cent mortality} = 1 - \frac{T_a \times C_b}{T_b \times C_a} \times 100$$

where,

$T_a = \text{Number of mites in the treatment after spraying}$

$T_b = \text{Number of mites in the treatment before spraying}$

$C_a = \text{Number of mites in the untreated check after spraying}$

$C_b = \text{Number of mites in the untreated check before spraying}$

Two-way analysis of variance (ANOVA) was conducted on the mortality data to test the level of significance of the difference in response between the treatments. A correlation was calculated between the number of mites and chlorophyll content of leaves.

**RESULTS AND DISCUSSION**

**Efficacy of Beauveria bassiana against Tetranychus urticae:** The data on the effect of three doses of B. bassiana (strain ITCC- 4668) ($0.3 \times 10^9$, $0.3 \times 10^8$and $0.3 \times 10^7$ conidia ml$^{-1}$) against mite, T. urticae showed reduction in mite infestation under field conditions in okra (Table 1). B. bassiana was found to be potent against T. urticae as it caused 55.59, 56.69 and 62.36 percent reduction at $0.3 \times 10^9$ conidia ml$^{-1}$, $0.3 \times 10^8$ conidia ml$^{-1}$ and $0.3 \times 10^7$ conidia ml$^{-1}$ concentration, however ethion showed 61.97 percent reduction in T. urticae population when the post treatment count were correlated with pre- treatment. The values of reduction in terms of pre- treatment count were 37.63, 43.31 and 44.05 percent in $0.3 \times 10^7$, $0.3 \times 10^8$ and $0.3 \times 10^9$ conidia ml$^{-1}$. All the treatments along
TABLE 1: Efficacy of Beauveria bassiana (strain ITCC- 4668) against Tetranychus urticae

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average number of mites/ sq. cm. leaf after days of treatment</th>
<th>Reduction after treatment(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre – count treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 Mean</td>
<td></td>
</tr>
<tr>
<td>Ethion 50EC (0.05%)</td>
<td>18.80 14.08 10.26 8.30 6.75 5.25 4.45 3.62 3.32</td>
<td>61.97</td>
</tr>
<tr>
<td>Control (water + Tween80)</td>
<td>11.07 10.93 10.35 10.21 9.86 10.32 10.96 11.23 11.23</td>
<td>4.69</td>
</tr>
<tr>
<td>Mean (Duration)</td>
<td>13.21 11.44 a 10.14 b, c, d 8.71 b, c, d, 7.76 c, d, 7.32 c, d, 7.17 d</td>
<td></td>
</tr>
</tbody>
</table>

CD (p= 0.05) for Treatment (T) = 1.48 (21.63); Duration (D) = 1.59; T x D = 3.91
Figures denoted by same superscript do not differ significantly
Figures in parentheses represent percent reduction in mite count as compared to pre- treatment count

The cumulative corrected percent mortality data for T. urticae due to B. bassiana (strain ITCC- 4668) also showed significantly higher mortality of mite with 0.3× 10^9 conidia ml^-1 concentration (67.67%) as compared to lower concentrations i.e. 0.3× 10^8 conidia ml^-1 (63.31%) and 0.3× 10^7 conidia ml^-1 concentration (59.49%) (Table 2). Statistically, higher B. bassiana treatments (0.3× 10^9 and 0.3× 10^8 conidia ml^-1) provided comparable reduction in T. urticae population with acaricide treatment (Ethion @0.05%) (67.83%). Concentrations 0.3× 10^8 and 0.3× 10^7 conidia ml^-1 also do not differ significantly with each other (CD= 6.51; p= 0.05).

Effect of Tetranychus urticae on chlorophyll content of okra leaf: In uninfected leaves higher chlorophyll content was recorded (1.016 µg/ml) as compared to mite infested (0.166 µg/ml) as well as treated leaves (0.346 µg/ml) (Table 3). In B. bassiana treated leaves better leaf colour was observed as compared to T. urticae infested leaves in which chlorotic patches were observed. After the treatment of B. bassiana strains an increase in chlorophyll was observed. A significant negative correlation (r = -0.766) was found between the average number of mites after B. bassiana treatments and total chlorophyll of okra leaves. The Chlorophyll a content was also higher in uninfested leaves (0.646 µg/ml) as compared to infested leaves (0.061 µg/ml). An improvement in the chlorophyll a (0.079 µg/ml) and Chlorophyll b content (0.267 µg/ml) was observed because of the B. bassiana treatments as compared
TABLE 2: Cumulative corrected percent mortality of Tetranychus urticae to Beauveria bassiana

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cumulative corrected percent mortality of mites after days of treatment</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>0.3×10^8 (conidia ml^-1)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0.3×10^8 (conidia ml^-1)</td>
<td>25.79</td>
<td>45.98</td>
</tr>
<tr>
<td>(0.3×10^7 (conidia ml^-1)</td>
<td>28.01</td>
<td>40.92</td>
</tr>
<tr>
<td>Ethion 50 EC (0.05%)</td>
<td>33.18</td>
<td>51.93</td>
</tr>
<tr>
<td>Control (water + Tween 80)</td>
<td>3.77</td>
<td>10.07</td>
</tr>
<tr>
<td>Mean</td>
<td>22.95</td>
<td>37.73</td>
</tr>
</tbody>
</table>

CD (p= 0.05) for Treatment (T) = 6.51; Duration (D) = 7.70; T x D = NS

Figures denoted by same superscript do not differ significantly

TABLE 3: Effect of mite infestation on chlorophyll a and chlorophyll b contents of leaf

<table>
<thead>
<tr>
<th>Leaf grade</th>
<th>Average number of mites per sq. cm. leaf</th>
<th>Chlorophyll a(µg/ml)</th>
<th>Chlorophyll b(µg/ml)</th>
<th>Total chlorophyll (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre- treatment</td>
<td>After treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfested leaves</td>
<td>0</td>
<td>0</td>
<td>0.646</td>
<td>0.370</td>
</tr>
<tr>
<td>Infested leaves</td>
<td>13.00</td>
<td>17.54</td>
<td>0.061</td>
<td>0.105</td>
</tr>
<tr>
<td>B. bassiana treated leaves</td>
<td>23.25</td>
<td>8.75</td>
<td>0.079</td>
<td>0.267</td>
</tr>
<tr>
<td>Correlation</td>
<td></td>
<td></td>
<td></td>
<td>0.766^*</td>
</tr>
</tbody>
</table>

(Correlation is between numbers of mites after treatment × Total chlorophyll)

to infested leaves. New cells emerged in due course which improved the chlorophyll content and color of plant. Bounfour et al. (2002) recorded significant decrease in total chlorophyll content of red raspberry leaves, 4.31 and 4.51 mg/dm² in T. urticae and E. carpini borealis infested leaves respectively after two weeks of study. Jayasinghe and Mallik (2010) recorded significant defoliation in tomato plants which were infested by mites for longer duration (12 weeks) and under higher mite population (400 mites/plants initially released) during early growth stages.

REFERENCES


