Study on β-exotoxin (thurigiensin) effect on biology and growth parameters of *Tetranychus urticae* Koch (Acari: Tetranychidae) on common bean

Reza Vafaei Shoushtari, Seyed Saeid Modarres Najafabadi Abbas ali Zamani

Abstract: 
The direct toxicity of β-exotoxin (thurigiensin) of *Bacillus thuringiensis* Berliner on different stages (reproduction and population development) of *Tetranychus urticae* Koch were evaluated in laboratory conditions. Immature stages of *T. urticae* were more susceptible to thuringiensin than adults. Fecundity of *T. urticae* was significantly reduced when females were exposed to residues for 3 days. At the LC₅₀, larvae and protonymphs were significantly more susceptible to thuringiensin than deutonymphs and adults. Thuringiensin not effect on egg stage. The LC50 on larvae, protonymphs, deutonymphs and adults was 148, 176.6, 293.4 and 318.5 ppm respectively. The daily eggs laid exhibited significant differences among six concentrations of β-exotoxin (thurigiensin) and non-significant difference in the percentage of eggs hatching for all concentrations. The maximum reduction of fecundity for *T. urticae* was 75% (in 500 ppm).

Key words: β–exotoxin, *Bacillus thuringiensis*, *Tetranychus urticae*, common bean.

Introduction: 
Two spotted spider mite, *Tetranychus urticae* Koch (TSSM) is economically important pest on a wide range of agricultural and horticultural crops (Gotoh, 1997; Kishimoto, 2002). The name ‘spider mite’ emanates from the silk webbing made by mites and not because they appear like small spider (Zhang, 2003). They are not the only web-spinning pests to feed on plants, but they are the most common. TSSM is a phytophagus mite and causes significant yield losses in many horticultural, ornamental and agronomic crops globally (van de Vrie *et al.*, 1972; Jeppson *et al.*, 1975). Due to its rapid proliferation and very short life cycle coupled with favourable climatic conditions, many generation of *T. urticae* can be completed in a growing season (Crooker, 1985; Helle and Sabelis, 1985a). Common bean (*Phaseolus vulgaris* L.) is one of the most important and widely grown crop in the world, and commercially produced in Markazi, Lorestan, Fars and Zanjan provinces of Iran. This crop are grown on more than 105000 ha annually in Iran and two-spotted spider mite is a major pest on bean, soybean and cotton in most countries around the world and Iran, causing serious damage in many bean-growing areas of Iran (Rott and Ponsoby, 2000; Fikru and Leon, 2003; Ragkou *et al.*, 2004; Khanjani, 2005; Khanjani and Haddad, 2006). Chemical control of both mites is commonly achieved with a narrow range of acaricides. However, the use of conventional acaricides has been severely restricted by resistance, intolerable residues on export products, toxicological and environmental problems (Vargas *et al.*, 2001). To overcome these problems, the search for alternatives to conventional pesticides has intensified over the last two decades (Roush and Tabashnik, 1990). Control of pest insects with the bioinsecticide *Bacillus thuringiensis* Berliner (B.t.) has increased in frequency in the last decade. Insecticide formulations with highly effective B.t. strains are now available. Also, transgenic crop lines have recently been developed and introduced that produce toxic B.t. proteins in plant tissue. Thuringiensin (β-exotoxin) has several characteristics that may make it suitable for use in spider mite control programmes. Thuringiensin is a water-soluble, dialysable...
nucleotide composed of adenine, ribose, glucose, and allaric acid with a phosphate group (Farkas et al. 1969; Vargas et al. 2001). The objectives of this study were to compare the direct toxicity of thuringiensin on immature and adult stages of *T. urticae* and to determine the effects of thuringiensin on *T. urticae* reproduction and population development.

**Materials and Methods:**

This research was done during 2009-2011 at Islamic Azad University, Arak, Iran. In addition, experiments were done to determine the effects of thuringiensin on fecundity, development and survival of two-spotted spider mite (TSSM) *Tetanychus urticae* Koch. The primary colony of *T. urticae* Koch was collected from common bean fields of the Khomein region, Iran. This colony was transferred to laboratory and was reared on bean plants (*Phaseolus vulgaris* L., var. Chiti Bean Khomein) that was planted on plastic pots (20 cm diameter × 25 cm height) in a greenhouse (for several generations before conducting the experiments). All experiments were carried out at 27±2°C, 70±5% humidity and a photoperiod of 16:8 h (L:D) in a germinator. An experimental formulation of thuringiensin (provided by Abott Laboratories) (Vargas et al. 2001) was used in all experiments that took of Plant Protection Research Institute of Iran.

**Experiments:** On each occasion 3 mL of thuringiensin suspension were sprayed at 40 kPa; followed by a 10s settling period. This technique resulted in a wet deposit of 2.00 ± 0.03 mg/cm² (all experiments performed on leaf discs that each leaf disc was 4 cm² of area center of leaves that this unit separated by plastic padding 2 cm × 2 cm. Each leaf disc was placed on moistened cotton in a plastic Petri dishes (8 cm diameter × 1.5 cm height with a hole in its center (1 cm diameter)). Up to 40 mites were placed into each arena. At least four replicates for each concentration were tested (one replicate per leaflet). Controls with a similar number of water-treated mites were included in tests at each stage. For a concentration-mortality response to be estimated using the probit model, preliminary experiments with a small number of mites were done to select a series of five concentrations that would produce 5-95% mortality (Robertson et al., 1984)

a) **Effect of thuringiensin:** The leaf disc method (2 cm × 2 cm) was used to determine the direct toxicity of thuringiensin to TSSM. After mites were placed in the arenas, the leaf discs were sprayed with a range of six thuringiensin concentrations; 0, 50, 100, 200, 400 and 500 ppm (in 6 treatments and 4 replications). In each developmental stages of TSSM, for each concentration of thuringiensin 40 mites were used (adults, eggs, larvae, protonymphes and deutonymphes) in 4 replications and each replication was one plastic Petri dishes (8 cm diameter × 1.5 cm height with a hole in its center (1 cm diameter)). Mortality records were made after 1, 2 and 3 days

b) **Fecundity.** The thuringiensin effect on *T. urticae* fecundity was determined by female mites for varying periods. In this stage, 50 female mites (1-old day) were placed on Chiti Bean Khomein leaf discs for each concentration of thuringiensin. When females began to lay eggs, their eggs were counted and removed daily until all experimental females died. The daily fecundity and total fecundity of individual females and egg hatch was counted daily. Each female was considered a replicate.
Statistical analysis. The proportion of immature mites surviving, longevity and fecundity of TSSM were analysed with analyses of variance (ANOVA) using the MINITAB-13.1 statistical software (Minitab Inc. 1994 Philadelphia, PA) and means comparison were done based on Duncan’s multiple range test \( (P<0.01) \) (DMRT 1%) (Maia et al., 2000) and using the SAS System Software V6.12 (SAS Institute, 2003). The responses of the test subjects to different thuringiensin concentrations were analysed by log-probit analysis POLO (Russell et al., 1977).

Results:
Effect of \( \beta \)-exotoxin (thuringiensin): Effects of \( \beta \)-exotoxin (thuringiensin) on the mortality of eggs, larvae, protonymphs, deutonymphs and adults are shown in Table 1. Our results showed that the percentage of mortality were significantly different in all concentrations. So, in all developmental stages excepting egg stage, non-significant difference between 400 and 500 ppm. The relative susceptibility of \( T. urticae \) life stages was calculated. At the LC50, larvae and protonymphs were significantly more susceptible to thuringiensin than deutonymphs and adults. Thuringiensin not effect on egg stage. The LC50 on larvae, protonymphs, deutonymphs and adults was 148, 176.6, 293.4 and 318.5 ppm respectively.

<table>
<thead>
<tr>
<th>Thuringiensin concentration (ppm)</th>
<th>Percentage of mortality ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Egg</td>
</tr>
<tr>
<td>0</td>
<td>0.00±0.05a</td>
</tr>
<tr>
<td>50</td>
<td>0.00±0.04a</td>
</tr>
<tr>
<td>100</td>
<td>0.00±0.05a</td>
</tr>
<tr>
<td>200</td>
<td>0.00±0.07a</td>
</tr>
<tr>
<td>400</td>
<td>0.00±0.09a</td>
</tr>
<tr>
<td>500</td>
<td>0.00±0.07a</td>
</tr>
<tr>
<td>LC50 (ppm)</td>
<td>0</td>
</tr>
</tbody>
</table>

Means followed by similar letters in columns are not significantly different

Fecundity and egg hatching: The number of daily eggs laid by each female and the percentage of eggs hatching of TSSM after 3 days are given in Table 2. The daily eggs laid exhibited significant differences \( (P<0.01) \) among six concentrations of \( \beta \)-exotoxin (thuringiensin). Two spotted spider mite, laid the highest daily number of eggs in check treatment which was significantly more than on the other concentrations. So, non-significant difference in the percentage of eggs hatching for all concentrations \( (P<0.01) \).
Table 2: The number of daily eggs and the percentage of eggs hatching of T. urticae after 3 days.

<table>
<thead>
<tr>
<th>Thuringiensin concentration (ppm)</th>
<th>Mean eggs/fem/d (±SEM)</th>
<th>% Hatch (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13.31± 0.05a</td>
<td>93.32± 0.04a</td>
</tr>
<tr>
<td>50</td>
<td>12.75± 0.04ab</td>
<td>91.25± 0.03a</td>
</tr>
<tr>
<td>100</td>
<td>9.58± 0.05c</td>
<td>91.14± 0.07a</td>
</tr>
<tr>
<td>200</td>
<td>7.21± 0.07cd</td>
<td>89.98± 0.09a</td>
</tr>
<tr>
<td>400</td>
<td>5.94± 0.09de</td>
<td>89.00± 0.10a</td>
</tr>
<tr>
<td>500</td>
<td>3.32± 0.07f</td>
<td>88.25± 0.09a</td>
</tr>
</tbody>
</table>

Means followed by similar letters in columns are not significantly different

Discussion:

The β-exotoxin of Bacillus thuringiensis Berliner (thuringiensin), has been accepted as a typical feature of this toxicant (Krieg, 1968; Hall et al., 1971; Perring and Farrar, 1986; Royalty and Perring, 1987; Royalty et al., 1990, 1991; Vargas et al., 2001). In the T. urticae actual mortality is likely to have occurred at moulting when larvae were due to enter the next stage. Mortality assessments for other immature stages were also done when mites in the control group reached adulthood. In this research, average mortality of protonymphs and deutonymphs occurred 24-48 h after exposure to thuringiensin, reflecting the slightly longer developmental period for these stages. The mortality of adult mites was considerably slower and they could survive for up to 10-12 days after treatment. Beebee and Bond (1973a, b) believed that the adult mortality might be a consequence of the disruption of a different biochemical mechanism to that in juvenile mites. Our results, strongly suggest that thuringiensin may achieve effective control of immature stages of TSSM in a relatively short time, which is agreement with Vargas et al. (2001) results and in disagreement with information previously cited about the slow activity of thuringiensin on T. urticae (Royalty et al., 1990, 1991). Royalty et al. (1990) showed that the immature stages of developmental period not be important in the mortality responses of T. urticae but, this study has shown that regressions for residual exposure for all stages were significantly different and that the LC50 increased with the developmental stage (Table 1) that is agreement with Vargas et al. (2001). However, these results clearly demonstrate that early immature stages are more susceptible to thuringiensin than are the later immature and adult stages. Exposure of T. urticae to direct spraying significantly affected fecundity, which was inhibited after 3 d (Table 2). In this research, the maximum reduction of fecundity for T. urticae was 75% (in 500 ppm) but this value recorded by other researchers 25% (Royalty et al., 1990) and 92.93% (Vargas et al. (2001).

References:


