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INSECTICIDAL ACTIVITY OF METHANOLIC EXTRACT OF SILVERLEAF NIGHTSHADE AGAINST *TRIBOLIUM CASTANEUM*

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A B S T R A C T

Methanol extracts of the seeds and leaves of *Solanum elaeagnifolium* were tested in laboratory conditions for their insecticidal activities against larvae and adults of the red flour beetle *Tribolium castaneum*. Insects were exposed to two extracts by topical application and ingestion. Highest mortalities were recorded on larvae treated with seeds extract. Moreover, seed and leaf extracts have a strong anti-feeding activity against the larvae. While the leaf extract showed a low anti-feeding activity against adults. Furthermore, a repellent effect was observed after two hours of exposure to the methanolic extracts. Thus, the treatment of the botanical insecticide may be promising in protecting stored grains from coleopteran pest attacks.

Keywords: Insecticidal activity, methanol extract, treatment method, *Solanum elaeagnifolium*, *Tribolium castaneum*.

INTRODUCTION

The overzealous and indiscriminate use of most of the synthetic pesticide has created different types of environmental and toxicological problems. Recently, in different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapeutants in plant protection. The popularity of botanical pesticides is once again increasing and some plant products are being used globally as green pesticides (Tokunaga *et al.,* 2004; Gurjar *et al.*, 2012). Silverleaf nightshade, *Solanum elaeagnifolium*, from the south-western United States, is reported invasive in 21 countries (Mkula, 2006). In Tunisia, this common weed is found on cultivated land, orchards, meadows and manmade environments such as banks of canals, roads, railways, wasteland (Parsons, 1981; Heap *et al*., 1997). Biochemical studies have shown the presence of secondary metabolites known for their insecticidal activity and toxic alkaloids (Jonasson and Olsson, 1994; Sanford *et al*., 1997; Mkula, 2006). The aim of the present study is to evaluate the insecticidal activity of the methanol extracts from *S. elaeagnifolium* against larvae and adults of *T. castaneum*. Length of larvae, mortality of larvae and adults, anti-feeding and repellent activities are assessed.

MATERIALS AND METHODS

Plant material: Weeds of *Solanum elaeagnifolium* were collected from Chott Mariem region. Seeds and leaves were separated, dried at 40°C and powdered. Powdered plant tissues (100 g) were macerated three times in methanol for 24 h. Each extract was filtrated through Whatman filter paper n°1 to remove peel particles. After filtration, the methanol extracts were let to evaporate at room temperature during 48 h and stored at 4°C until tested.

Insects: The red floor beetle *T. castaneum* was reared on artificial diet of semolina mixed with corn flour and beer yeast (100/50/5, w/w/w) at a constant temperature of 30 ± 1°C in the dark. Adult insects of 10 to 14 days old and third instar larvae were used for toxicity tests. All bioassays were carried out under the same environmental conditions as the cultures.

Bioassays:

Repellent activity: The repellency was tested according to McDonald *et al.* (1970). Half filter paper discs (Whatman n° 40, 9 cm diam.) were prepared and 20 mg of each extract was diluted in 1 ml of methanol at the concentration of 2%. A volume of 200 µl of each concentration was applied separately to one half of the filter paper as uniformly as possible with a micropipette.

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The other half (control) was treated with 200 µl of methanol. Both the treated and the control halves were allowed to dry out as exposed in the air for 10 min. Each treated half-disc was then attached lengthwise, edge-to-edge, to a control half disc with adhesive tape and placed in a Petri dish (9 cm diameter).

Twenty adult insects were released in the middle of each filter-paper circle. Each extract was replicated five times. Insects that settled on each half of the filter paper disc were counted after 15 min, 30 min, 1h and 2h.

The average of the counts was converted to percentage repellency (PR) using the formula of McDonald *et al.* (1970):

$$PR = \left[\frac{Nc - Nt}{Nc + Nt}\right] X\ 100$$

Where **Nc:** number of insects in control test; **Nt:** number of insects in treated test.

The mean repellency value of each extract was calculated and assigned to repellency classes from 0 to V: class 0 (PR ≤ 0.1%), class I (PR = 0.1 - 20%), class II (PR = 20.1 - 40%), class III (40.1- 60%), class IV (60.1 - 80%) and class V (80.1 - 100%).

Antifeedant studies: Semolina disks were prepared according to the methods of Xie *et al.* (1996) and Huang *et al.* (1997). Discs were treated with a single dose (5 µl) at concentration of 2% of each methanolic extracts (seeds and leaves extract).

Controls discs were treated only with methanol. After evaporation of the solvent, the discs were weighed and placed each one in a Petri dish containing 5 adults or 5 third instars larvae whose length has been measured. Twenty one days after, the length of larvae, the mortality and the loss in weight of each set of discs were determined. Formula described by Simmonds *et al.* (1989) was used for calculating the antifeedant index "AIF":

$$AIF = \left(\frac{C - T}{C + T}\right) X\ 100$$

Where **C:** Amount of food consumed from the control discs; **T:** Amount of food consumed from the treated discs with extracts. The following criteria were adopted to categorize the antifeedant index according to Liu *et al.* (2007):

FDI% < 20%: (-) No feeding deterrence, 50% > FDI% ≥ 20%: (+) Weak feeding deterrence, 70% > FDI% ≥ 50%: (++) Moderate feeding deterrence, FDI% ≥ 70%: (+++) Strong feeding deterrence.

Toxicity by ingestion: The mortality rate was recorded after 1, 7, 14 and 21 days. The assessment of mortality rate was corrected for control mortality according to Abbott's correction formula (1925):

$$Mc = \left(\frac{Mo - Me}{100 - Me}\right) X\ 100$$

With **Mc:** corrected mortality rate (%), **Mo:** mortality rate of treated adults or larvae (%), **Me:** mortality rate of control (%).

Toxicity by topical application: Twenty mg of each crude extract was dissolved in distilled water to obtain the final concentration of 2%. One micro-liter of each solution (seeds, leaves) was applied on the abdomen of 10 larvae and 10 adults. The control received 1 µl of distilled water only (five replications).

The length of larvae and the mortality rate was recorded after 1, 2 and 7 days. The mortality rate was calculated and corrected for control mortality according to Abbott's correction formula.

Statistical analysis: The experiment results were statistically analyzed by the mean of one-way analysis of variance ANOVA and when results were statistically significant at $p = 0.05$, Student-Newman-Keuls test was used.

RESULTS

Repellent activity: During the experiment, the average of repellency values for leaves and seeds extracts of *S. elaeagnifolium* on *T.castaneum* noted after 15, 30, 60 and 120 min were reported in Table 1. The highest effect was observed for seed extract with 94% after 2 hours of exposure and categorized in class V. A significant repellent effect was also noted for the leaf extract reaching 74% and having the class IV.

Feeding deterrent activity: Results related to the feeding deterrent activity of the two extracts tested against larvae and adults of the red flour beetle are shown in Table 2. In fact, an antifeeding effect was observed for seeds and leaves extracts at a concentration of 2%. This effect was more important against *T. castaneum* larvae and was classified as strong feeding deterrence (FDI% ≥ 70%).For adults, seeds extract exhibited a significant activity with 83.15%. However, leaves extract had a weaker effect with 33.57% (50% > FDI% ≥ 20%) (Table 2).

Table 1. Repellent activity of *S. elaeagnifolium* methanolic extracts of seeds and leaves against *T. castaneum* adults.

Methanolic extract of *S. elaeagnifolium*	Exposure time (min)	Mean repellency (%)	Repellency Class
Seeds	15	42	III
	30	72	IV
	60	86	V
	120	94	V
Leave	15	40	II
	30	60	III
	60	68	IV
	120	74	IV

Table 2. Feeding deterrent activity of seeds and leaves extracts of *S. elaeagnifolium* against *Tribolium castaneum* larvae and adults.

Methanolic extract of *S. elaeagnifolium*	Antifeedant Index (%)	
	Larvae	Adult
Seeds	89,70 ± 4,72 (+++)	83,15 ± 2,45 (+++)
Leave	84,15 ± 6,70 (+++)	33,57 ± 5,97 (+)

(+) : Weak feeding deterrence; (+++) : Strong feeding deterrence.

Toxicity by ingestion: The toxic activity of leaves and seeds extract of *S. elaeagnifolium* against *T.castaneum* showed an evolution of adult mortality rate. In fact, since the seventh day, a significant mortality rates for seeds extracts were recorded (p≤0.05). The highest level was showed by the seeds extract with a mortality rate 88%. However, a lower toxicity was observed on adults treated with leaf extract that showing a significant mortality rate (p≤0.05) after 21 days of treatment (Figure 1).

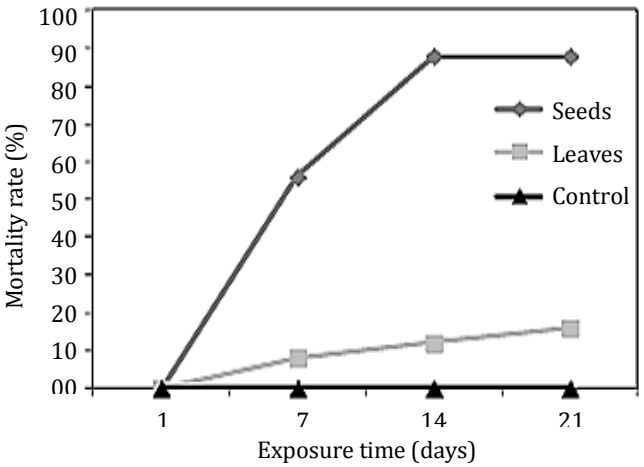

Figure 1. Mortality rate of *Tribolium castaneum* adults treated by ingestion with methanol extracts of *S. elaeagnifolium* seeds and leaves as compared to the untreated control.

Mortality rate was corrected using Abbott's formula (1925).

The toxic property of methanolic extracts of *S. elaeagnifolium* was confirmed by their effect on *T.castaneum* larvae. After 7 days of treatment, we noted significant (p≤0.05) mortality for leaves and seed extracts reaching 88% and 84%, respectively (Figure 2).

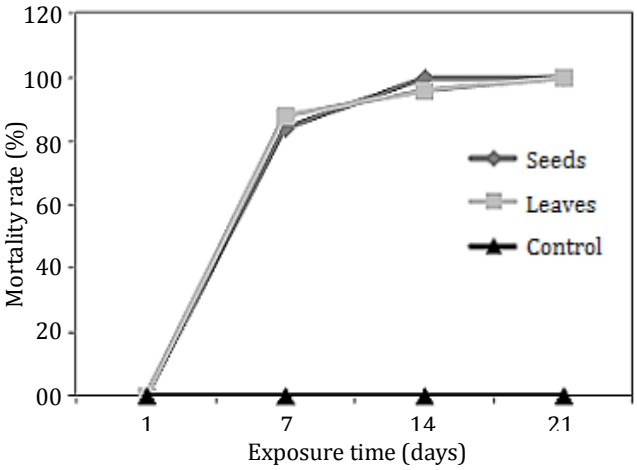

Figure 2. Mortality rate of *Tribolium castaneum* larvae treated by ingestion with methanol extracts of *S. elaeagnifolium* seeds and leaves as compared to the untreated control.

Mortality rate was corrected using Abbott's formula (1925).

Larval length inhibition: Figure 3 shows that the two extracts decreased significantly (p≤0.05) the larvae length gain as compared to the untreated control which reached 1.076 mm. The total inhibition was mainly due to the feeding-deterrent activity (Figure 3).

Toxicity by topical application: During the experiment, mortality rate of *Tribolium castaneum* larvae increased with increasing time until reaching 34% for seeds

extract and 6% for leaves extract after 7 days (Figure 4). Therefore, statistical analysis showed significant effect of seeds extract ($p \leq 0.05$) on pest mortality. Moreover, topical application showed another significant effect ($p \leq 0.05$) on larvae length gain. This effect appeared to be lower than observed in the disc bioassay (Figure 5).

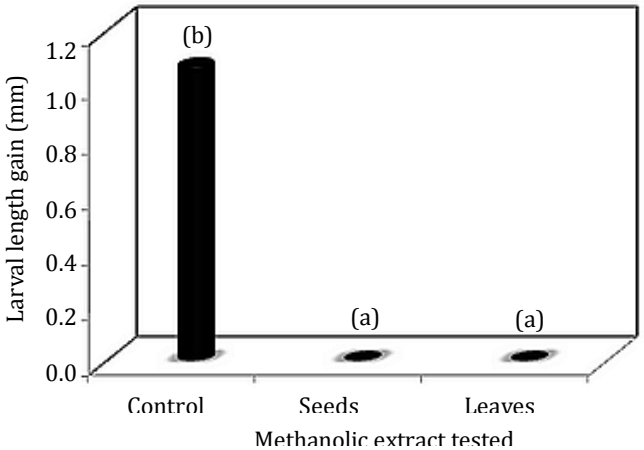

Figure 3. Length gain of *Tribolium castaneum* larvae treated by ingestion with methanol extracts of *S. elaeagnifolium* seeds and leaves as compared to the control. Control: Larvae were fed on disks treated with methanol.

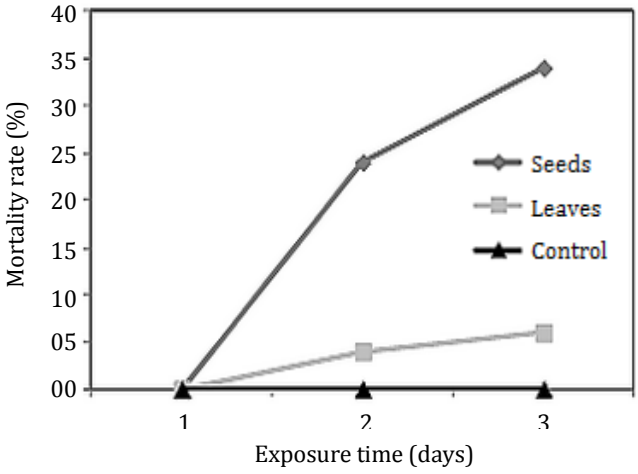

Figure 4. Mortality rate of *Tribolium castaneum* larvae treated by topical application with methanol extracts of *S. elaeagnifolium* seeds and leaves as compared to the untreated control. Mortality rate was corrected using Abbott's formula (1925).

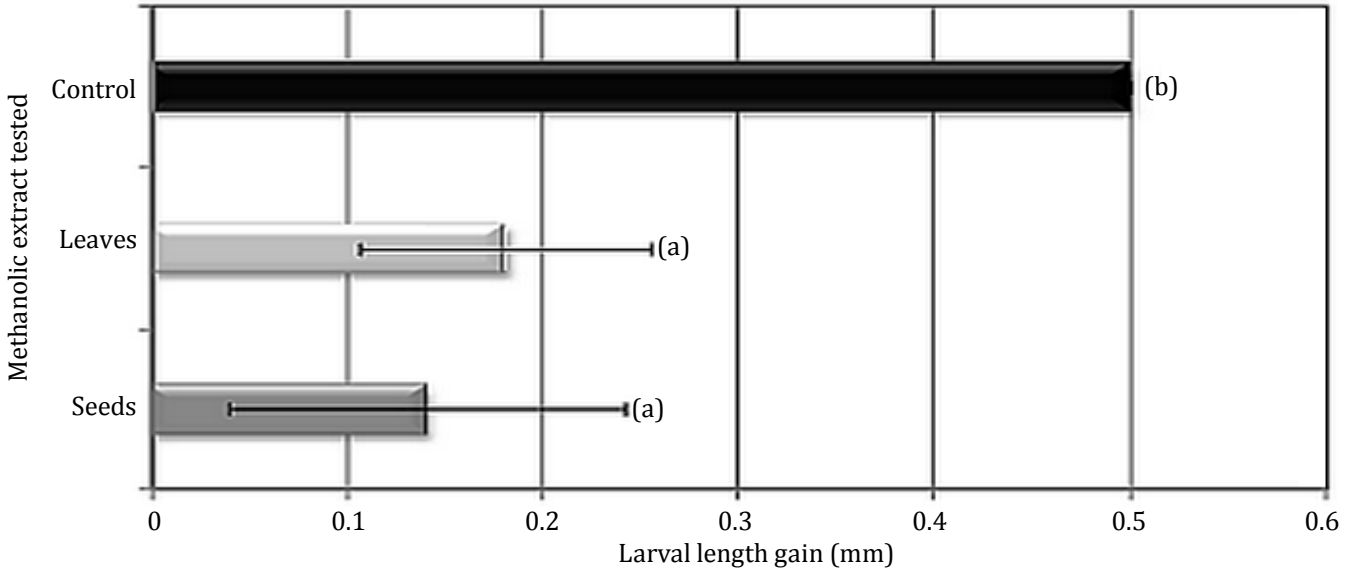

Figure 5. Length gain of *Tribolium castaneum* larvae treated by topical application with methanol extracts of *S. elaeagnifolium* seeds and leaves as compared to the control. Bars attributed by the same letter are not significantly different according to the Student-Newman-Keuls test ($p \leq 0.05$).

DISCUSSION

The present study revealed that methanolic extracts from seeds and leaves of silverleaf nightshade *S.elaeagnifolium* showed an insecticidal activity against *T. castaneum*. Similar results were observed by Markouk *et al.* (2000) on *Anopheles labranchiae* larvae. This activity is mainly attributed to the glycoalkaloids (solamargine, solasonine and solasodine) causing the mortality of red flour beetle (Weissenberg *et al.*, 1998). Antifeedant chemicals play an important role in the unsuitability of non-host plants as food for insects. In the present study, methanolic extract of seeds and leaves of *S. elaeagnifolium* was promising in reducing feeding rate of *T. castaneum*. This indicates that the active principals

present in this plant inhibit feeding behavior of the insect or make the food unpalatable or the substances directly act on the chemosensilla of the insect resulting in feeding deterrence. Wink (2006) showed that this feeding deterrence activity is caused by glycoalkaloids and steroidal saponins which are widespread in the genus *Solanum*. In addition to the antifeeding activity, use of the *S.elaeagnifolium* extracts led to a significant toxicity against *T. castaneum* larvae and adults at the concentration of 2%. The higher mortality rate was observed in the case of seed extract. The toxicity was revealed for the consumption of methanolic extracts and its topical application. Furthermore, similar results concerning inhibition of larval growth and development were observed for seed and leaf extracts. These results were similar of Weissenberg *et al.* (1998) that they have shown that glycoalkaloids extracted from *Solanum* inhibit *T. castaneum* larval development. Moreover, *S.elaeagnifolium* extracts exhibited a repellent activity against red flour beetle. This activity was observed on many pests and is mainly caused by the steroidal alkaloids (Saunders *et al.*, 1992). The same effect against *T. castaneum* was observed in *Solanum nigrum* and *Solanum sisymbrifolium* (Oben-Ofori and Freeman, 2001).

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