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Insecticidal effect of *Solanum elaeagnifolium* extracts under laboratory conditions

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Abstract
The insecticidal activity of methanolic extracts from leaves and seeds of *Solanum elaeagnifolium* against three pest species (*Myzus* persicae, *Phthorimaea operculella* and *Tribolium castaneum*) was investigated. Seed extract had the greatest effect in causing mortality of 23.6% for peach potato aphids and 34% for the red floor beetle, and inhibiting oviposition (95.9%) and egg hatching (98.6%) for potato tuber moth. Except the effect of leaf extract on *P. operculella*, no significant toxicity was observed on *T. castaneum* and *M. persicae*. The present study suggests that seeds and leaves from *S. elaeagnifolium* have insecticidal and repellent properties against several crop pests and could be employed as alternatives for chemical pesticides.

Keywords: *Solanum elaeagnifolium*, methanolic extracts, insecticide activity

1. Introduction

Crop and stored product protection using chemicals is a profit-induced poisoning of the environment. It is very commonly used in large parts of the agricultural world. Plants have been a source of bioactives for use against pest, disease, and weeds since early agricultural times. Some plant extracts are toxic to the target, some are repellent and others work as attractants. Moreover, they have developed defense mechanisms against environmental aggressions; one of the most important is the defense of vegetables against parasites and predators. It is postulated that most of the defensive mechanisms of plants have a chemical character and their existence is due to secondary metabolites [1].

The genus *Solanum* is the largest genus of the family Solanaceae in the west side of the country [2]. *Solanum elaeagnifolium* belongs to the Solanaceae family, commonly called silverleaf nightshade, bitter apple and tomato weed. This species is widely distributed in America and propagated in Australia, Egypt, Greece, India, Israel, Zimbabwe, Sicily, Greece, South Africa, the Maghreb countries and Spain [3]. Many studies have identified some potential uses for *S. elaeagnifolium* as plant extracts have shown molluscicidal and nematicidal activity, as well as cancer inhibiting activity [4]. Most species of the genus have trichomes in stems, leaves, and inflorescences [5], which produce chemical substances with anti-insect activity [6, 7, 8, 9, 10, 11].

In Tunisia, many investigations have been performed on local plants showing a toxicity effect on insects or modifying their behavior [12, 13].

The peach potato aphids, *Myzus persicae* Sulzer, is a highly polyphagous species capable of infecting plants in more than 40 different plant families including many economically important plants like peach, potato and cabbage which it can vector up to 100 phytoviruses [14].

Phthorimaea operculella (Zeller) is a serious pest of potatoes both in the field and storage. It attacks also many solanaceous crops either in the field or in stores [15].

Tribolium castaneum is one of the most serious pests in stored grain and related products. It is considered a secondary pest, which can easily infest damaged or broken kernels and apart from grain. It is particularly destructive to flour and other processed grain products [16, 17].

The aim of this work was to evaluate the insecticidal activity of methanolic extracts of *S. elaeagnifolium* leaves and seeds on the peach potato aphids, the potato tuber moth and the red floor beetle.

2. Materials and Methods

2.1 Plant material

Weeds of *Solanum elaeagnifolium* were collected from Chott Mariem region in January 2013, separated and dried at 40°C. Powdered plant tissues (100 g) were extracted three times by maceration in methanol. 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.

2.2 Insects

The peach potato aphids, used in the bioassay, were collected from pepper crop grown in greenhouses of the Regional Centre of Research on Horticulture and Organic Agriculture.
The potato tuber moths were reared on potato tubers in the laboratory of entomology in the Regional Centre of Research on Horticulture and Organic Agriculture. *P. operculella* colonies were kept in standard conditions at 27 °C and 65% humidity.
The red floor beetle 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.

2.3 Laboratory bioassays

2.3.1 Insecticidal activity assay against *M. persicae*

Twenty mg of each extract was dissolved in distilled water to obtain the final concentration of 2%. 5 µl of each solution (seed and leaf extracts) were sprayed directly on pepper leaves infested by *M. persicae*. The control received 5 µl of distilled water. Leaves containing the insects were placed in petri dishes measuring 9 cm*1.3 cm coated with filter paper and then maintained in a climatic chamber at 25 ± 2ºC, relative humidity of 70 ± 10% and photoperiod of 12 h. The assessment of mortality rate was recorded after 24 hours under binocular microscope. When no antennal or leg movements were observed, insects were considered dead. The calculation of mortality rate was corrected for control mortality according to Abbott's correction formula [18]:

$$\mathbf{Mc} = \frac{\mathbf{Mt\text{-}Mc}}{\mathbf{100\text{-}Mc}} \times \mathbf{100}$$

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

2.3.2 Insecticidal activity assay against *P. operculella*

To examine the percentage of larval penetration of *P. operculella*, the first larval instar was used because it searches and mines into the host. At first, each potato tuber was dipped in 1 ml of 5% methanolic extracts of *S. elaeagnifolium*. When solvent was evaporated and tubers were dried, they (five potato tubers per treatment) were transferred into plastic boxes with ventilated lids kept at 25±2ºC, relative humidity of 70 ± 10% and photoperiod of 16: 8 (Light : Dark). Infested tubers were introduced on each box. Therefore, larval penetration was recorded with the number of individuals moving into potatoes. For the oviposition-preference activity, the number of eggs was determined under a binocular microscope.

2.3.3 Insecticidal activity assay against *T. castaneum*

20 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 3rd instars larvae. The control received 1 µl of distilled water only. Ten larvae were used for each test with 5 replications. The mortality rate was recorded after 7 days. The assessment of mortality rate was corrected for control mortality according to Abbott's correction formula [18] as in the case of *M. persicae* assay.

2.4 Statistical analysis

Five replications were performed for each test. For statistical comparison among several means, all the data were subjected to a one-way analysis of variance (ANOVA) followed by mean comparisons (at P = 0.05) and Student-Newman-Keuls (SPSS 11.0).

3. Results

3.1 Insecticidal activity of *S. elaeagnifolium* methanolic extracts against *M. persicae*

Percentage mortality in adults of *M. persicae* attacking pepper plants treated with seed extract differed significantly compared to other treatments ($P \leq 0.05$). Aphids in this treatment suffered the highest mortality with 23.6 %. However, no significant difference was observed between leaf extract and untreated control (Table 1).

Table 1: Mortality rate of *M. persicae* adults treated by foliar application with methanol extracts of *S. elaeagnifolium* as compared to the untreated control. Same letter after the mortality rate are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$).

Treatment	Numbers tested (n)	Mortality (%)
Control	76	0 (a)
Seed	123	23.60 ± 7.92 (b)
Leaf	133	9.35 ± 7.52 (a)

3.2 Insecticidal activity of *S. elaeagnifolium* methanolic extracts against *P. operculella*

Methanolic extracts of seeds and leaves tested at concentration of 2% had a high preventive effect on potato tuber moth larval penetration. In fact, the number of larvae differed significantly for all treatments with 98.6% for seed extract and 79.4% for leaf extract less than the untreated control (Table 2).
Moreover, applying methanolic extracts of Silverleaf nightshade on potato tubers reduced the number of egg laid by *P. operculella*. The number of eggs laid on tubers treated with seed and leaf extracts decreased significantly with 95.9% and 58.9%, respectively (Table 2). Therefore, the pest preferred to oviposit on non-treated tubers with a mean of 14.6 eggs/tuber.

Table 2: Effect of methanolic extracts of *S. elaeagnifolium* seeds and leaves on the mean numbers of larvae and eggs of *P. operculella* into potato tubers as compared to the untreated control. The same letters after means are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$).

Treatment	Mean numbers of larvae/Potato tuber	Mean numbers of eggs/ Potato tuber
Control	14.6 ± 2.5 (x)	14.6 ± 4.8 (a)
Seed	0.2 ± 0.3 (y)	0.6 ± 0.7 (b)
Leaf	3 ± 1.8 (y)	6 ± 1.2 (c)

3.3 Insecticidal activity of *S. elaeagnifolium* methanolic extracts against *T. castaneum*

Table 3 represented the mortality rate of *T. castaneum* 3rd instars larvae caused by methanolic extracts after 7 days. This experiment showed a mortality rate of 34% in the case of larvae treated with seed extract and only 6% for leaf extract.

Therefore, statistical analyses showed a significant effect of seed extract on pest mortality. However, no significant difference was observed between leaf extract and untreated control.

Table 3: 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). The same letters after the mortality rate are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$).

Treatment	Numbers tested (n)	Mortality (%)
Control	50	0 (a)
Seed	50	34 ± 7.01 (b)
Leaf	50	6 ± 7.01 (a)

4. Discussion

The present study revealed that methanolic extracts from seeds and leaves of silverleaf nightshade *S. elaeagnifolium* showed an insecticidal activity against *T. castaneum* and *M. persicae*. Among the two extracts tested, only seeds extract has a toxic effect. It causes 23.6 % of mortality on *M. persicae* and 34% on *T. castaneum*. Similar results were observed by Ben Hamouda *et al.* [19] on *T. castaneum*, Markouk *et al.* [20] on *Anopheles labranchiae* larvae and [21] on many other pests. This activity is mainly attributed to the glycoalkaloids (solamargine, solasonine and solasodine) causing the mortality of red flour beetle [22].

Many studies showed that *Solanum* is a rich source for several classes of compounds such as alkaloids [23], steroids [24] and phenolic compounds [25]. The pesticidal components of *S. elaeagnifolium* have been shown to include glycoalkaloids as solamargine, solasonine, solasodine … that are responsible for the mortality of *T. castaneum* [26].

Moreover, Wink [27] showed repellent activity of different plant extracts from the genus *Solanum*. The same effect against *T. castaneum* was observed in *S. elaeagnifolium*, *S. nigrum* and *S. sisymbriifolium* [28, 19]. Present studies data showed the oviposition deterrence against *P. operculella* for both leaf and seed extracts. However, seeds show the most effective activity. Only 4.1% of eggs were laid on tubers treated by seeds extract and 1.36% of first instars larvae were penetrated into treated tubers. It can be concluded that the dose of 2% could be used for achieving the desired level of potato tubers protection and reduce egg laying of this moth.

These preliminary data suggest that the methanol extracts of seeds and leaves of the Silverleaf nightshade should be further investigated in order to determine its chemical composition and to elucidate more its insecticidal potentialities.

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