# Repellency and Insecticidal Activities of *Thapsia garganica* Crude Extract against Some Important Pests

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## ABSTRACT

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Repellency and insecticidal activities of *Thapsia garganica* leaf methanolic extract were investigated against *Tribolium castaneum*, *Myzus persicae*, *Phthorimaea operculella*, and *Spodoptera littoralis*. Repellency and toxic activities (by ingestion and topical application) were evaluated on *T. castaneum* nymphs and adults. Topical application treatment caused total larval growth inhibition at 10%, until mortality after 7 days. The highest mortality was recorded with 94% at the same concentration. Methanolic extracts incorporation into *T. castaneum* larvae artificial diet at 10% caused 100% mortality after 3 days. The extract at 1% cause high repellent effect on *T. castaneum* after 60 min of exposure, while *M. persicae* was less sensitive. *P. operculella* female's showed sensitivity by a repellent effect at oviposition. Egg's number laid on treated tubers at 1% and 2% decreased significantly to 32% and 72%, respectively. In addition, methanolic extracts had a preventive effect on *P. operculella* larval penetration. In fact, the number of larvae was reduced by 30.46% and 76.12% in the treated tubers at 1% and 2%, respectively. For *S. littoralis*, a low antifeeding effect was recorded. However, the relative growth rate (RGR), conversion of ingested and digested food to biomass, were decreased. The approximate digestibility increased. Moreover, a delay in larval development was observed. This study suggests that the leaf extract of *T. garganica* could be applied as bio-insecticide.

*Keywords*: Antifeeding proprieties, crude extract, insecticidal activity, *Thapsia garganica*

Food provision has always been a challenge facing mankind; but the competition from insect pests is the main factor in this challenge. Hence, insect pests are responsible for large losses to stored products and crops through feeding damage; but also as vectors of plant pathogens such as viruses. The application and the development of synthetic insecticides have made it possible to suppress populations of pests in order to achieve an adequate supply of food. However, the use of chemicals has been associated with environmental pollution and adverse effects on human health and non-target organisms and frequent applications of insecticides have led to the

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occurrence of resistance in many insect populations (Sparks et al. 2021; Wang et al. 2022). Researchers are therefore turning towards natural active substances capable to interfere with the development and physiology of insect pests (Tlak Gajger and Dar 2021; Yactayo-Chang et al. 2020).

Plants are a rich source of bioactive compounds against pests. For example, the alkaloid nicotine, extracted from *Nicotiana tabacum*, was one of the first natural products used as an insecticide (Kortbeek et al. 2019). Another example is azadirachtin, which was isolated from neem seeds. This compound was long used in agriculture (Kortbeek et al. 2019).

The present article is focused on four relevant insect species: *Tribolium castaneum* (Coleoptera, Tenebrionidae), *Myzus persicae* (Hemiptera, Aphididae), *Phthorimaea operculella* (Lepidoptera, Gelechiidae) and *Spodoptera littoralis* (Lepidoptera, Noctuidae). *T. castaneum* is one of the most important insects damaging stored grains and related products (particularly flour and the other processed grain products) (Hamouda et al. 2015a). *M. persicae* is highly polyphagous species. It is able to feed on over 400 host plant species, including several major crop types (Hemming et al. 2022). Regarding *P. operculella*, it is a cosmopolitan insect of solanaceous crops. It causes relevant damage to potato (storage and field) (Hamouda et al. 2015a). Concerning *S. littoralis*, it attacks around 44 plant families and deciduous fruit trees (Lopez et al. 2006). The voracious caterpillars attack plants (leaves, flowers, and stems) (Alford 1995).

The aim of this work was to evaluate the insecticidal activity of *T. garganica* leaf methanolic extract on the insect species previously cited. *T. castaneum* was subjected to assays evaluating the effect of the extract through topical application, repellency, and ingestion. The insecticidal potential of the methanolic extract was also evaluated on *M. persicae*, and *P. operculella* through larval penetration and oviposition-preference activity tests, and on *S. littoralis* through feeding assays and morphological malformations evaluation.

## MATERIALS AND METHODS

### Plant material.

Leaves of *T. garganica* were collected in February 2021 from the region of Sousse (35° 49' 31" N and 10° 38' 13" E) in the Sahel of Tunisia. *T. garganica* was in the vegetative growth stage when sampling. The collected plant was identified using the flora of Tunisia (Pottier-Alapetite, 1981). Leaves were dried at room temperature in complete darkness and grind. Powdered plant tissues (100 g) were macerated in methanol. To remove peel particles, the extracts were filtered through 0.22-µm-pore paper in a Büchner funnel coupled to a vacuum pump. They were evaporated to dryness under reduced pressure at 45-50°C, using Rotavapor R-114 (Buchi, France) and stored at 4°C until use (Jmii et al. 2020).

### Insects.

*M. persicae* was collected from pepper crop grown in greenhouses located in the Regional Center of Research on Horticulture and Organic Agriculture (CRRHAB), Chott Mariem, Sousse, Tunisia.

*P. operculella* was reared on potato tubers in the laboratory of entomology in the CRRHAB. Insects were kept at (27 °C), constant day/night cycle (photoperiod of 12 h), and ambient relative humidity of 65%.

*T. castaneum* was reared on wheat semolina (*Triticum durum*) mixed with beer yeast and corn flour (100/5/50, w/w/w) at 30°C in complete darkness.

Toxicity assays were carried out both on nymph and adult insects. Tested larvae were in the third instar of development (3 mm of length), and the age of adult insects analyzed was 14 days old.

*S. littoralis* was reared starting from caterpillars collected from pepper crops grown in greenhouses, and maintained in the laboratory of entomology in the RCRHOA. Caterpillars were reared in Petri dishes at 25°C, 70% humidity, and a 16h:8h photoperiod (L:D). They were fed with artificial diet composed of 800 mL water, 150 g chickpea powder, 20 g agar, 40 g beer yeast, 1 g benzoic acid, 5 g ascorbic acid, and 1 g nipagin. When caterpillars reached third instar, they were reared individually to avoid cannibalism. At the last (the sixth) instar, they were transferred to round plastic bowls (15 cm in depth and 70 cm in diameter) furnished with moistened saw dust to serve as pupating medium. Resulted pupae were daily collected and transferred to adult rearing cages (nesting cages). Cages were cubic (measuring 50 × 50 × 50 cm). The top, the floor, and the three sides of each cage were wooden, and covered with kraft paper serving as oviposition support. The fourth side of the cage was composed of wire screen (acts as a door and allows the ventilation of the cage). In the cages, small pots containing pieces of cotton wool soaked with 10% sugar feeding solution were placed. The kraft paper (on which the eggs were deposited) was cut into pieces. These pieces were placed in Petri dishes containing the artificial diet of chickpea. Newly emerged third instar caterpillars were used for toxicity assays.

**Insecticidal activity against *T. castaneum*.**

**Topical application test.** The dry extract was dissolved in distilled water to obtain final concentrations of 0.1, 1 and 10%. 1 µL of each solution was applied on the topical part of the thorax of nymphs and adults. The control insects received 1 µL of distilled water. For each assay, 10 larvae and adults were used. The mortality was assessed daily via direct observation for a period of 7 days. Insect mortality was calculated using the Abbott correction formula for natural mortality in untreated controls (Abbott 1925). The increase in length was also determined for both treated and control groups.

**Repellency assay.** An area preference method was adopted to assess the repellent activity of *T. garganica* essential oils against *T. castaneum* adults. Repellency assay was carried out into Petri dishes measuring 9 × 1.3 cm (diameter/height). Each Petri dish contained a disc of Whatman paper N°1 of the same diameter, cut into two equal halves. The first semi-circle was soaked in methanol (200 µL) to be the control part, while the second semi-circle was treated with the same quantity of the extract at the concentrations of 0.1 and 1%. After the evaporation of the solvent, both treated and untreated halves were then attached with cellophane tape and placed in Petri dish. Twenty *T. castaneum* adults were deposited into the center of the dish (Hamouda et al. 2015b).

The measured parameter was the number of adults in each semi-circle, after 15, 30, 60, and 120 min. Repulsion percentage (PR) was calculated according to the formula (McDonald et al. 1970): PR = [(N control - N treated) / (N control + N treated)] × 100, where N control: number of insects on the control area and N treated: number of insects on the treated area.

The repulsion average percentage was calculated and attributed according to McDonald's classification (McDonald et al. 1970) to one of the different repellent classes varying from 0 to V:

| Repulsion Percentage (PR) | Class |
|---|---|
| PR ≤ 0.1 % | 0 |
| 0.1 % < PR ≤ 20 % | I |
| 20.1 % < PR ≤ 40 % | II |
| 40.1 % < PR ≤ 60 % | III |
| 60.1 % < PR ≤ 80 % | IV |
| 80.1 % < PR ≤ 100 % | V |

**Ingestion assay.** This assay aims evaluating the potential of the extract to be used as poisoned bait for the control of *T. castaneum*, through its incorporation into larvae's food (wheat semolina). The extract was first dissolved in methanol at 0.1, 1, and 10% concentrations, and 10 mL of each solution were added to 20 mg of wheat semolina. Once the solvent has evaporated, the treated diet was introduced in Petri dishes, containing 20 *T. castaneum* larvae. For control samples, semolina (20 mg) was treated with 10 mL of methanol following the same procedure, and larvae mortality and adults' emergence percentages were assessed daily via direct observation for a period of 21 days (Hamouda et al. 2015b).

**Insecticidal activity against *M. persicae*.**

The dry methanolic extract was dissolved in distilled water at the concentrations of 0.1, 1, and 2%. These solutions were tested by spray treatment, for which 5 µL were homogeneously sprayed on pepper leaves infested by *M. persicae*. The number of insects used was 120, 132, 127, and 141 for the control, and the different treatments were 0.1, 1, and 2%, respectively. In the control experiment, the insects received the same quantity of distilled water. The infested leaves were then introduced in Petri dishes (9 cm × 1.3 cm), coated with filter paper, and introduced into a climatic chamber. They were kept for 24 h with a photoperiod of 12h:12h (L:D), while maintained at a temperature of 25±2°C, and relative humidity of 70±10%.

The mortality rate assessment was then recorded using a binocular stereomicroscope. Insects were considered dead when no leg or antennal movements were observed after stimulation with a soft paint brush. The mortality rate assessment was corrected according to Abbott's (1925) correction formula.

**Insecticidal activity against *P. operculella.***

Each non infested potato tuber was dipped in 10 mL of 1% and 2% methanolic extract of *T. garganica*. When tubers dried and solvent evaporated, five potato tubers per treatment were transferred into plastic boxes with ventilated lids kept at 25°C, photoperiod of 16h:8h (L:D), and relative humidity of 70±10%. In each box, five infested tubers were introduced, and larval penetration was recorded with the number of larvae moving into potatoes. For the oviposition-preference activity, the egg number was determined under a binocular stereomicroscope (Hamouda et al. 2015a).

**Insecticidal activity against *S. littoralis.***

Third instar caterpillars were weighed and placed individually in Petri dishes, to be fed with artificial diet, previously treated with the methanolic extract. This food consisted of a 1 g disc prepared by mixing the diet based on chickpea with the methanolic extract. The weight of this extract was 0.1 g and 1 g per 100 g diet. For the negative control, caterpillars were fed with chickpea mixed with distilled water.

Every two days, each disc was weighed before being presented to the caterpillars, and reweighed and replaced by a new weighed disc, for a total time of 10 days. The five gravimetric indices defined by Waldbauer (1968) were calculated, which allow to understand the use of food by the caterpillars. The

measured parameters to perform these calculations were the weights of consumed food, the rejected excrement, and the biomass variations in the insect after the treatment period. The five indices were:

The relative ingestion rate (RIR) = Fi / (P × Td),

The approximate digestibility (AD) = 100 × (Fi - Fp) / Fi,

The efficiency of converting digested food into biomass (ECD) = 100 × WG / (Fi - Fp),

The efficiency of converting ingested food into biomass (ECI) = 100 × WG / Fi

The relative growth rate (RGR) = WG / (P × Td),

where Fi: Food ingested (mg), Fp: Faeces produced (mg), Wg: Weight gain (mg) = (Wf - Wi), Td: Development time (day), Wf: Final weight of the caterpillar (mg), Wi: Initial weight of the caterpillar (mg), P: Average weight of the caterpillar = ((Wf - Wi)/log ((Wf / Wi)).

The antifeeding activity of the methanolic extract was determined according to Simmonds et al (1989): AFI = (C - T / C + T) × 100, where C: Consumption of control caterpillar, T: Consumption of treated caterpillar.

The antifeeding activity classification was carried out according to Liu et al (2007):

* AFI < 20%: no antifeeding activity (–),
* 50% > AFI ≥ 20%: low antifeeding activity (+),
* 70% > AFI ≥ 50%: medium antifeeding activity (++),
* AFI ≥ 70%: strong antifeeding activity (+++).

Malformations (like head capsule persistence, pupae and larvae having reduced size) which could make *S. littoralis* vulnerable to several sorts of mortality (such as molting and exuviations difficulties) were also evaluated during this experiment.

**Statistical analyses.**

The data were reported as mean ± standard deviation (SD) in five replicates. ANOVAs followed by Duncan's test were performed by IBM SPSS Statistics version 20.0 to analyze the differences between treatments. Differences were considered statistically significant at the 5 % level ($p < 0.05$).

## RESULTS

### Contact effect of the methanolic extract on *T. castaneum*.

**Larval development.** Table 1 shows that *T. garganica* leaf methanolic extract (at 0.1%, 1%, and 10%) significantly ($p < 0.05$) inhibited larval length growth. The larva increase in length was only 0.32 mm at 0.1%, after 7 days of the treatment, versus 0.54 mm for the control. At the highest concentration, the larval growth was totally inhibited.

**Table 1.** Increase in length (mm) of *T. castaneum* larvae after 7 days of the topical application treatment with *T. garganica* leaf methanolic extract at 0.1%, 1%, and 10%, as compared to the control

| | Control | 0.1% | 1% | 10% |
|---|---|---|---|---|
| **Larva increase in length (mm)** | $0.54\pm0.05^{d}$ | $0.32\pm0.04^{c}$ | $0.12\pm0^{b}$ | $0\pm0^{a}$ |

Values are means ± standard deviations (SD) (n = 5). Means with the same letter are not significantly different at $p < 0.05$ (Duncan's test).

**Toxic effect.** The mortality rate of *T. castaneum* 3rd instars caused by *T. garganica* leaf methanolic extract after 7 days are presented in Table 2. This experiment showed a mortality rate of 48% in the case of larvae treated by topical application with the extract at 0.1%. In the case of larvae treated with methanolic extract at 1% and 10%, the mortality rate reached 96% and 100%, respectively

For *T. castaneum* adults treated with methanolic extract at 0.1% and 1%, the mortality rate reached 28% and 86%, respectively, after 7 days of the treatment. At the highest concentration, adults exhibited the highest mortality with 94% (Table 2).

**Table 2.** Mortality rate (%) of *T. castaneum* larvae and adults after 7 days of the topical application treatment with *T. garganica* leaf methanolic extract at 0.1%, 1%, and 10%, as compared to the control. Mortality rate was corrected using Abbott's formula (1925)

| Insect mortality rate | 0.1% | 1% | 10% |
|---|---|---|---|
| **Larvae** | $48\pm8.36^{a}$ | $96\pm5.47^{b}$ | $100\pm0^{c}$ |
| **Adults** | $28\pm13.03^{a}$ | $86\pm11.4^{b}$ | $94\pm8.94^{c}$ |

Values are means ± standard deviations (SD) (n = 5). Means with the same letter are not significantly different at $p < 0.05$ (Duncan's test).

**Repellency assay.**

The repellency percentage calculated for adults exposed to methanolic extract at 0.1% and 1% showed that the extract at 0.1% has a moderately repellent effect in the order of 53% after 60 min of exposure. Nevertheless, from the first 15 min, the extract at 1% noted a repellent effect with 68%. This effect became very repulsive (84%) after 30 min of exposure to reach its maximum after 60 min (100%) (Table 3).

**Ingestion assay.**

The methanolic extract incorporation into *T. castaneum* larva artificial diet at 10% caused 100% larvae mortality after 3 days. As a result, the percentage of emergence of *T. castaneum* adults from larvae subjected to treated food was 0% at the highest concentration (Table 4).

**Table 3.** Repulsion average percentages of *T. casataneum* adults exposed to *T. garganica* leaf methanolic extract at 0.1% and 1%

| Extract concentration | Exposure (min) | Average repulsion (%) | Repulsive class | Repellency effect |
|---|---|---|---|---|
| **0.1%** | 15 | $14\pm0.49^{a**}$ | I | Very weak repellent |
| | 30 | $14\pm0.49^{a**}$ | I | Very weak repellent |
| | 60 | $53\pm0.22^{b**}$ | III | Moderately repellent |
| | 120 | $53\pm0.22^{b**}$ | III | Moderately repellent |
| **1%** | 15 | $68\pm0.36^{a}$ | IV | Repellent |
| | 30 | $84\pm0.47^{b}$ | V | Very repellent |
| | 60 | $100\pm0^{c}$ | V | Very repellent |
| | 120 | $100\pm0^{c}$ | V | Very repellent |

Values are means ± standard deviations (SD) (n = 5). Means with the same letter are not significantly different at $p < 0.05$ (Duncan's test). ** means are significantly different using means variation test ANOVA at ($p < 0.01$).

**Table 4.** Percentage of emergence (%) of *T. castaneum* adults from larvae fed on artificial diet mixed with *T. garganica* leaf methanolic extracts at 0.1%, 1%, and 10%

| Extract concentration | Control | 0.1% | 1% | 10% |
|---|---|---|---|---|
| **Larvae mortality (%)** | $0\pm0^{a}$ | $21\pm1^{b}$ | $72\pm1^{c}$ | $100\pm0^{d}$ |
| **Percentage of emergence of *T. castaneum* adults from larvae subjected to ingestion assay (%)** | $100\pm0^{d}$ | $79\pm1.73^{c}$ | $28\pm2.64^{b}$ | $0\pm0^{a}$ |

Values are means ± standard deviations (SD) (n = 5). Means with the same letter are not significantly different at $p < 0.05$ (Duncan's test).

**Insecticidal activity against *M. persicae*.**

Percentage of mortality in adults of *M. persicae* attacking pepper plants treated with *T. garganica* leaf methanolic extract at 0.1%, 1%, and 2% is represented in Table 5. Aphids in this treatment showed the highest mortality with 7.09% at the highest concentration. However, the mortality rate did not exceed 1.51% and 4.72% at 0.1% and 1%, respectively.

**Table 5.** Mortality rate (%) in adults of *M. persicae* adults treated by foliar application with *T. garganica* leaf methanolic extract at 0.1%, 1%, and 2% as compared to control. Mortality rate was corrected using Abbott's formula (1925)

| Extract concentration | Numbers tested (n) | Mortality (%) |
|---|---|---|
| **0.1%** | 132 | $1.51\pm0.14^{a}$ |
| **1%** | 127 | $4.72\pm0.23^{b}$ |
| **2%** | 141 | $7.09\pm0.18^{c}$ |

Values are means ± standard deviations (SD). Means with the same letter are not significantly different at $p < 0.05$ (Duncan's test).

**Insecticidal activity against *P. operculella.***

Table 6 shows that the number of larvae decreased with 30.46% and 76.12% in tubers treated with leaf methanolic extract at 1% and 2%, respectively. Similarly, the number of eggs laid by *P. operculella* on treated tubers was reduced by 32% and 72% in the presence of the extracts at 1% and 2%, respectively.

**Table 6.** Mean numbers of larvae and eggs of *P. operculella* in potato tubers treated with *T. garganica* leaf methanolic extract at 1% and 2%, as compared to the control

| Extract concentration | Mean numbers of larvae/Potato tuber | Mean numbers of eggs/Potato tuber |
|---|---|---|
| **Control** | 15.33±0.57$^{c}$ | 16.66±0.57$^{c}$ |
| **1%** | 10.66±1.15$^{b}$ | 11.33±0.57$^{b}$ |
| **2%** | 3.66±0.57$^{a}$ | 4.66±0.57$^{a}$ |

Values are means ± standard deviations (SD) (n = 5). Means with the same letter are not significantly different at $p < 0.05$ (Duncan's test).

**Insecticidal activity against *S. littoralis.***

**Effect on nutrient utilization.** The methanolic extract incorporation into the artificial diet of *S. littoralis* caterpillars significantly affected all gravimetric indices. The caterpillars fed on artificial diet supplemented with distilled water (control) showed the highest relative growth rate (RGR = 0.48 mg/mg/day). In the presence of the methanolic extract, the relative growth rate decreases significantly ($p < 0.05$) especially at the concentration of 1% (RGR = 0.35 mg/mg/day) (Table 7).

The caterpillars subjected to a diet supplemented with methanolic extracts from *T. garganica* leaves at 0.1% and 1% showed a RIR which exceeded than the control group (RIR = 1.78 mg/mg/day). The incorporation of the extract into *S. littoralis* diet at the highest concentration had the highest RIR effect on caterpillars (RIR = 4.02 mg/mg/day) (Table 7).

The data of the AD showed a significant difference between treatments ($p < 0.05$). Caterpillars fed on artificial diet containing the methanolic extract at 1% had the highest approximate digestibility with 96.24%. However, the control showed the lowest approximate digestibility, which was around 82.95%. The extract at 0.1% induced an average rate of 89.95% (Table 7).

For ECI and ECD, statistical analysis made possible to classify these two parameters into three groups: The control presented the highest ECI and ECD, with 28.20% and 34% respectively, while the caterpillars fed on artificial diet supplemented with the methanolic extract at 1% had two lowest values with 9.49% and 9.89%. The treatment at 0.1%, demonstrated an ECI and ECD of 20.31% and 22.70%, respectively.

**Table 7.** Relative growth rate (RGR) (mg/mg/day), relative ingestion rate (RIR) (mg/mg/day), approximate digestibility (AD) (%), efficiency of converting ingested food into biomass (ECI) (%), and efficiency of converting digested food into biomass (ECD) (%) of *S. littoralis* caterpillars subjected to a diet supplemented with *T. garganica* leaf methanolic extracts at 1% and 2%, as compared to the control

| Extract concentration | RGR | RIR | AD | ECI | ECD |
|---|---|---|---|---|---|
| **Control** | $0.48\pm0.01^{c}$ | $1.78\pm0.36^{a}$ | $82.95\pm4.71^{a}$ | $28.20\pm5.67^{c}$ | $34\pm6.77^{c}$ |
| **0.1%** | $0.42\pm0.02^{b}$ | $2.23\pm0.52^{b}$ | $89.95\pm2.02^{b}$ | $20.31\pm5.94^{b}$ | $22.7\pm6.9^{b}$ |
| **1%** | $0.35\pm0.02^{a}$ | $4.02\pm1.01^{c}$ | $96.24\pm1.3^{c}$ | $9.49\pm2.97^{a}$ | $9.89\pm3.23^{a}$ |

Values are means ± standard deviations (SD) (n = 5). Means with the same letterare not significantly different at $p < 0.05$ (Duncan's test).

**Antifeeding activity.** The methanolic extract at 0.1% and 1% had low antifeeding effects (50% > AFI ≥ 20%) on *S. littoralis* caterpillars (Table 8).

**Table 8.** Antifeeding activity (%) of *T. garganica* leaf methanolic extract at 1% and 2% on *S. littoralis* caterpillars. Low antifeeding activity (+)

| Extract concentration | Antifeeding activity (AFI) |
|---|---|
| **0.1%** | 22.40 ± 9.29 (+) ** |
| **1%** | 31.16 ± 8.38 (+) |

Values are means ± standard deviations (SD) (n = 5). Data were analyzed by ANOVA test. **: Statistically significant differences ($p < 0.01$).

**Toxicity and malformations.** The incorporation of methanol extract in *S. littoralis* artificial diet caused a mortality of larvae and pupae. The development of larvae fed on diet treated by the extract at 0.1% and 1% was delayed. After 15 days of delay, the mortality rate of larvae (having a reduced size) fed on diet mixed with the extract at 0.1% reached 36%. The larvae died because of a molting difficulty. Only 52% of dwarf pupae were formed from dwarf larvae (Table 9). Twenty percent (20%) of dwarf adults were emerged from the dwarf pupae. In the case of the treatment at 1%, after more than one month of development delay, 74% of dwarf larvae died. All (100%) of malformed pupae (head capsule persistence) after exuviation difficulties died (Table 9).

**Table 9.** Larval and pupal mortality (%) of *S. littoralis* caterpillars subjected to a diet supplemented with *T. garganica* leaf methanolic extracts at 1% and 2%, as compared to the control

| Extract concentration | Larval mortality (%) | Pupal mortality (%) |
|---|---|---|
| **0.1%** | 36[b] | 48[b] |
| **1%** | 74[c] | 100[c] |
| **Control** | 0[a] | 0[a] |

Values are means ± standard deviations (SD) (n = 5). Means with the same letter are not significantly different at $p < 0.05$ (Duncan's test).

## DISCUSSION

*T. garganica* leaf methanolic extract showed an insecticidal activity against stored product pests *T. castaneum*, and against crop pests *P. operculella* and *S. littoralis*. Apiaceae is one of the most known and used plants for their richness of secondary metabolites. Our previous work (Jmii et al. 2020) showed that *T. garganica* leaf methanolic extract is a source of four molecules: Two flavonoid glycosides, luteolin 7-*O*-glucoside and apigenin7-*O*-glucoside and two sesquiterpene lactones, thapsigargin, and 10β-acetoxy-8α-butyryloxy-11α-hydroxy-2β-((2-methyl-butanoyl) oxy)-1β*H*,6α*H*,7α*H*,11β*H*-guaian-3-en-12,6-olide. These molecules could be responsible for the toxicity, causing *T. castaneum* larvae total mortality (following ingestion and topical application treatment), repellent activity (on *T. castaneum* and *P. operculella*), larval growth delay until mortality and pupal malformations (in the case of *S. littoralis*). In fact, plants have developed an array of defensive strategies by producing biochemical defenses that restrict insect pests, to avoid herbivore damage. The biochemical traits include various toxic secondary metabolites (Ben-Khalifa et al. 2018). These authors indicated that many several compounds synthesized as secondary metabolites have repellent and antifeeding activity against insect pests. Such metabolites can also disturb the insect growth and development and inhibit their oviposition (Ben-Khalifa et al. 2018). It was reported for example that flavonoids influence the insect behavior, growth, and development. A number of flavones such as isoflavonoids, proanthocyanidins, flavonols, and flavonones have been investigated as feeding deterrents. A flavonoid isolated from *Tephrosia vogelii* (5-methoxyisoronchocarpin) has been found as feeding deterrent against *S. littoralis*. Judaicin was also found to be deterrent to the same insect (War et al. 2012). Additionally, it has been found that tannins and procyanidin polymers act as feeding deterrents, affecting the growth of some insects (such as *Euproctis chrysorrhoea* for tannins and *Aphis Craccivora* for procyanidin polymers). Condensed tannins from *Betula Neoalaskana* (Alaska paper birch), coated on birch leaves at 3% dry weight, reduced *Rheumaptera hastata* larva survival and pupal mass (War et al. 2012). Glycoalkaloids (solamargine, solasonine, and solasodine) were responsible for *T. castaneum* mortality (Hamouda et al. 2015a). Saunders et al (1992) determined that steroidal alkaloids possess insect repellent activity.

Methanolic extracts addition to *S. littoralis* diet caused developmental disturbances, demonstrated by weight gain inhibition and malformations at pupation. These results highlight two hypotheses: (1) the antifeeding proprieties of *Thapsia* leads to a reduction in the weight of caterpillars and therefore of the dwarf nymphs, and (2) reduced size can also be explained by the disruption of the insect's hormonal balance, thus causing early pupation (without going through all larval stages) (Chaieb 2005). Certainly, the leaves showed low antifeeding activity; so, the second hypothesis is more likely. RIR increase can prove also the low antifeeding effect of *T. garganica* leaf methanolic extracts. Caterpillars feeding on treated artificial diet showed an AD greater than control. Digestion increase could be a detoxifying way to get rid of toxins contained in *T. garganica*. Thus, Haubruge and Amichot (1998) showed that the insect contact with an insecticide leads to increased activity of xenobiotic degradation systems. ECD decrease could be explained by a decrease in the ability to detoxify toxic compounds present in the methanolic extracts that affect the conversion of the absorbed food into biomass. The reduction in ECD associated with secondary metabolite ingestion is a frequent phenomenon (Reese 1978; Lindroth et al. 1988; Koul et al. 1990; Appel and Martin 1992). This may be due to the interaction of the secondary metabolites with certain metabolic processes (Slansky 1992) or an indirect slowdown in growth, thereby by passing a greater proportion of absorbed food in the breath (Appel and Martin 1992). Amr (2001) found that the reduction in the efficiency of *S. littoralis* caterpillars to convert digested and ingested food causes a weight reduction. This explanation was confirmed by Reese and Beck (1976 a, b) and Dahlman (1977) results who suggested that ECI reduction is the result of TRC decrease.

A low mortality rate in *M. persicae* treated adults could be explained by their resistance to secondary metabolites contained in *T. garganica* leaf methanolic extract. In fact, it was reported that *Shistocerca gregaria* has the ability to tolerate tannins by hydrolyzing them rapidly, to avoid their damaging effects. In addition, they have the ability to adsorb them (for example on the thick peritrophic membrane) to restrict their passage (War et al. 2012). Furthermore, various detoxifying enzymes (increased activities of cytochrome P450 and esterase, involvement of glutathione S-transferases in the metabolism of secondary metabolites) could be induced by insects to avoid the damaging effects of reactive toxicants. It is the case for example for aphids (War et al. 2018).

To conclude, the present study showed that leaves from *T. garganica* have repellent and toxic activity against larvae and adults of *T. castaneum. P. operculella* showing sensitivity by a repellent effect at oviposition and a decrease of potato tuber moth larval penetration. Leaves were also able to delay larval growth until mortality and induce pupal deformities in *S. littoralis*. *M. persicae* was less sensitive to the treatment. Secondary metabolites contained in *T. garganica* leaf methanolic extract may prove to be important for the formulation of effective bio-insecticides. *T. garganica* methanolic leaf extract should be further investigated in order to elucidate more their insecticidal potentialities and to identify the bioactive molecules.

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**RESUME**

**Jmii G., Haouala R., Gharsallaoui S., Chaieb I. et Laarif, A. 2022. Activités répulsives et insecticides de l'extrait brut de *Thapsia garganica* contre quelques ravageurs importants. Tunisian Journal of Plant Protection 17 (1): 29-42.**

L'activité répulsive et insecticide des extraits méthanoliques de feuilles de *Thapsia garganica* (Apiacées) a été étudiée contre *Tribolium castaneum*, *Myzus persicae*, *Phthorimaea operculella* et *Spodoptera littoralis*. Une activité répulsive et toxique (par ingestion forcée et application topique) contre les larves et les adultes de *T. castaneum* a été démontrée. Le traitement par application topique a provoqué une inhibition totale de la croissance larvaire à 10%, jusqu'à la mortalité après 7 jours. La mortalité la plus élevée a atteint 94% à la même concentration. L'incorporation d'extraits méthanoliques dans l'alimentation artificielle des larves de *T. castaneum* à 10% a causé 100% de mortalité après 3 jours. L'extrait à 1% a eu un effet répulsif élevé sur *T. castaneum* après 60 min d'exposition tandis que *M. persicae* a été moins sensible. Le taux de mortalité a atteint 7,09 % à la concentration de 2%. Les femelles de *P. operculella* ont montré une sensibilité par un effet répulsif lors de la ponte. Le nombre d'œufs pondus sur les tubercules traités à 1% et 2% a diminué de manière significative avec 32% et 72%, respectivement. De plus, les extraits méthanoliques ont eu un effet préventif sur la pénétration larvaire de *P. operculella*. En fait, le nombre de larves a diminué de 30,46 % et 76,12 % dans les tubercules traités à 1% et 2%, respectivement. Pour *S. littoralis*, un faible effet anti-appétant a été enregistré sur ces chenilles. Cependant, la conversion des aliments ingérés et digérés en biomasse a diminué. De même, le taux de croissance a diminué. Quant à la digestibilité approximative, elle a augmenté. De plus, un retard de développement larvaire a été observé. Cette étude suggère que l'extrait des feuilles de *T. garganica* pourraient être employées comme bio-insecticides.

*Mots clés*: Activité insecticide, extrait brut, propriétés antiappétantes, *Thapsia garganica*

**ملخص**

**جميعي، غفران وربيعة حوالة وسمير غرسلاوي وإقبال الشايب وأسماء لعريف. 2022. الأنشطة الطاردة والمبيدة للحشرات للمستخلص الخام من *Thapsia garganica* ضد بعض الآفات الهامة.**

**Tunisian Journal of Plant Protection 17 (1): 29-42.**

تم تقييم الأنشطة الطاردة والمبيدة للحشرات للمستخلصات الميثانولية لأوراق *Thapsia garganica* ضد الحشرات *Tribolium castaneum* و *Myzus persicae* و *Phtorimaea operculella* و *Spodoptera littoralis*. تم إثبات الأنشطة الطاردة والمبيدة للحشرات (عن طريق الابتلاع القسري والتطبيق الموضعي) ضد *T. castaneum*. تسببت المعاملة الموضعية في عرقلة نمو اليرقات بنسبة 10% حتى النفوق بعد 7 أيام. تم تسجيل أعلى معدل وفيات بنسبة 94% وبنفس التركيز. أدمجت المستخلصات الميثانولية في النظام الغذائي الصناعي ليرقات *T. castaneum* بنسبة 10% مما تسبب في وفيات بنسبة 100% بعد 3 أيام. كان للمستخلص بنسبة 1% تأثير طارد عالي على *T. castaneum* بعد 60 دقيقة من التعرض، بينما كان *M. persicae* أقل حساسية. بلغ معدل الوفيات 7.09%بتركيز 2%. أظهرت *P. operculella* الأنثى حساسية من خلال تأثير طارد في وضع البيض. انخفض عدد البيض الذي تم وضعه على درنات بطاطا معاملة بنسبة 1% و 2% بشكل ملحوظ بنسبة 32% و 72%، على التوالي. بالإضافة إلى ذلك، كان للمستخلصات الميثانولية تأثير وقائي على اختراق يرقات *P. operculella*. لكن، انخفض عدد اليرقات بنسبة 30.46% و 76.12% في الدرنات المعاملة بنسبة 1% و 2%، على التوالي. بالنسبة للحشرة *S. littoralis*، تم تسجيل تأثير منخفض ضد التغذية على اليرقات، حيث انخفض تحويل الطعام المهضوم والمبتلع إلى كتلة حيوية. نفس الشيء بالنسبة إلى معدل النمو الذي أظهر انخفاضًا، لكن قابلية الهضم التقريبية ازدادت، إضافة إلى ذلك حدوث تأخير في نمو اليرقات. تشير هذه الدراسة إلى أنه يمكن استخدام مستخلص أوراق *T. garganica* كمبيد حشري حيوي.

*كلمات مفتاحية*: خصائص مضادة للتغذية، مستخلص خام، نشاط مبيد للحشرات، *Thapsia garganica*

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