

Potentials of the Extracts of Algerian Saharan Plant *Cotula cinerea* for the Management of Two Insect Pests, *Aphis fabae* and *Tribolium castaneum*

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ABSTRACT

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The insecticidal potential of the Saharan plant *Cotula cinerea*, was evaluated on two insect species namely *Aphis fabae* and *Tribolium castaneum* by topical application (contact toxicity) and repellency test. A crude ethanolic extract of aerial part of the plant was prepared and tested in the laboratory on adults of both species. For contact toxicity, five doses were tested on each of the two species 1.56, 3.12, 6.25, 12.5 and 25 mg/ml for *A. fabae* and 25, 50, 250, 350 and 500 µg/insect for *T. castaneum*. The repellency of the extract was studied at the dose 500 µg/insect for *T. castaneum* and 25 µg/ml for *A. fabae*. Results showed that the repellency of the extract increased with exposure time and the highest rates were observed after 4 h of exposure ($72.33 \pm 22\%$ for *T. castaneum* and $87 \pm 3.6\%$ for *A. fabae*). For insecticidal activity, at the highest doses (25 mg/ml and 500 µg/ml), 100% mortality is obtained 72 h after treatment for *A. fabae* and after 48 h for *T. castaneum*. The extract of this plant was found to be more toxic against *T. castaneum* adults. LD₅₀ calculated 24 h after treatment for the two species is estimated at 1.7 mg/ml for *A. fabae* and at 30.3 µg/insect for *T. castaneum*. The extract of this plant inhibited the activity of acetylcholinesterase (AChE) in both insect species. This result suggests that this plant has a neurotoxic effect on *A. fabae* and *T. castaneum*. The results of phytochemical study showed that the plant is mainly rich in flavonoids, gallic tannins, alkaloids, saponosides and glucosides. The insecticidal effect obtained in this study could be due to the synergetic action of all constituents of the extract. Results suggest the possibility of using the extracts of this plant in integrated pest management to replace the chemical insecticides.

Keywords: *Aphis fabae*, *Cotula cinerea*, ethanolic extract, repellent, toxicity, *Tribolium castaneum*

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One of the major causes of crop losses is phytophagous insect species (Isman 2000). Insect infestation has a direct impact on agricultural food production and stored products as they may account for 20-30% production loss and in severe cases, they cause total loss (De Geyter et al. 2007). Aphids can cause damage to hundreds of host plants in both the field and under protection (Mardani-Talaei et al. 2016), by sucking plant sap, transmitting plant viruses, and excreting honeydew that induces fungal growth (Sprawka et al. 2011). The black bean aphid, *Aphis fabae* (Homoptera: Aphididae) is one of the most important green house and open-field crop pests with a wide range of hosts, which causes damages through feeding on vegetable sap and transmitting viral diseases (Ravan et al. 2019).

On the other hand, in stored grain mill, insect damage may account for 10-40% yield loss worldwide (Matthews 1993). The red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae), is a cosmopolitan and serious pest of many stored products and other food and animal-based products (Brandt et al. 2019; Hagstrum and Subramanyam 2009; Hill 2003). Larvae and adults cause extensive damage to cereals and oilseeds (Kumar 2017). *T. castaneum* caused up to 40% reduction in grain weight (Ajayi and Rahman 2006; Rees 2007). It consumes endosperm of the seeds leaving them with coagulating consistency and moldy smell (Keskin and Ozkaya 2013). Qualitative damage is due also to loss of nutritional, industrial and marketing properties (Padin et al. 2002). In addition, recent researches have shown that the red flour beetle transmits some phytopathogenic fungi and bacteria such as *Bacillus cereus*, *Pseudomonas aeruginosa*, *Aspergillus flavus* and *A. niger* (Bosly and El-Banna 2015; Prabha-Kumari 2011).

In many agricultural systems, the insect pest management is mainly based on synthetic insecticides including organophosphorus, carbamates, neonicotinoid such as imidacloprid for aphids (Jiang et al. 2018) and fumigation with methyl bromide and phosphine for managing a wide range of stored grains pests (Bell 2000). However, application of chemical insecticides has been reported to have dangerous effects on human health and environment due to the toxic residues in treated products and environmental pollution. Increased applications of insecticides result also in the development of insects' resistance and increasing costs of synthetic insecticide (Dubey et al. 2008; Isman 2006). To preserve human health, minimize the effects of chemicals on non-target organisms and attain maximum food security, there is a need to develop some safe pesticides and suitable agents. An alternative approach is the use of botanicals against pests (Zaka et al. 2019). Moreover, plant-based products do not accumulate into the environment and are least toxic to non-target organisms, including humans (Benelli 2015; Govindarajan et al. 2016).

Several plant extracts, essential oils and their active compounds have been reported to be effective against different groups of insect pests including stored grain pests (Deb and Kumar 2020; Maazouna et al. 2017; Matosa et al. 2020; Zaka et al. 2019) and aphids (Gouvêa et al. 2019; Jiang et al. 2018; Smith et al. 2018). In Algeria, numerous studies were conducted on plant-based products to find a better alternative to chemical insecticides. In this regards, different extracts, essential oils from various aromatic and medicinal plants have been tested and have shown to be very promising in biological control of different insect pests models (Acheuk et

al. 2012; Benabdallah et al. 2018; Bouguerra et al. 2018; Brahmi et al. 2016; Chenni et al. 2020; Djeghader et al. 2018; Draouet et al. 2020; Dris et al. 2017a,b).

Cotula cinerea (Asteraceae) is a small xerophytic plant widely distributed in sandy and desert ground from Algeria and Morocco (Djellouli et al. 2013; Markouk et al. 1999). This plant is usually known as “Guertofa” among the local people in Northern Sahara and is commonly used in traditional medicine in the southwest of Algeria. Several diseases are treated by this plant like colic, cough, diarrhea, headache, and digestive disorders. All parts of the plant are used in different forms (maceration, decoction, infusion or inhalation) according to the treated diseases (Djellouli et al. 2013, 2015). This plant contains a variety of chemical compounds such as: flavonoids, germacranolide (Dendougui et al. 2012), terpenes and essential oils (Boussoula et al. 2016). Plant’s chemical composition has been shown to vary as a function of geographic location and time of collection. Essential oils from the Algerian plant showed the presence of 22 compounds during the flowering period with 3-carene as a major compound and 21 chemical compounds during the fruiting period with the dominance of thujone (Atef et al. 2015), while the Moroccan plant was characterized by dominance of the 3-iso-thujanol (Boussoula et al. 2016). However, for Egyptian plant, camphor was identified as a main constituent (El-Fiky et al. 2017). The activity of *C. cinerea* has been studied extensively and several biological properties have been reported for this plant, antibacterial (Boussoula et al. 2016; Chouikh et al. 2015; Larbi et al. 2018), antifungal (Boussoula et al. 2016), antioxidant (Kasrati et al. 2015), cytotoxic (Larbi et al. 2018), analgesic and molluscicide activities (Markouk

1999). But only a limited number of studies have assessed its insecticidal activity. According to our bibliographic investigations, the insecticidal activity of this plant has been the subject only of a few researches, the study of Kasrati et al. (2015) and that of Markouk et al. (2000). However, in order to complete the insecticidal investigations of this plant, the aim of the present study is to investigate, under laboratory conditions, the insecticidal activity, the repellency, and some biological effects of the ethanolic extracts of the Algerian endemic plant *C. cinerea* against two major insect pests, *T. castaneum*, which is one of the main pests of stored grain products, and *A. fabae*, one of the key field and greenhouse insect pest causing direct damage and transmission of viral diseases. In addition, the effect of this extract on the activity of acetylcholinesterase (AChE), which is an important enzyme of the insect nervous system, will also be determined.

Plant material.

C. cinerea was harvested during the spring season, in March 2018 in Touggourt area at the Algerian Sahara (33° 04' 13" North, 06° 05' 49" East). The freshly collected plant was washed with water, dried in the shade and then ground into a fine powder. The recovered plant powder is stored in glass boxes, hermetically closed until extraction.

Crude ethanolic extract preparation and phytochemical screening.

The crude ethanolic extract of the aerial part of *C. cinerea* was prepared, according to Acheuk et al. (2012) method with small modifications, by macerating the powder (100 mg) for 3 days in ethanol (100 ml), followed by filtration and evaporation at 40 °C. The dried extract was kept at 4 °C until further use. The

ethanolic extract was tested for the main secondary metabolites usually sought for plants, alkaloids, sugar, phenolic compounds, flavonoids, saponins, tannins, iridois and coumarins. Phytochemical screening of the extract was carried out according to the standard method of Dohou et al. (2003). Visible color change or precipitate formation was taken into consideration for presence (+) or absence (-) of particular active constituents.

Insect rearing.

For *A. fabae*, stock of adult wingless aphids used was collected randomly from infested faba bean field in Ain Taya, Algiers area. Aphids was reared on the leaf blades of faba bean *Vicia faba* Plants leaves were changed each two days and fresh leaves infested with aphids taken from the discarded leaves. Aphid's population was kept in fine mesh cages of size 45×45×45 cm under laboratory at temperature of 22 ± 2 °C, 40-80% RH and a long photoperiod of 14 h light. Adult apterous females (1-2 old days) were selected for all subsequent experiments.

For *T. castaneum*, initial stock culture of *T. castaneum* was obtained from Entomology Laboratory of National Institute of Plant Protection, El-Harrach, Algiers. Beetles were reared, at Zoology Laboratory of Boumerdes University, in glass containers (0.5 liter) containing wheat flour mixed with brewer's yeast (10:1; w/w). The culture was maintained in the dark in growth incubators at 28-30 °C and 70-80% RH without exposure to any insecticide. Adult insects of 1-5 days post emergence were used in experiments.

All bioassays were carried out under the same laboratory conditions as the cultures.

Insecticidal bioassays.

Repellent activity. To assess the repellent activity of *C. cinerea* crude ethanolic extract against *T. castaneum* and *A. fabae* adults, an area preference method of McDonald et al. (1970) was adopted (with slight modifications). The test was carried out under the same conditions described above for the mass rearing for each insect species using glass Petri dishes as containers. Filter paper (Whatman N° 1.9 mm) was cut in two halves. The test extract was tested at the concentration of 500 µg/insect for *T. castaneum* and 25 mg/ml for *A. fabae*. Test compounds were dissolved in acetone for *T. castaneum* (Hu et al. 2019; Olivero-Verbel et al. 2013; Zapata and Smagge 2010) and in ethanol for *A. fabae* (Gouvêa et al. 2019). A volume of 500 µl of the test solution was applied uniformly to half filter paper disc. Another half was treated with the solvent only. Treated and untreated halves were air dried, carefully fixed and placed in Petri dishes. For each test twenty adults were introduced at the center of the Petri dishes. Numbers of insects on the two halves disks were recorded after 2 and 4 h from the beginning of the test. Three replicates were made for each trial. The percentage of repellency was calculated as follows: $PR (\%) = (N_c - N_t) / (N_c + N_t) \times 100$, with N_c : Number of insects on control part, N_t : Number of insects on treated part. The average values were then categorized according to the scale in Table 1.

Table 1. Classification of the repellency rate according to McDonald et al. (1970)

Repellency rate (%)	Class
0.01 to 0.1	0
0.1 to 20	I
20.1 to 40	II
40.1 to 60	III
60.1 to 80	IV
80.1 to 100	V

Contact toxicity. For *A. fabae*, bioassays were conducted in Petri dishes under the same laboratory conditions as mass rearing. Forty aphids were transferred to Petri dishes on fresh faba bean leaves serving as a support for the aphids. Wet cotton discs were placed under the faba bean leaves to keep them fresh during the test period. Five concentrations (25, 12.5, 6.25, 3.12 and 1.56 mg/ml), were prepared with the initial dry crude extract, using ethanol as solvent. One microliter (1 μ l) of each insecticide solution was applied topically (contact) onto the pronotum of each adult aphid using micropipette. Controls were treated with only absolute ethanol. Mortality percentages were determined for each treatment after 2, 4, 24, 48, 72 and 96 h. The corrected mortality rates were measured using Abbott's formula (Abbott, 1925). Corrected mortality (%) = $(M1 - Mc)/(100 - Mc) \times 100$, where M1 (%) is the mortality of the treated groups and Mc (%) the mortality of the control groups.

Probit analysis (Finney 1971) was conducted to estimate lethal dose (LD₅₀, 24 h after treatment) with its 95% confidence interval.

For *T. castaneum*, bioassays were carried out using five concentrations of the crude ethanolic extract: 25, 50, 250, 350 and 500 μ g/insect. Test solutions were prepared using acetone as solvent.

Unsexed adult insects were immobilized 15 min before the beginning of the test, using cold. Aliquots of 5 μ l of each tested concentration were topically applied onto the thorax of insects using micropipette. For each concentration, 20 insects were used in 3 replicates. Acetone was used for the control test. After treatment, insects were transferred into glass Petri dishes containing a mixture of wheat flour and brewer's yeast given as food. All treated and control insects were kept under the same conditions as described for the insect rearing. Insect's mortality was recorded daily. Probit analysis (Finney 1971) was conducted to estimate lethal dose (LD₅₀, 24 h after treatment) with its 95% confidence interval.

Enzymatic assays.

The acetylcholinesterase (AChE) activity was carried out following the method of Ellman et al. (1961) using acetylthiocholine as a substrate. Aphids or *T. castaneum* adults were sampled, 2 h after treatment, from control and treated groups (at low dose: 1.56 mg/ml for aphids and 25 μ g/insect for *T. castaneum*). Pools of twenty adult aphids were homogenized in the solution containing 38.03 mg of ethylene glycol tetra-acetic (EGTA), 1 ml triton X-100, 5.845 g NaCl and 80 ml tris buffer (10 Mm, pH 7). The homogenate was centrifuged (5000 g for 5 min at 4 °C),

and the resulting supernatant was used for enzymatic assay. The AChE activity was measured in aliquots (100 µl) of resulting supernatants added to 100 µl of 5-5' dithiobis-(2-nitrobenzoic acid) (DNTB) in tris buffer (0.01 M, pH 8) and 1 ml tris (0.1 M, pH 8). After 5 min, 100 µl of acetylthiocholine was added. Measurements were conducted at a wavelength of 412 nm with a run time of 20 min. Enzyme activities were expressed as µmol/min/mg proteins.

Statistical analysis.

Results were expressed as means ± standard deviation (SD). Bioassays data were performed using Probit analysis (Finney, 1971) to find out the median lethal dose (LD₅₀) with their 95% fiducial limits using SPSS version 20.0 for

Windows. To identify significant effects of the treatments on the measured variables, all experimental results were submitted for an adequate statistical analysis (ANOVA, Student test, Mann-Whitney U test), depending on the normality or not of the results, using the SPSS software version 20.0 for Windows.

RESULTS

Phytochemical screening.

Results of the phytochemical screening of the ethanolic extract of *C. cinerea* presented in Table 2, indicate that the plant is mainly rich in flavonoids, gallic tannins, alkaloids, saponins and glucosides. Moreover, results revealed that the plant does not contain anthocyanins, coumarins and iridoids.

Table 2. Qualitative phytochemical screening of the crude ethanolic extract of the aerial part of *Cotula cinerea*.

Alkaloids	Anthocyanins	Coumarins	Tannins	Saponins	Iridoids	Flavonoids	Glucosides
++	-	-	+++	++	-	++	+++

(-): Absence, (+): Low presence, (++) : Moderate presence, (+++): Strong presence

Repellent activity.

The results of repellency assays for *C. cinerea* ethanolic extract are presented in Table 3. Data showed that at tested concentrations (25 mg/ml for *A. fabae* and 500 µg/insect for *T. castaneum*), the studied extract exhibited promising repellent effect against the two insects. The activity increased significantly ($P < 0.05$) with prolonged

exposure time. The highest repellent activity was obtained after 4 h of exposure and is 72.33 ± 22 and $87 \pm 3.6\%$, respectively for *T. castaneum* and *A. fabae*. *A. fabae* was previously more sensitive than *T. castaneum* to the extract since the V repellency class with > 80% repellency was obtained only for this insect species.

Table 3. Repellent activity of the crude ethanolic extract of the plant *Cotula cinerea* against adults of *Tribolium castaneum* and *Aphis fabae* at different exposure times

Insects species	<i>T. castaneum</i>		<i>A. fabae</i>	
Extract doses	500 µg/insect		25 mg/ml	
Time	2 h	4 h	2 h	4 h
Repulsion (%)	26.66 ± 11.54*	72.33 ± 22*	35 ± 5.0*	87 ± 3.6*
Class	II	IV	II	V

For *T. castaneum*, * denotes significant difference at $P < 0.05$ (Two way ANOVA test: $p = 0$). For *A. fabae*, 2h, * denotes significant difference at $P < 0.05$, (Student test: $t(4), 15.56, p = 0$) and 4 h, * denotes significant difference at $P < 0.05$, (Student test: $t(4), 10.61, p = 0$). $N = 20$ insects/replicate and 3 replicates were made for each trial.

Insecticidal activity.

Results of the insecticidal activity of ethanolic extract of *C. cinerea* against *A. fabae* and *T. castaneum* are

presented in Figs. 1, 2 and Table 4. Results showed that the tested extract had significant ($P < 0.05$) insecticidal efficiency against aphid and beetle adults.

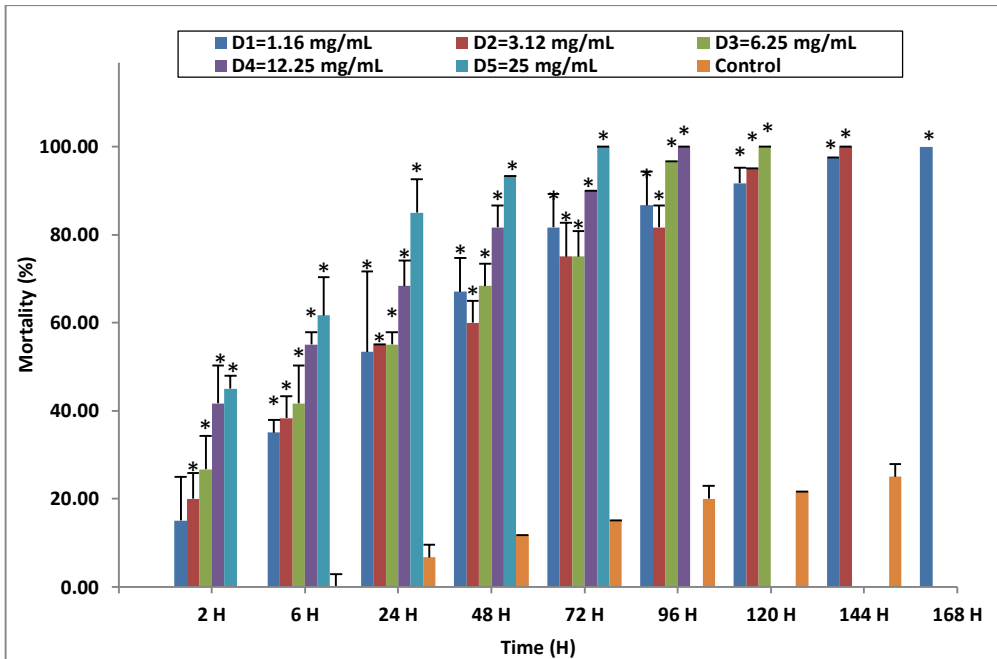


Fig. 1. Concentration-response relationships for the contact toxicity effect of crude ethanolic extract of *Cotula cinerea* against *Aphis fabae* adults. $N = 40$ insects/replicate, 3 replicates. *: denotes significant different at $P < 0.05$ compared to the control (ANOVA test).

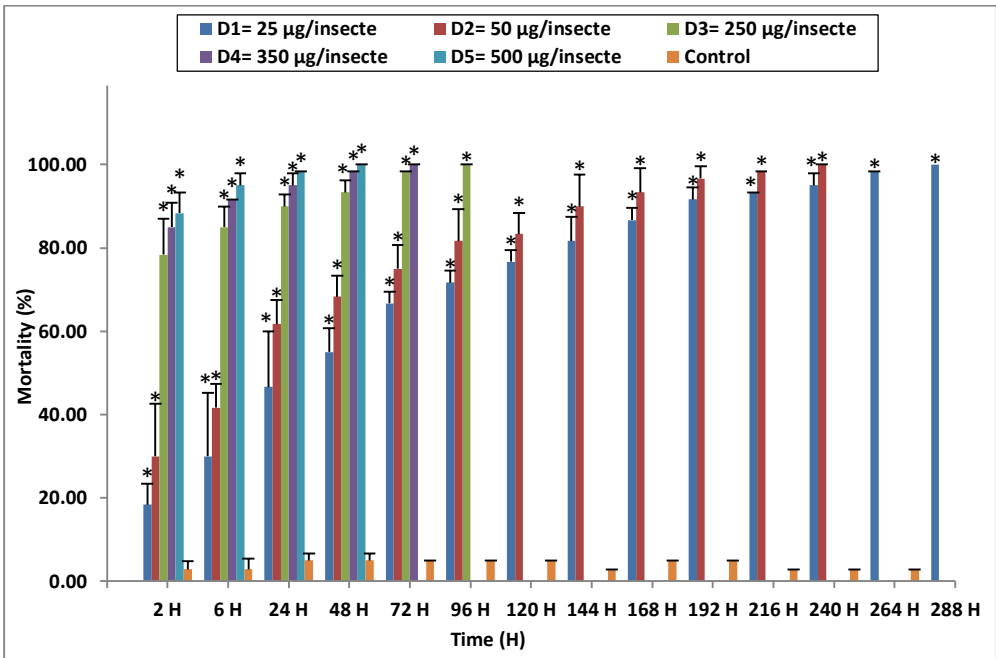


Fig. 2. Concentration-response relationships for the contact toxicity effect of crude ethanolic extract of *Cotula cinerea* against *Tribolium castaneum* adults. N = 20 insects/replicate, 3 replicates. *: denotes significant different at $P < 0.05$ compared to the control (ANOVA test).

Table 4. Contact toxicity (LD_{50} values) of the crude ethanolic extract of *Cotula cinerea* against adults of *Aphis fabae* and *Tribolium castaneum* 24 h after treatment

Insect species	LD_{50} values	Confidence limits (95%)	Slope	Chi square (χ^2)	df
<i>T. castaneum</i>	30.3µg/insect	20.04 - 40.93	2.48	8.456	13
<i>A. fabae</i>	1.70 mg/mL	0.44 - 3.084	1.22	9.241	13

N = 40 insects/replicate for *A. fabae* and 20 insects/replicate for *T. castaneum*, 3 replicates for each insect species.

Toxicity significantly increased ($P < 0.05$) with increasing of doses and time exposure for the two insects *A. fabae* and *T. castaneum*. The total dead adults (100% mortality) was obtained three day after treatment with the higher tested dose (25 mg/ml) for *A. fabae* (Fig. 1) and after

2 days for *T. castaneum* at 500 µg/insect (Fig. 2). Results of Probit data analysis (Table 4) showed that the two insects were sensitive to the extract. LD_{50} calculated 24 h after treatment was 30.3 µg/insect for *T. castaneum* and 1.7 mg/ml for *A. fabae*.

Enzymatic activity.

The enzymatic activity of AChE was measured for both insects. Results are shown in Figs. 3, 4. The extract of *C. cinerea* was neurotoxic to both insect

species: *A. fabae* (Fig. 3) and *T. castaneum* (Fig. 4). AChE activity was significantly ($P < 0.05$) reduced in the treated series compared to the control.

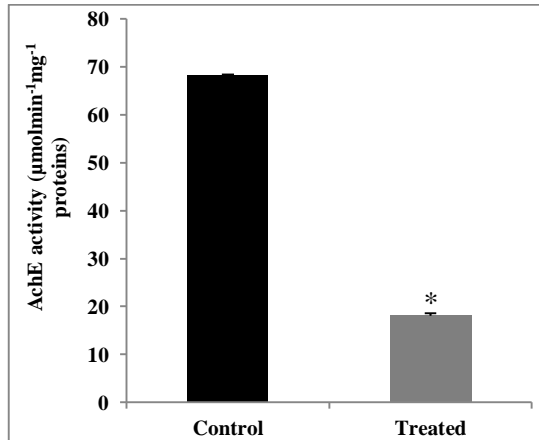


Fig. 3. Effect of crude ethanolic extract of *Cotula cinerea* on AChE activity of *Aphis fabae* (Mean \pm SD). N = 20 aphids, 3 replicates. * denotes significant difference at $P < 0.05$ compared to the control Student test.

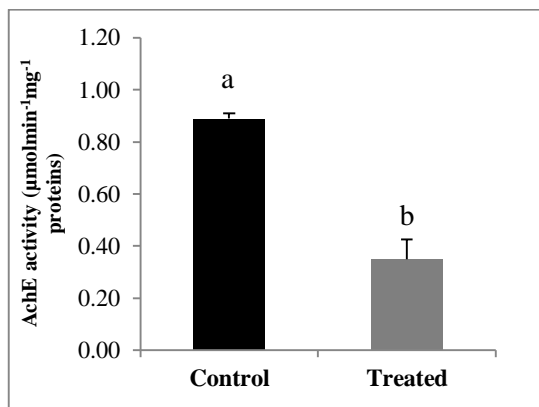


Fig.4. Effect of crude ethanolic extract of *Cotula cinerea* on AChE activity of the adults of *Tribolium castaneum* (Mean \pm SD). N = 20 insects, 3 replicates. Different letters denote significant difference at $P < 0.05$ of the treatment compared to the control by Mann-Whitney U test.

DISCUSSION

Several species of Asteraceae are known for their insecticidal activities against insect pests. *C. cinerea* is one of the Asteraceae species whose active compounds are known for their therapeutic, bactericidal, antifungal, antioxidant and antitumoral properties (Fathy et al. 2017). All these activities have been extensively studied in most cases, but only a limited number of studies have assessed their insecticidal activity. To our knowledge, there has been only two previous reports evaluating the insecticidal properties of its essential oils against *T. castaneum* (Kasrati et al. 2015) and its organic extract on *Anopheles labranchiae* mosquito larvae (Markouk et al. 2000). Indeed, the present work is undertaken to complete the insecticidal investigation of this plant. The obtained results have demonstrated that the studied extract had a potent repulsive and insecticidal effect against *T. castaneum* and *A. fabae* adults. Results clearly showed a significant enhancement of repellency with increasing exposure period on both insect species. The highest repellent activity was obtained after 4 h of exposure and is $72.33 \pm 22\%$ and $87 \pm 3.6\%$, respectively for *T. castaneum* and *A. fabae*. Moreover, considering the potential of aphids as an important vector of plant viruses, the use of plant extracts and essential oils with repellent properties could be a useful method of preventing viral transmission (Czerniewicz et al. 2018). Previous studies have reported that Asteraceae plants may be a promising source of aphid repellent. Results obtained by Czerniewicz et al. (2018) demonstrated that in laboratory bioassays, essential oils from plants belonging to Asteraceae family such as *Achillea millefolium*, *Artemisia absinthium*, *Santolina chamaecyparissus*, *Tanacetum patula* and *Tanacetum vulgare* displayed

anti-settling activity against the green peach aphid females. This inhibition is mainly caused by their repellency. Also, it was found that aniseed, peppermint and lemongrass essential oils at $0.15 \mu\text{l}/\text{cm}^2$, were repellent against apterous females of the bird cherry-oat aphid, *Rhopalosiphum padi* L (Pascual-Villalobos et al. 2017).

Many plant products, such essential oils (Gallardo et al. 2012; Taban et al. 2017), crude extracts (Phankaen et al. 2017) and pure compounds (Yu et al. 2017) have been screened for their repellent activity against stored grain pests, obtained results of these research have shown that *T. castaneum* can be repelled by many of them. Based on our results, *C. cinerea* extract was shown to possess repellent activity towards *T. castaneum* adults. Similar observations have also been reported that significant repellency was observed with hexane, dichloromethane, ethyl acetate and methanol extracts from *Coffea arabica* against *T. castaneum*. The active ingredient isolated from dichloromethane extract was identified as caffeine and it was responsible of the high repellency of the total extract (Phankaen et al. 2017). Using the same method than that of our study, filter paper arena test, Zapata and Smagghe (2010) have found that essential oils extracted from the leaves and bark of *Laurelia sempervirens* and *Drimys winteri* had a very strong repellent activity towards *T. castaneum*. More than 90% repellency has been achieved after 4 h exposure with *L. sempervirens* oils at low concentrations of $0.032 \text{ liter}/\text{cm}^2$. In recent studies, Hu et al. (2019) reported that the repellent property of essential oils of *Artemisia brachyloba* against *T. castaneum* adults was 73.33-96.67%, while the repellent property of α -terpineol of $0.315 \mu\text{l}/\text{cm}^2$ was 75-100%. Moderate repellency (46.67-61.67%) was achieved by $0.315 \mu\text{l}/\text{cm}^2$ davanone. On

the other hand, Rajabpour et al. (2019) reported that both ethanolic and aqueous extracts of *Conocarpus erectus*, were significantly repellent for adults of *T. castaneum*, while there was no significant repellency effect on larval stages.

The crude ethanolic extract of the studied plant showed also significant strong contact toxicity against *A. fabae* and *T. castaneum* adults with LD₅₀ of 30.30 µg/insect and 1.7 mg/l respectively for *T. castaneum* and *A. fabae*, 24 h after treatment. Results clearly showed a significant enhancement of mortality on increasing dose and exposure period on both insect species. In other studies with *C. cinerea* extract, Markouk et al. (2000) suggested that high concentration of ethyl ether and ethyl acetate extract of *C. cinerea* are needed to achieve larvicidal activity against *Anopheles labranchiae* mosquito larvae. In contact assays, Feng et al. (2020) showed that supercritical CO₂ fluid extract of *Valeriana officinalis* exhibited strong toxicity to *T. castaneum* (LD₅₀ = 10.0 µg/adult). In accordance with our results, Habib and Karim (2016) found that effectiveness of ethyl acetate extract of *Calotropis gigantean* flower was increased with exposure time. The maximum residual toxicity was observed with LD₅₀ of 0.39 and 0.716 mg/cm² for 6th instar larvae and adults of *T. castaneum*, respectively, after 48 h of exposure. The presence of methoxy-4-vinyl phenol in this extract justified its effective insecticidal activity. Also, the results obtained by Rajabpour et al. (2019) showed that the aqueous and ethanolic extracts of *Conocarpus erectus* leaves had relatively high toxic effects on adults and larvae of the *T. castaneum*.

For insecticidal effect, our results against *A. fabae* are in accordance with previous results that demonstrated insecticidal activity of plant extracts or essential oils on aphids. In this respect,

Jiang et al. (2018) reported that petroleum ether fraction of *Robinia pseudoacacia* seeds exhibited strong insecticidal activity against two aphid species: cotton aphid and cabbage aphid with LD₅₀ values of 7.04 ng/insect and 6.87 ng/insect, respectively (at 24 h post-treatment), using a topical application method. Also, the seed extract formulation showed notable efficacy on aphids in a field test. Mortality was above 95% by 7 days after treatment in the oilseed rape field. Similarly, in the case of *Solanum incanum* plant, it was found that the aqueous crude fruit extract of this plant, showed insecticidal and deterrent activities against green peach aphids *Myzus persicae* (Umar et al. 2015). In the same context, investigation of insecticidal effect of ethanolic extract of *Ungernia severtzovii* bulbs against the grain aphid *Schizaphis graminum*, conducted by Chermenskaya et al. (2012), showed that 100% mortality of aphid nymphs and near 90% for aphid females were caused by 1% concentration. LC₅₀ value was 2.35 g/l for female aphids. Also, results found by Ravan et al. (2019) showed that essential oil of *Teucrium polium* at lowest concentration (0.88 µl/l air) caused 18% mortality after 24 h compared to 82.5% mortality at the highest concentration (12 µl/l air).

In the present study, the toxic and repellent properties of *C. cinerea* extract against *A. fabae* and *T. castaneum* may be related to high alkaloids and phenolic compounds (flavonoids and tannins) in the plant. Results of phytochemical screening showed that *C. cinerea* extract is a mixture of several secondary metabolites and confirmed the presence of many bioactive molecules such as flavonoids, tannins, alkaloids, saponins and glucosides which form plants' defense system (Singh et al. 1997). The same constituents were found by

Djoulouli et al. (2013) for *C. cinerea* harvested from the region of Bechar (South of Algeria).

It is recognized that plant essential oils and extracts possess multiple pest control properties, but their mode of action has not been completely elucidated. It should be emphasized that knowledge of the target sites, the physiological mode of action, and the extract doses needed to kill insect pests are important for their effective and safe application in insect control (Czerniewicz et al. 2018). The insecticidal effect obtained in our study against the two insects could be due to the synergistic relationship between many constituents of the extract. It is recognized that plant secondary metabolites have significant biological activities associated with the presence of alkaloids, iridoids, monoterpenes, sesquiterpene, lactones, di- and triterpenes, naphthoquinones, anthraquinones, coumarins, phenylpropanoids, flavonoids, and other types of phenolics. These compounds can act as insecticidal, ovicidal, ovipositional deterrents, feeding deterrents and growth retardants to pests through acute toxicity, interference with the consumption and/or utilization of food and enzyme inhibition (Céspedes et al. 2006; Selin-Rani et al. 2016). Impact of photochemical products on detoxification enzymes has been investigated in several studies. Recent studies proved that essential oils, alkaloids and extracts of some plants are potent neurotoxic and giving symptoms similar to those produced by organophosphates and carbamates insecticides. Inhibition of AChE activity could be a possible mode of action of these compounds.

Data in our study showed that *C. cinerea* was neurotoxic to *A. fabae* and *T. castaneum* and its extract was able to interfere with AChE enzyme and had

significant inhibitory effect on insect's AChE activity. Our results were in agreement with previous studies that revealed high AChE inhibitor potential of plant extracts on aphids and stored product insect pests. Mami-Maazoun et al. (2017), reported that *Urginea maritima* bulb extract inhibited AChE activity of *Sitophilus oryzae* and this inhibition could possibly be due to its high content in phenolic compounds and alkaloids. Similarly, Phankaen et al. (2017) showed that, in vivo study, dichloromethane, ethyl acetate and methanol extracts from *Coffea arabica* inhibited AChE activity on *T. castaneum* adults. Hu et al. (2019) reported that *Artemisia brachyloba* essential oils and its major compound, α -terpineol, can significantly inhibited the activity of AChE on *T. castaneum* adults at high concentrations (100 and 200 μ l/l air). Inhibition of AChE is known as a target enzyme for stored product insect control chemicals, which can block the neurotransmitter acetylcholine at the synaptic cleft (López and Pascual-Villalobos 2010). Against aphids, Czerniewicz et al. (2018) reported that highest reduction in AChE activity (about 50% inhibition) was shown at 24 h and 48 h after treatment with essential oils from the Asteraceae plants, *Achillea millefolium* and *Santolina chamaecyparissus*. The neurotoxic action measured by the activity of AChE could be due to the effect of alkaloids present in abundance in this plant, which were the cause of the early mortalities of the adults of the two insects.

Further studies on the phytochemical composition and mechanism of action are needed to integrate the extracts of this plant in insect pest management program in replacement of chemical insecticides.

RESUME

Acheuk F., Abdellaoui K., Lakhdari W., Chahbar N., Dehliz A., Belaid M., Baouche N., et Bouazouz, H. 2020. Potentialités de l'extrait de la plante saharienne algérienne *Cotula cinerea* pour la gestion de deux insectes ravageurs, *Aphis fabae* et *Tribolium castaneum*. *Tunisian Journal of Plant Protection* 15 (2): 41-57.

Le potentiel insecticide de la plante saharienne *Cotula cinerea*, a été évalué sur deux espèces d'insectes *Aphis fabae* et *Tribolium castaneum* par application topique (toxicité par contact) et par effet répulsif. Un extrait éthanolique brut a été préparé et testé au laboratoire sur des adultes des deux espèces. Pour la toxicité par contact, 5 doses ont été testées sur chacune des deux espèces: 25, 12,5, 6,25, 3,12 et 1,56 mg/ml pour *A. fabae* et 25, 50, 250, 350 et 500 µg/insecte pour *T. castaneum*. L'effet répulsif de l'extrait a été évalué à la dose de 500 µg/insecte pour *T. castaneum* et à 25 mg/ml pour *A. fabae*. Les résultats obtenus ont montré que la répulsion de l'extrait augmente avec le temps d'exposition et les taux les plus élevés ont été observés après 4 h d'exposition ($72,33 \pm 22\%$ pour *T. castaneum* et $87 \pm 3,6\%$ pour *A. fabae*). Pour l'activité insecticide sur les pucerons, une mortalité de 100% a été obtenue 72 h après le traitement à la dose la plus élevée (25 mg/ml). L'extrait de cette plante s'est avéré plus toxique contre les adultes de *T. castaneum*; une mortalité de 100% a été atteinte 48 h après traitement avec la dose la plus élevée (500 µg/insecte). La DL_{50} calculée 24 h après traitement pour les deux espèces est estimée à 1.7 mg/ml pour *A. fabae* et à 30.3 µg/insecte pour *T. castaneum*. L'extrait de cette plante a inhibé l'activité de l'acétylcholinestérase (AChE) chez les deux espèces d'insectes. Ce résultat suggère que cette plante a un effet neurotoxique sur le puceron et le tribolium. Les résultats de l'étude phytochimique ont montré que la plante est principalement riche en flavonoïdes, tanins galliques, alcaloïdes, saponosides et glucosides. L'effet insecticide obtenu dans cette étude pourrait être dû à l'action synergique de l'ensemble des constituants composant l'extrait brut. Les résultats suggèrent la possibilité d'utiliser les extraits de cette plante dans les programmes de lutte intégrée contre les ravageurs pour remplacer les insecticides chimiques.

Mots clés: *Aphis fabae*, *Cotula cinerea*, extrait éthanolique, répulsif, toxicité, *Tribolium castaneum*

ملخص

عاشق، فاطمة وخميس عبداللوي ووسيمة اخضاري ونورة شهبازي وعبد الزحمان دهليز ومسعودة بلعيد ونوال بعوش وحنان بوغزوز. 2020. إمكانات المستخلصات للنبتة الصحراوية الجزائرية *Cotula cinerea* لإدارة حشرة من الفول الأسود (*Aphis fabae*) وحشرة سوسة الطحين الحمراء (*Tribolium castaneum*).

Tunisian Journal of Plant Protection 15 (2): 41-57.

تم تقييم الإمكانية الطاردة والمبيدة للمستخلص الإيثانولي الخام للنبتة الجزائرية الصحراوية *Cotula cinerea* ضد نوعين من الحشرات هما من الفول الأسود (*Aphis fabae*) وسوسة الطحين الحمراء (*Tribolium castaneum*). في دراسة السمية، تم اختبار خمس جرعات عن طريق التطبيق الموضعي على كل من النوعين 25، 12.5، 6.25، 3.12 و 1.56 مغ/مل بالنسبة إلى حشرة المن و 25، 50، 250، 350 و 500 مكغ/حشرة بالنسبة إلى حشرة السوسة. وفي خصوص دراسة الفعالية الطاردة، تم اختبار جرعتين هما 500 مكغ/حشرة بالنسبة إلى حشرة السوسة و 25 مغ/مل بالنسبة إلى حشرة المن. أظهرت النتائج التي تم الحصول عليها أن النشاط الطارد للمستخلص يزداد مع مدة التعرض ولوحظت أعلى المعدلات بعد 4 ساعات من المعاملة ($72.33 \pm 22\%$ للنسبة و $87 \pm 3.6\%$ للمن). في خصوص نشاط الإبادة الحشرية للمستخلص، وصلت نسبة الوفيات الكلية للمن 100% بعد 72 ساعة من المعاملة وذلك باستعمال أعلى تركيز (25 مغ/مل). وكان هذا المستخلص أعلى سمية على الحشرات البالغة للسوسة حيث وصلت نسبة الوفيات 100% بعد 48 ساعة من المعاملة بأعلى تركيز وهو 500 مكغ/حشرة. قدرت الجرعة القاتلة 50% بعد 24 ساعة من المعاملة ب 1.7 مغ/مل للمن و 30.3 مكغ/حشرة بالنسبة إلى السوسة. أعاق المستخلص الخام لهذا النبات نشاط أنزيم الأستيلكولينستراز لكلا النوعين. ومن خلال هذه النتيجة يتبين أن لهذا النبات تأثير عصبي سام على المن والسوسة.

أوضحت الدراسة الكيميائية لهذا النبات أنه غني أساسا بالفلافونويد والتانينات والغالكية والقلويدات والصابونوزيدات والغلوكوزيدات. يمكن لمفعول الإبادة الحشرية الذي تم الحصول عليه في هذه الدراسة أن يكون راجعا إلى العمل التكاملي لجملة مكونات المستخلص الخام. تشير النتائج إلى إمكانية استعمال مستخلصات هذا النبات في برامج الإدارة المتكاملة للأفات لتعويض المبيدات الكيميائية.

كلمات مفتاحية: سمية، طارد، مستخلص إيثانولي، *Aphis fabae*، *Cotula cinerea*، *Tribolium castaneum*

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