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TOXICITY, ACETHYLCOLINESTERASE AND GLUTATHIONE S-TRANSFERASE EFFECTS OF HALOCNEMUM STROBILACEUM CRUDE EXTRACT AGAINST TRIBOLIUM CASTANEUM

SUMMARY

Halocnemum stobilaceum (Chenopodiaceae) is one of many halophyte plants from Algerian Sahara. In this study, we investigate the phytochemical composition and the insecticidal activity of the crude ethanolic extract of this plant against adults of the red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae). Te effect of this extract on the detoxification enzyme Glutathione S-transferase (GST) and the neuroenzyme Acethylcolinesterase (AChE) was also investigated. The repellent effect was evaluated, at the concentration of 1000 µg/ insect, using the preferential zone method on blotting paper. The insecticidal effect was investigated by testing 5 doses: 100, 200, 400, 800 and 1000 µg/insect.

The obtained results show that *H. stobilaceum* is very rich in saponins, gallic tannins, flavonoids, antocyans, coumarins and alkaloids. The plant is poor in irridoids. On insecticidal level, the extract tested at dose 1000 µg/insecte has a good repellent effect on adults of *T. castaneum*. The repulsively rate calculated after two hours of exposure was 60%. The extract was toxic too. Indeed, the five tested doses caused mortalities of 15, 33.3, 41.6, 48.3 and 70%, respectively, after 6 hours of exposure. The highest dose (1000 µg/insect) generated 100 deaths after 96 h of exposure. The LD_{50} calculated 24 h after treatment was 225.4 µg/insect. Furthermore, the extract of this plant, at the concentration of 1000 µg/insect, inhibited acetylcholinesterase (AChE) activity. The obtained results suggest that extracts of this plant can be used to protect the stored products against insect secondary pest.

Keywords: Crude ethanolic extract, Enzymatic assays, *Halocnemum stobilaceum*, Repellency, Toxicity, *Tribolium castaneum*

INTRODUCTION

Grains and milling products constitute a major part of the daily of human and animal populations among. The most important risk associated with flour and

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cereal product consumption is insect contamination, which is an important quality control problem of concern milling industries (Taban et al., 2017). Currently, there are different kinds of preventive and curative control measures to get protection from this pest and chemical pesticides are one of them (Habib and Karim, 2015).These insecticides bring about such serious problems as contamination of the environment, lethal effects in non-targeted organisms which can lead to a failure of biological control programs and insect resistance. Toxic residues on stored grain for human consumption are other problems related to chemical pesticides (Rajendran and Sriranjini, 2008). However, with growing evidence that many conventional pesticides can adversely affect the environment and the human safety, requirement for safer means of pest management have become crucial (Rozman et al., 2007).One such alternative is the use of botanical insecticides (Baskar et al., 2009).

In the co-evolution of plant-insect interactions, plants have been able to synthesize a wide range of products to defend themselves against insect attacks, including primary metabolites (e.g. proteinase, amylase inhibitor and lectins) and secondary metabolites (e.g. alkaloids, tannins and rotenoids) (Zanini-Martins et al., 2012). Indeed, plants may provide potential option to currently used insect-control agents because they constitute a rich source of bioactive chemicals. Plant secondary compounds have been, than, the subject of investigation for the past 20 years in an effort to discover new sources of botanical insecticides, repellents and antifeedants (Akhtar and Isman, 2004).The main advantage of phytochemical insecticides is that they could be prepared easily by farmers, small-scal industries and are potentially less expensive (Nikkon et al., 2009).

In Algeria, research on the discovery and exploitation of natural resources for the development of botanical insecticides, for pest control, has been undertaken for several years (Acheuk et al., 2012 ; 2017 a & b; Acheuk and Doumandji-Mitiche, 2013 ; Dane, 2016).

Halophytes are an important group of plants and play key roles in the ecosystem. While they have been used by local communities for millennia, their full potential is still untapped. Some halophytes are now being harvested commercially to obtain gums, oils and resins for production of household goods, food processing, and heavy industrial applications. Other halophytes are well known for their bioactive derivatives and are essential ingredients for pharmaceuticals, agricultural pesticides, traditional medicines and natural cosmetics (Al-Oudat and Qadir, 2011).

Halocnemum strobilaceum is a halophilic *Chenopodiaceae* plant of saline and marshy areas, it's grows in damp salt soil (salinity > 90 dS/m). The plant was grazed by sheep and camels, but Bedouins believe that overfeeding on this plant causes lung disease in camels (Al-Oudat and Qadir, 2011).

Currently, no data concerning the biological or the insecticidal effects of this plant is available. In the order to find alternative bioactive compounds to control *Tirbolium castaneum*, the present study has screened the repellency and the efficacy of the crude ethanolic extract of *H. strobilaceum* as potential

botanical insecticide against this pest under laboratory conditions. Te effect of this extract on the detoxification enzyme Glutathione S-transferase (GST) and the neuroenzyme Acethylcolinesterase (AChE) was also investigated.

MATERIAL AND METHODS

Plant collection and preparation of crude ethanolic extract

The plant *H. strobilaceum*was collected from Sidi Mehdi, Touggourt region (Southeastern part of Algeria) during autumn season in 2015. The plant was taxonomical identified and confirmed by Dr Benhouhou from the National High College of Agriculture, Algiers, Algeria. Aerial parts of *H. strobilaceum* were air dried in the shade and grounded into fine powder using electrical blender. The powder was stored at room temperature in hermetically sealed plastic boxes until extraction.

Insect rearing

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 L) 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. Adults of 1-5 days post emergence were used in experiments.

Preparation of crude ethanolic extract and phytochemical screening

The crude ethanolic extract of the aerials parts of *H. strobilaceum* was prepared by macerating the powder for 3 days in ethanol, 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 plant secondary metabolites, alkaloids, 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.

Repellent activity

To assess the repellency activity of *H. strobilaceum* crude ethanolic extract against *T. castaneum* adults, an area preference method of McDonald et al. (1970), with sligh modifications, was adopted. The test was carried out under the same conditions described above for the mass rearing using glass Petri dishes as containers. Filter paper (Whatman N° 1, 9 mm) was cut in half.

The test extract was tested at the concentration of 1000 µg. Test compounds were dissolved on acetone and 500 µl of the test solution were applied uniformly to half filter paper disc. Another half was treated with acetone only.

Treated and untreated halves were air dried, carefully fixed and placed in Petri dishes. For each test twenty adults insects (1-5 days post emergence) were introduced at the centre for the Petri dishes. The number of insects on the two halves disks were recorded after 2 and 4h from the beginning of the test. The percentage of repellency was calculated as follows:

$$PR\ (\%) = (Nc-Nt)/\ (Nc+Nt)\ x\ 100$$

Nc: Number of insects on control part
Nt : Number of insects on treated part

The average values were then categorized according to the following scale:

Class	Repellency rate (%)
0	>0.0l to 0.1
I	0.1 to 20
II	20.1 to 40
III	40.1 to 60
IV	60.1 to 80
V	80.1 to 100

Contact toxicity test

The bioassay was carried out using five concentrations of the crude ethanolic extract: 100, 200, 400, 800 and 1000 µg/insect. Test solutions were prepared using acetone as solvent. Unsexed adults insects were immobilized 15 min before the beginning of the test. Aliquots of 5 µL of each tested concentration were topically applied onto the thorax of insects using micropipette applicator. For each concentration twenty insects were used in 5 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 and LD_{50} was calculated.

Acetylcholinesterase and Gluthatione S-transferase assays

The AChE activity was carried out following the method of Ellman et al. (1961) using acetylthiocholine as a substrate. Adults of *T. castaneum* were sampled from control and treated groups (1000 µg/insect). Pools of twenty adults were homogenized in the solution containing 38.03 mg of ethylene glycol tetraacetic (EGTA), 1mL Triton X-100, 5.845 g NaCl and 80 mL Tris buffer (10Mm, 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 minutes.

GST activities were determined with the soluble fraction as enzyme source. GST activities toward 1-chloro-2, 4-dinitrobenzene (CDNB) were measured according to Habig et al. (1974). Treated (1000 µg/insect) and control insect were homogenized in sodium phosphate buffer (0.1 M, pH 6) and centrifuged (14000 g, 30 min). Two hundred microliter of the resulting supernatant was added to 1.2 mL of reaction mixture containing 1Mm of CDNB

and 5 Mm of reduced glutathione (GST) in the homogenization buffer. Changes in absorbance were recorded at 340 nm. Total protein content was determined according to method of Bradford (1976) using bovine serum albumin as a standard. Enzyme activities were expressed as $nMmin^{-1}mg^{-1}$ proteins. The percentage of activation or inhibition was calculated for each enzyme.

Statistical analysis

Results are expressed as means ± standard deviation (SD). To identify significant effects of the treatments on the variables measured, data were submitted to a monofactorial ANOVA using XLSTAT 7.5.2. Means were compared using Tukey's HSD test ($P< 0.05$).

RESULTS AND DISCUSSION

Phytochemical screening

The crude ethanolic extract of *H. strobilaceum*was subjected to qualitative phytochemical screening to identify presence or absence of selected chemical constituents using classical methods of analysis. From phytochemical screening (Table1) we observed that the studied plant contains different groups of secondary products. The plant is rich in flavonoids, tannins, alkaloids, coumarins, antocyans and saponins. The study also shows that this plant does not contain iridoids.

Table 1.Qualitative phytochemical screening of crude ethanolic*H. strobilaceum.*

Alkaloids	Antocyans	Comarins	Tannins	Saponins	Iridoids	Flavonoids
+	+++	++	++	+++	-	+++

(-): Absent ; (+): Low presence ; (++): Moderate presence ; (+++): Strong presence

Contact toxicity

The tested extract exhibited obvious toxicity against adults of *T. castaneum* (Fig. 1). Indeed, the five tested doses caused mortalities of 15, 33.3, 41.6, 48.3 and 70%, respectively, after 6 hours of exposure. The highest dose (1000 µg/insect) generated 100 deaths after 96 h of exposure. The LD_{50} calculated 24 h after treatment was 225.4 µg/insect.

Repellent effect

The extract tested at dose of 1000 µg/insecte has a good repellent effect on adults of *T. castaneum* (Table 2). Repellent action varied depending on the time exposure. Indeed, the repulsively rate calculated after two hours of exposure was 60%. After 4 h of exposure, the PR value was 89.26 ±5.2 %.

Table 2.Repellent activity of the crude ethanolic extract of the plant *H. strobilaceum* against adults of *T. castaneum* at differents exposure times.

Exposure time	2 h	4 h
% of repellency	60 ± 02^{b}	89.26 ± 5.2^{a}
Class	III	V

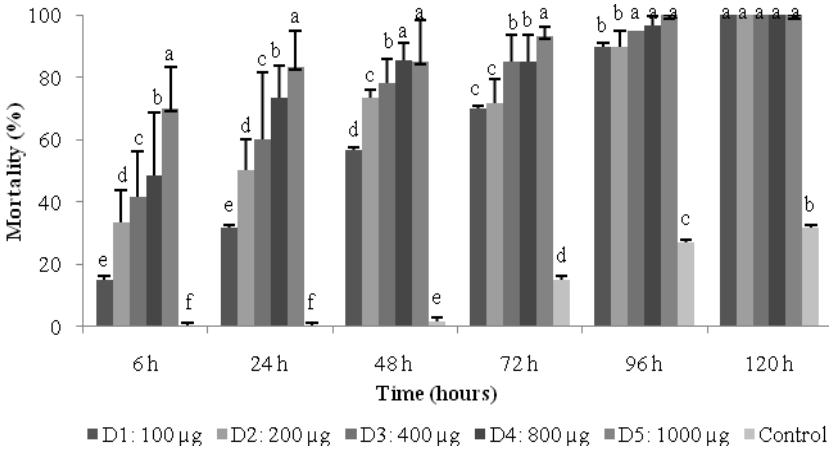

Figure 1. Toxicity of the crude ethanolic extract of *H. strobilaceum* applied topically to the adults of *T. castaneum* (Mean ± SD). N = 20 insects/replicate. Values followed by the same letter are not significantly different at P < 0.05according to Tukey's test.

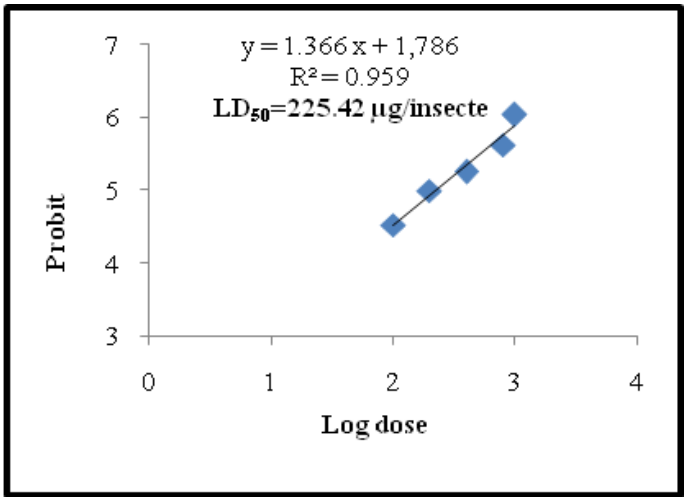

Figure 2. Effect of crude ethanolic extract of *H. strobilaceum* applied topically on the adults of the red flour beetle *T.castaneum* noted after 6 h of exposure (Mean ± SD). N = 20 insects/replicate.

Enzymatic assays

For enzymatic activities (Figs. 3 and 4), the results showed that the crude ethanolic extract of *H. strobilaceum*, applied at the dose of 1000 µg/insect inhibited significantly the AChE activity.

However, the extract has no significant effect on the GST activity. Indeed, the values of the enzyme activity are comparable between the control and treated series.

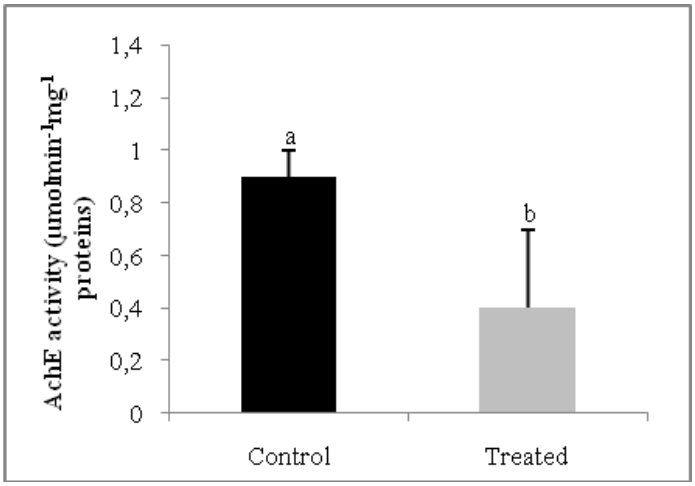

Figure 3. Effect of crude ethanolic extract of *H. strobilaceum*on AChE activity of the adults of the red flour beetle *T. castaneum* (Maen ± SD), N=20 insects. Different letters denote significant differences (Tukey's test, $p < 0.05$).

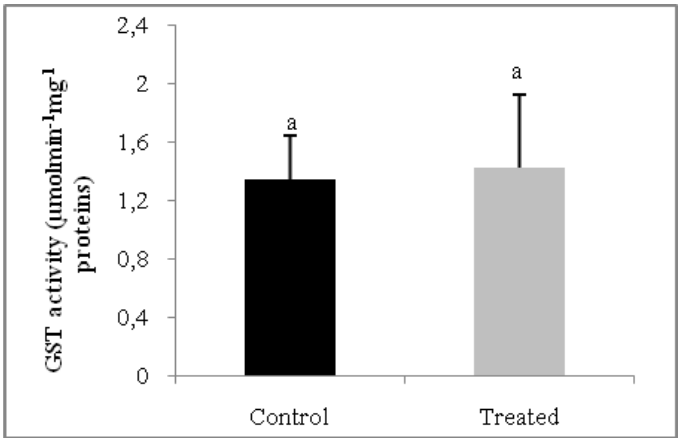

Figure 4. Effect of crude ethanolic extract of *H. strobilaceum*on GST activity of the adults of the red flour beetle *T. castaneum* (Maen ± SD), N=20 insects. Different letters denote significant differences (Tukey's test, $p < 0.05$).

Plant secondary compounds are an important biochemical basis for the plant defense against herbivores insects. Those compounds such as phenolics, alkaloids and proteins amino-acids are deleterious to insects and other herbivores by divers ways. Thus, they play a key role in plant defensive response to pests through acute toxicity and enzyme inhibition (Zhang et al. 2013). Phytochemical study of the studied plant showed the presence of various groups of natural products. The plant is rich in flavonoids, tannins, alkaloids and coumarins. The study also shows that this plant does not contain iridoids. The phytochemistry of this halophyte remains very poorly known, only two studies indicate the isolation

of bio-actives compounds from this plant. Four coumarins: coumarin, hydroxy-3-methylcoumarin, oreoselone, and heraclenin where isolated from the aerial part of *H. strobilaceum* (Miftakhova et al. 2001). Also, a new n-alkyl ester of 3,4-dihydroxycinnamic acid (caffeic acid) has been isolated from this plant by Gibbons et al. (1999). Aerial plant parts contain 6.9% protein, 2.15% fat, 17% fiber and 40.1% ash (El Shaer et al. 1991).

On insecticidal level, positive results for contact and repellent activity of the tested extract were obtained against the adults of the red flour beetles. Indeed, it was very clear that the percentage of mortality was directly proportional to the concentration of the test extract. Mortality increased from higher to lower concentration. The five tested doses caused mortalities of 15, 33.3, 41.6, 48.3 and 70 %, respectively, after 6 hours of exposure. The highest dose (1000 µg/insect) generated 100 deaths after 96 h of exposure. The LD_{50} calculated 24 h after treatment was 225.4 µg/insect. The insecticidal properties of the crude extract of this plant could be rationalized by synergistic action of its all compounds, major compounds and some other minor compounds. Many plant extracts and essential oils have been reported to be effective against pests in stored products. The study conducted by Zardi-Bergaoui et al. (2008) indicate that the ethyl acetate crude extract and eight fractions A_3, A_4, P_8, P_{10}, F_2, F_3 and F_7 of *Anacyclus cyrtolepidoides* showed a significant inhibitory effect of *T. confusum* growth. 100 % mortality of adults was achieved 12 days after treatment using fractions A_4, P_8 and F_7. In a recent study of Phankaen et al. (2017), a significant mortality was recorded on fumigation assay with the dichloromethane extract of *Coffea arabica*. However, the active ingredient isolated of this extract did not induce similar toxicity as the dichloromethane extract. Results obtained by Saidana et al. (2010) indicated that the methanolic extract of tunisian halophyte *Tamarix boveana* caused significant and early mortalities and growth inhibition of the insect *Trogoderma granarium* at the concentration of of 50 µg/disc.

In the present study, the plant extract showed potent repellent effect on *T. castaneum* adults. The PR value was 89.26 ±5.2 % after 4 h of exposure at the testing concentration (1000 µg/insect). Many researchers have reported on the repellency of essential oils and plant extracts against insects, especially those infesting stored products. Essentials oils extracted from *Stureja* spp were strongly repellent against *T. castaneum* adults at the concentration of 1% (v/v) after 4 hours of exposure (Taban et al., 2017). Phankaen et al. (2017) obtained a very strong repellency with the active compounds isolated from *C. Arabica* against *T. castaneum* adults.

Plant secondary compounds such as phenolics, alkaloids and non-protein amino acids are deleterious to insects and other herbivores in diverse ways. Thus, they play a key role in plant defensive response to pests through acute toxicity and enzyme inhibition (Zhanget al., 2013). The understanding of the mechanism of action of the extract of our plant on certain target enzymes remains important for a possible formulation of a bio-insecticide. The measurement of the activity of the GST and the AChE was carried out in this perspective. For enzymatic

activities, data showed that the crude ethanolic extract of *H. strobilaceum*, at the dose of 1000 µg/insect, has no significant effect on the GST activity. However, the extract inhibited the AChE activity. Our results were in agreement with those of Mami-Maazoun et al. (2017) wich showed that *Urginea maritima* bulbs extract was able to interfere with *Sitophylus oryzae* acetylcholinesterase enzyme andexhibited significant inhibitory effect on acetylcholinesterase activity. This inhibition could possibly be due to its high content in phenolic compounds and alkaloids. Once they penetrated inside the insect body, alkaloids and phenolic compounds reached nervous system and inhibited activity of acetylcholinesterase.

CONCLUSION

Based on the present study, it can be concluded that the crude ethanolic extract of the Saharian halophyte plant *H. strobilaceum* tested for its repellent activity and contact toxicity, exhibited obvious effects against the red flour beetle *T. castaneum*. For its acethylcolinesterase inhibition, this extract can be used as an insecticide against the pests of stored products. However, further tests are needed to develop a formulation of this natural insecticide.

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