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PHYTOCHEMISTRY, BIOCHEMICAL AND INSECTICIDAL ACTIVITIES OF *RUTA CHALEPENSIS* ESSENTIAL OILS ON *TRIBOLIUM CONFUSUM*

SUMMARY

The confused flour beetle, *Tribolium confusum* (Coleoptera: Tenebrionidae) is a common pest insect known for attacking and infesting stored flour and grain. Biodegradable and ecologically natural products such as essential oils are emerging candidates for replacement of usually applied chemical pesticides. The essential oils of *Ruta chalepensis* flowering aerial parts were investigated for their contact toxicity and physiological aspects on *T. confusum*.

Essential oils were obtained by hydrodistillation and 56 components were identified by GC-MS. Our results clearly indicated that these compounds exhibited toxicity against *T. confusum* pupae and adults with an LC_{50} value of 0.08 and 0.055 µL/cm^2, respectively after 7 days of treatment. In repellency assay, essential oils repelled *T. confusum* adults significantly even at 0.06 µL/cm^2 concentration in an area preference test. Result also showed that maximum exposure time resulted in maximum repellency of the pest at all the concentrations. The repellent activity could be related to the abundance of the 2-undecanone (25.94%) in the oils. In other experiments, the essential oils were investigated on the activities of acetylcholinesterase (AChE) and glutathione *S*-transferases (GSTs). Biochemical analysis demonstrated that the essential oils of *R. chalepensis* induced GSTs and reduced the activity of AChE. Based on these results, essential oils of *R. chalepensis* origin could have greater potential in future in stored-product pest management.

Keywords: Essential oils, phytochemistry, toxicity, Acetylcholinesterase, glutathione S-transferases.

INTRODUCTION

Stored products of agricultural and animal origin are attacked by more than 600 species of beetle pests, 70 species of moths and about 355 species of mites causing quantitative and qualitative losses (Rajendran and Sriranjini, 2008).

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Stored-grain insect pests have been damaging food grains in granaries and store houses and accounts for 10-40% loss worldwide (Chaubey, 2012). The confused flour beetle, *Tribolium confusum* (Coleoptera: Tenebrionidae) is a very common pest infesting many flour mills, warehouses and grocery stores. It is cosmopolitan and considered one of the major stored-product insects (Garcìa et al., 2005). In Tunisia and North Africa, Jarraya (2003) reported that this insect is amid the most important and destructive pests in mills.

Infestations of stored product insects are typically controlled by the use of synthetic insecticides and fumigants. Currently, phosphine and methyl bromide are the common product worldwide used for stored protection (Suthisut et al., 2011). These insecticides are the most effective applications for the protection of flour mills, grocery shops, warehouses, and other agricultural commodities from stored insect infestation (Park et al., 2004). However, they have a number of associated disadvantages, such as environmental pollution, development of insect resistance and potential toxicity to non target organisms. The use of methyl bromide is being restricted because of its potential to damage the ozone layer (Butler and Rodriguez, 1996; MBTOC, 1998). The Montreal Protocol of the United Nations Environment Program (UNEP 1995) recommends the phasing out of methyl bromide by 2005 in developed countries and by 2015 in developing countries (MBTOC, 1998). Insect resistance to phosphine is a global issue now and control failures have been reported in field situations in some countries (Rajendran and Sriranjini, 2008). Collins et al. (2005) and Pimentel et al. (2009) reported that phosphine is not effective against some insect populations in India, Australia and Brazil, because of resistance. Therefore, today there is a need to develop alternatives that is capable of reducing the large-scale utilization of synthetic pesticides for crop protection.

Research in recent years has been turning more towards selective biorational pesticides. Among these botanical insecticides have attracted the greatest attention and have been reviewed extensively (Abdellaoui et al., 2013). Plants have always been rich source of chemicals and drugs for human and synthesize a wide array of compounds that are generally thought to be involved in plant-insect interactions (Amason et al., 1981). Thus, organic molecules of botanical origin may offer a safe source of compounds for pest management, being environmentally friendly, and an excellent alternative to persistent synthetic insecticides (Abdellaoui et al., 2015).

Among the natural compounds produced by plants, the essential oils appear to directly or indirectly influence the patterns of growth and reproduction of associated phytophagous insects. Essential oils, secondary metabolites that plants produce for their own needs other than for nutrition, have traditionally been utilized to protect stored grain and legumes (Isman, 2000). In recent years, they received a great deal of attention as pest control agents. These complex mixtures, and their individual constituents, have been shown to possess multiple pest control properties, including toxic, repellent, ovicidal, antifeedant and antioviposition effects (Ho et al., 1996; Bakkali et al., 2008; Rajendran and

Sriranjini, 2008). Liu et al. (2013) reported that many essential oils from plants including medicinal herbs, spices and fruits have been evaluated and shown to be effective as pesticides against stored product insects. In addition, they are low toxic to human and animals, volatile and can function as fumigants, and may also be applicable to the protection of stored products (Sahaf et al., 2008).

In an effort to identify novel active natural products derived from plants as alternatives to conventional insecticides, we have studied the contact toxicity and repellency effect of *R. chalepensis***,** medicinal plant which occur in the Tunisia flora, essential oils against *T. confusum*.

MATERIAL AND METHODS

Insect rearing

T. confusum was reared in 2-L plastic containers containing wheat flour mixed with yeast (10:1 w/w). The cultures were maintained in darkness in a growth chamber set at 30±1°C and 60-70% r.h.

Plant materials

Aerial parts of *R. chalepensis* were collected at full-flowering stage during spring season (March-April) from the region of Kairouan (N: 35.67°, E: 10.09°) in the Center of Tunisia. The samples were dried in the shade at room temperature for two weeks. After drying, the samples were ground to a fine powder used for the extraction of essential oils.

Essential oil extraction and identification

The essential oils have been extracted from 100 g air-dried flowering aerial parts of *R. chalepensis* by hydrodistillation for 4 h, using a Clevenger-type apparatus. The essential oils were dried using anhydrous sodium sulphate and then stored in sterile tubes at 4°C until analyses.

Chemical components of essential oils were identified with gas chromatography-mass spectrometry instrument (GCMS-QP 2010 Plus Shimadzu, Japan). RTX-5 ms capillary column 30m x 0.25mm x 0.25µm film thickness was used. The column temperature was initially set at 50°C for 2 min, then gradually ramped at 7°C/min to 250°C and then left at 250°C for 5 min. Injection and detector temperatures were kept respectively at 250 and 280°C. The carrier gas was helium (99.995% purity), the flow through the column was 1.2 mL/min and the split ratio was set to 1:50 with injection of 1 µL of oil sample. The mass spectrometer conditions were as follow: ionization voltage 70 eV, ion source temperature 200°C and the scan range was from 50 to 550 m/z. The identification of the components separated by GC-MS was made by comparing the obtained mass spectra for each component with the values stored in NIST Mass Spectral Library (NIST 08). The percentage composition of the oils was calculated in peak areas using the normalization method.

Contact toxicity

In an effort to determine the toxicity by contact of the *R. chalepensis* essential oils and the median effective time to cause mortality in 50% of tested insects (LT_{50} values), three solutions of essential oils were prepared in acetone.

500 µL of each solution was applied to the surface of filter papers (Whatman No. 1, cut into 5 cm diameter pieces) and homogeneously dispersed, giving a range of concentration of 0.06, 0.12 and 0.25 µL/cm^2. Controls were treated with solvent alone. The solvent was allowed to evaporate for 10 min prior to the introduction of 20 *T. confusum* adults (1-6 days old) or pupae (0-1 day old) selected randomly. In the adult boxes, 0.5 g of food (artificial diet of wheat flour mixed with beer yeast: 10/1 w/w) was added. Control and treated groups were kept under the same conditions described above for mass rearing. Each treatment was replicated four times. The mortality was assessed daily via direct observation for a period of 7 days, and when no antennal movements were observed, the insects were considered dead. Probit analysis (Finney, 1971) was conducted to estimate the LC_{50} and LC_{95} values with their 95% confidence limits. Time-mortality data for each experiment were analyzed via the method developed by Finney (1971), with time as the explanatory variable to derive the estimated time for 50% mortality (LT_{50}).

Repellency bioassay

An area preference method (Zhang et al., 2011) was adopted to assess the repellent activity of *R. chalepensis* essential oils against *T. confusum* adults. Experiments were carried out in glass Petri dishes (diameter 8.5 cm and height 1.2 cm) using concentrations of 0.06, 0.12 and 0.25 µL/cm^2 prepared in acetone. Whatman filter paper was cut into two equal halves and each test solution (500 µL) was applied to filter paper half as uniform as possible using micropipette. The other half of filter paper was treated with acetone only as a control. The treated and control half disks were air-dried to evaporate solvent completely. Both treated and untreated halves were then attached with cellophane tape and placed at the bottom in Petri dish. Twenty adults of *T. confusum* (7-10 days old) were released at the centre of each filter paper disk and then Petri dishes were covered, sealed with parafilm and kept in dark. Five replicates were performed for each tested concentration. The number of insects on both treated and untreated halves was recorded after 1, 2 and 4 h of exposure. Percentage repellency (PR) was calculated according to Nerio et al. (2009) as follows: PR = [(Nc - Nt)/(Nc + Nt)] x 100, where Nc was the number of insects on the untreated area and Nt was the number of insects on the treated area.

Enzymatic assays

Larvae were homogenized in 1 mL of 0.1 M phosphate buffer (pH 7.4) using a Teflon glass tissue homogenizer. Homogenates were centrifuged (15,000 g for 20 min at 4°C) and supernatants were used for enzyme assays.

The acetylcholinesterase activity (AChE) was determined according to the method of Ellman et al. (1961) using the Jenway 6105 spectrophotometer. The reaction medium included sodium phosphate buffer (0.1 M, pH 7.2), DTNB (1.6 mM), AcSChI (156 mM) and sample (S9). Kinetics was recorded at 412 nm and the assay was carried out at 25°C. The enzymatic activity was expressed as nmol of acetylthiocholine hydrolyzed per min per mg of proteins.

The glutathione *S*-transferase (GST) activity was measured by the method of Habig et al. (1974) using 10 mg of cytosolic protein, 1 mM 1-chloro- 2,4-dinitrobenzene (CDNB) (Sigma-Aldrich, Saint Louis, MO, USA) and 4 mM glutathione (reduced form; GSH) in 100 mM sodium phosphate buffer, pH substrate 7.5. GST activity was determined by kinetic measurement at 20°C using a Jenway 6105 spectrophotometer (λ=340 nm). The results were expressed as nmol GSH-CDNB produced per min and per mg proteins. Proteins in the S9 fraction were quantified according to the Bradford (1976) method using Coomassie Blue reagent.

Data analysis

Statistical analysis was performed using Probit analysis (Fienny, 1971) to determine LC_{50} and LC_{95} values with their 95% confidence limits. Data from repellency and enzymes activities were expressed as means ± standard deviation (SD) and subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS: version 18.0). The significance between control and treated series was made by Student-Newman-Keuls (*SNK*) test at the 5% level.

RESULTS AND DISCUSSION

Chemical composition of essential oils

The essential oils yield of *R. chalepensis* flowering aerial parts was 0.87 ± 0.05% (V/W) on the basis of dry matter weight. The results of the chemical analysis are shown in Table 1. A total number of 56 components were identified by GC-MS. The main components of *R. chalepensis* essential oils were 2-Octanol acetate (30.98%), 2-Undecanone (25.94%), 2-Nonanone (16.28%) and 5-Dodecanone acetate (9.35%). Major compounds were followed by others components with lower percentages which are 2-Nonanol (2.54%) and 2-Decanone (2.42%) (Table 1).

Contact toxicity bioassay

Contact toxicity after 7 days of treatment was determined. LC_{50} and LC_{95} values, and their 95% confidence limits expressed as microliter per square centimeter are shown in Table 2. The evaluation of contact toxicity data revealed that *R. chalepensis* essential oil was toxic for both pupae and adults of *T. confusum*. Probit analysis showed that *T. confusum* pupae were more susceptible to *R. chalepensis* essential oil than adults. The corresponding LC_{50} and LC_{95} values were respectively 0.05 and 0.12 $\mu L/cm^2$ against 0.08 and 0.2 $\mu L/cm^2$ (Table 2). Our experiments also showed that the essential oil of *R. chalepensis* flowering aerial parts exhibited acute toxic effects especially when applied at the two highest concentrations (0.12 and 0.25 $\mu L/cm^2$).

Bioassay was also designed to determine median effective time to cause mortality of 50% of treated insects (LT_{50}). Considerable differences in LT_{50} values were noted with different concentrations and developmental stage. Results in Table 3 show that pupae were significantly more susceptible than adults. For pupa stage, LT_{50} values ranged from 3.75 days for the lowest dose (0.06 $\mu L/cm^2$)

to 1.04 days for the highest dose (0.25 µL/cm^2). With adults, the LT_{50} values ranged from 7.6 days to 1.16 days for the lowest and highest doses, respectively.

Table 1. Chemical constituents of the essential oils from *R. chalepensis* flowering aerial parts collected from Kairouan, Tunisia.

No.	Compounds	Retention time	%
1	2-Hexanal, (E)-	5.135	0.18
2	Butanoic acid, 2-methyl-,1-methylethyl ester	5.873	0.16
3	2-Octanone	8.301	0.35
4	2-Octanol, (R)-	8.524	0.12
5	2-Heptanol, acetate	9.508	0.43
6	2-Nonanone	10.848	16.28
7	2-Nonanol	11.039	2.54
8	Nonanal	11.110	0.19
9	1,3-Cycloheptadiene	11.995	0.69
10	2-Decanone	13.053	2.42
11	2-Octanol, acetate	14.133	30.98
12	2-Tridecanone	14.468	1.72
13	2-Undecanone	15.389	25.94
14	Acetic acid, nonyl ester	15.596	0.13
15	2-Heptanol, acetate	16.035	0.37
16	2-Dodecanone	16.706	1.84
17	5-Dodecanol acetate	18.034	9.35
18	Lauryl acetate	19.278	0.16
19	(E)-2-Decenyl acetate	19.893	0.39
20	6-Dodecanol acetate	20.300	0.78
21	4-(3,4-Methylenedioxyphenyl)-2-butanone	21.238	0.28
22	Propanedioic acid, (phenylmethyl)-, diethyl ester	23.677	0.36
23	1, 3-Benzodoxole, 5-propyl-	24.802	0.89
24	1-Adamantaneacetic acid	28.179	0.31
25	Phytol	29.077	0.12
26	Pyrrolo [3, 2-g] quinolone, 9-methoxy-2, 3, 5, 7-tetramethyl-	30.295	1.47
27-56	Other components	-	1.59
EO yields (%)			0.87 ± 0.05

Table 2. Toxicity of *T. confusum* pupae and adults treated with *R. chalepensis* essential oil in contact toxicity bioassay.

Insects	LC_{50} [a, b]	LC_{95} [a, b]	Chi square ($\chi 2$)	df	*P*-value
Adults	0.08 (0.01- 0.16)	0.20 (0.13 - 0.64)	7.52	2	0.02
Pupae	0.055 (0.02 - 0.09)	0.12 (0.1 - 0.29)	6.22	2	0.04

[a] Units LC_{50} and LC_{95}=µL/cm^2.
[b] 95% lower and upper fiducial limits are shown in parenthesis.

Table 3. LT_{50} values of *R. chalepensis* essential oil against pupae and adults of *T. confusum.*

Insects	Concentration (µL/cm^2)	LT_{50} (days)[a]	df	Chi square ($\chi 2$)
Adults	0.06	7.6 (6.82-8.81)	5	2.04
	0.12	4.34 (3.99-4.72)	5	1.25
	0.25	1.16 (0.41-1.70)	5	3.37
Pupae	0.06	3.75 (3.06-4.39)	5	3.66
	0.12	1.48 (0.84-1.95)	5	8.05
	0.25	1.04 (0.77-1.25)	5	9.68

[a] 95% lower and upper fiducial limits are shown in parenthesis.

In our observation, *R. chalepensis* essential oil was characterized by typical neurotoxic symptoms including hyperactivity, convulsion, and paralysis with mortality of treated adults. In the pupal stage, we observed other syndromes of intoxication resulting in malformations that can affect the whole body of the insect, and a change in color that becomes darker with the appearance of some necrotic areas (Fig. 1A).

Figure 1. Toxicity effects of *R. chalepensis* essential oils on *T. confusum* pupae (A) and disruption of adult emergence due to the impossibility to reject the old integuments (B).

We also noted exuviations difficulties due to the impossibility to reject the old integuments causing mortality at the beginning of the adult stage and increased percentage of abnormal insects emerging (Fig. 1B).

Repellency bioassay

The results of the repellent activity of the essential oils from *R. chalepensis* flowering aerial parts against *T. confusum* adults were presented in Table 4. The percentage repellency (PR) was determined as function of the oil concentrations and the periods of exposure. As illustrated in table 4, in the binary choice bioassays, the essential oil affects significantly the distribution of insects between the treated and untreated areas and caused acute repellent activity even at the low concentrations tested. The PR values were higher than 50% at the testing concentrations from the first hour of exposure. The lowest concentration (0.06 $\mu L/cm^2$) led to percentage repellency of 52 ± 8.36% at 1 h after exposure, but it increased to 72 ± 10.95% after 4 h. The repellent activity becomes more evident by increasing the concentration of the oil and the maximum PR, independently to the period of exposure, was observed with the highest concentration tested (0.25 $\mu L/cm^2$). Indeed, with this latter, repellency was arranged between 78 ± 14.83 and 100% for respectively 1 and 4 h of exposure (Table 4). The analysis of variance with the oil concentrations as classification criteria shows a significant difference among treatments and the *SNK*-test gives heterogeneous groups represented by different letters in Table 4. Moreover, it was found that PR values are widely correlated to the exposure times and the most effect was noted after 4 h of exposure with all concentrations applied. The ANOVA analysis indicated that for each tested concentration, significant differences ($P<0.05$) were obtained between the time periods evaluated. Therefore, the repellent activity of essential oils could be related to the testing concentrations and the exposure duration (Table 4).

Table 4. Percentage repellency (mean ± SD) of *R. chalepensis* essential oils against *T. confusum* adults after various periods of exposure.

Concentrations ($\mu L/cm^2$)	1h	2h	4h
0.06	$52 \pm 8.36^{a,A}$	$66 \pm 5.47^{a,B}$	$72 \pm 10.95^{a,B}$
0.12	$74 \pm 13.41^{b,A}$	$84 \pm 11.4^{b,AB}$	$98 \pm 4.47^{b,B}$
0.25	$78 \pm 14.83^{b,A}$	$94 \pm 8.94^{b,B}$	$100 \pm 0^{b,B}$
F-value	6.25	12.58	26.14
P-value	0.014	0.001	<0.001

Within column, comparison was made between concentrations (letter in lowercase). Within rows, comparison was made between exposure times for each concentration (letter in uppercase). Means followed by same letter were not statistically different by *SNK* test at $P<0.05$.

AChE inhibition assay

R. chalepensis essential oils were screened for their AChE inhibitory effects at different concentrations. Results are summarized in Table 5. In control,

the mean AChE activity remained constant during the experimental period. Values ranged from 1.039 ± 0.08 nM/min/mg proteins at the first day to 1.14 ± 0.06 nM/min/mg protein at day 3. However, in the treated series, ANOVA revealed a significant effect (P=0.000 after 72h of exposure) of the essential oils on the AChE activity which varied as function of the dose and the duration of treatment. As compared to control, the mean values recorded during the experimental period decreased by raising the concentration of the oil until reaching 40.6 and 50.88 % inhibition after 72 hours of exposure at 0.12 and 0.25 µL/cm^2, respectively. Data also indicated that the highest concentration had the greatest inhibitory activity at all the time periods evaluated and there was no significant difference between the two elevated concentrations (Table 5).

Table 5. Effect of *R. chalepensis* essential oils on the activity of acetylcholinesterase (means ± SD, nM/min/mg proteins) in surviving insects as function of the concentration and the exposure time.

Exposure time	Control	EO Concentrations (µL/cm^2)			*P*-value
		0.06	0.12	0.25	
24h	1.03±0.08^a (100)	0.93±0.12^a (89.5)	0.73±0.02^b (70.25)	0.68±0.05^b (65.44)	0.003
48h	1.01±0.07^a (100)	0.92±0.1^a (91.08)	0.72±0.07^b (71.28)	0.60±0.04^b (59.4)	0.001
72h	1.14±0.06^a (100)	0.64±0.13^b (56.14)	0.70±0.06^b (61.4)	0.56±0.05^b (49.12)	<0.001

For each exposure time, mean values followed by different letters are significantly different (P<0.05). Values in parentheses indicate per cent change with respect to control taken as 100%.

Glutathione *S*-transferases activity

As illustrated in table 6, the *R. chalepensis* essential oils caused significant change in the GST activity with a dose-response relationship.

Table 6. Effect of *R. chalepensis* essential oils on the activity of of glutathione *S*-transferases (means ± SD, nM/min/mg proteins) in surviving insects as function of the concentration and the exposure time.

Exposure time	Control	EO Concentrations (µL/cm^2)			*P*-value
		0.06	0.12	0.25	
24h	2.36±0.38^a	5.34±0.42^b	7.86±1.32^c	9.49±0.94^d	<0.001
48h	1.32±0.22^a	5.77±0.88^b	8.08±0.84^c	11.1±1.27^d	<0.001
72h	3.3±0.24^a	8.01±1.31^b	9.06±0.54^{bc}	10.53±1.21^c	<0.001

For each exposure time, mean values followed by different letters are significantly different (P<0.05).

A significance increase in the GST activity was recorded with the three tested doses starting day 1 of oils application. The enzymatic activity of the treated larvae varied significantly ($P<0.05$) among themselves and also when compared to the control. The maximum increase, independently to the exposure duration, was observed with the highest concentration tested (0.25 µL/cm^2). Indeed, the GST activity recorded in the treated group at this concentration is 11.1 ± 1.27 and 10.53 ± 1.21 nM/min/mg proteins respectively for 48 and 72 h of exposure. However, we measured in control insects 1.32 ± 0.22 and 3.3 ± 0.24 nM/min/mg proteins, respectively to the same exposure times (Table 6).

DISCUSSION

Some plant extracts and phytochemicals are known to possess insecticidal activity against various insect pests in stored-product and the essential oils extracted from aromatic plants have been widely investigated in this connection. Use of plant oils and its components as pesticide has received much attention of the scientific communities in pest management programme (Chaubey, 2012). In the previous studies, Tapondjou et al. (2005) evaluated contact toxicity of the essential oils extracted from *Eucalyptus saligna* and *Cupressus sempervirens* leaves by impregnation on filter paper discs against *T. confusum*. They claimed that these chemicals caused significant mortality with LC_{50} values of 0.48 and 0.74 µL/cm^2, respectively for *Eucalyptus* and *Cupressus* oils. Similarly, Russo et al. (2015) indicated that essential oils from *E. globulus* showed interesting insecticidal properties against *T. confusum* adults. Contact toxicity increased according to exposure time and concentration. Indeed, data showed that, at 4h exposure, essential oils applied at the lowest concentration (0.5 µL/cm^2), exhibited the toxicity of 63.33%, while a dose of 0.75 µL/cm^2 was required to obtain 100% mortality. However, after 12h of exposure, both concentrations killed all tested insects. In their study on the insecticidal activity of the essential oils of three *Chrysanthemum* species growing in Tunisia (*C. coronarium*, *C. fuscatum*, and *C. grandiflorum*) against *T. confusum*, Houas et al. (2012) reported that these chemicals caused an antifeeding effect and a high mortality (80%) of *T. confusum* larvae. The most effective essential oil was obtained from the leaves of *C. grandiflorum*.

Consulting many papers on this thematic, we can deduce that compared with the other essential oils in the literature, the essential oil of *R. chalepensis* flowering aerial parts possessed stronger contact toxicity against *T. confusum* adults, e.g. essential oils of *E. saligna* and *C. sempervirens* (LC_{95} = 1.2 and 2.42 µL/cm^2 calculated for mortality within 3 days of exposure, respectively) (Tapondjou et al., 2005).

In the present study, *R. chalepensis* essential oils have been also evaluated for their repellency. It appears that these compounds repelled *T. confusum* adults significantly even at the lowest concentration used. Similar repellent effects from other plant extracts have been reported. For example, essential oils extracted from *E. camaldulensis* (Myrtaceae) repelled *T. confusum* adults in an area

preference test (Huang et al., 1997). *Evodia rutaecarpa* (Rutaceae) essential oils also had a repellent effect on *T. castaneum* adults and reduced the growth rate of larvae (Liu and Ho, 1999). Also, Tripathi et al. (1999) reported that fruit oil of *Piper retrofractum* exhibited high repellency against *T. castaneum* (52, 76 and 90%) at 0.5, 1 and 2% concentrations. In the same context, Naseem and Khan (2011), reported in their study on the repellency of the essential oils of *P. nigrum* and *E. camaldulensis* against *T. castaneum* under laboratory conditions that maximum exposure time resulted in maximum repellency of the pest at all the concentrations.

Repellency of *R. chalepensis* essential oils against *T. confusum* adults appears to be related to the presence of 2-undecanone among the major compounds. In 2007, the arthropod repellent BioUD® (7.75% 2-undecanone) was registered by the US Environmental Protection Agency for use against mosquitoes and ticks. The active ingredient in BioUD® is the 11-carbon methyl ketone, 2-undecanone that was originally isolated from the glandular trichomes on the stems and foliage of the wild tomato plant, *Lycopersicon hirsutum* Dunal *f. glabratum* (Farrar and Kennedy, 1987). Resistance of *L. hirsutum f. glabratum* to insect herbivory is due in part to the presence of 2-undecanone (Kennedy, 2003). Witting-Bissinger et al. (2008) showed that BioUD® was an efficacious repellent against *Dermacentor variabilis* (Arachnida: Ixodidae) in laboratory studies on treated human skin, cloth, and filter paper. Additional research was conducted by Witting-Bissinger et al. (2009) to evaluate the repellency of BioUD® against three tick species (Acari: Ixodidae): *Amblyomma americanum, D. variabilis*, and *Ixodes scapularis*, important in disease pathogen transmission and to determine quantitative differences with DEET (*N,N* diethyl-*m*-toluamide) (98.11%), the most extensively used active ingredient in commercial arthropod repellents. A two-choice bioassay between repellent-treated and untreated filter paper surfaces was used under controlled laboratory conditions. The results showed that BioUD® is at least 2-4 times more active as a repellent than DEET against the three species of ixodid ticks.

In a second series of experiments, the essential oils of *R. chalepensis* were investigated on the activities of acetylcholinesterase (AChE) and glutathione *S*-transferases (GSTs). Data showed that the compound induced GSTs and reduced the activity of AChE. The GSTs are known to play a central role in the detoxification of both endogenous and xenobiotic compounds and are involved in intracellular transport and various biosynthetic pathways (Che-Mendoza et al. 2009). They play an important role in insecticide resistance and are implicated in the metabolism of organophosphorus and organochlorine compounds (Fang, 2012). Other xenobiotics such as plant secondary metabolites induce GST activity in phytophagous insects, and similarly in predators that feed on these herbivores (Vanhaelen et al. 2004, Acheuk et al., 2018). AchE has a key role in neurotransmission by hydrolyzing the neurotransmitter acetylcholine in cholinergic synapses of the nervous system and is the target site of several neurotoxic insecticides. The essential oils of *R. chalepensis* exhibited a

neurotoxic action in *T. confusum* resulting in convulsions, lack of mechanical coordination and tremors. Several previous studies reported that rapid action of essential oils against insect pests is an indicative of neurotoxic actions. Bessette et al. (2013) noted that in direct contact, essential oils may penetrate via insect's cuticle and contact the nerve endings in the invertebrate pest's trachea, and cause neurotoxic activity and more rapid death. The neurotoxic modes of action on insects are mainly related to AChE levels and several reports have demonstrated the interference of essential oils or its constituents with AChE enzyme activity in insects (Yeom et al. 2013). However, it is suspected that, in addition to AChE inhibition, the monoterpenes may act on other vulnerable sites (e.g. cytochrome P450-dependent monooxygenases) (Rajendran and Sriranjini, 2008). In fumigant toxicity tests with monoterpenes (limonene, linalool, menthol, menthone, α-pinene, *β*-pinene) against *Sitophilus oryzae* adults, Lee et al. (2001) did not find a direct correlation between insect toxicity and AChE inhibition.

As we have shown previously, *R. chalepensis* essential oils induced GSTs activity in *T. confusum*. Similarly, Vanhaelen et al. (2004) showed that Brassicacea secondary metabolites induced GST activity in *Myzus persicae* and several Lepidopteran species such as *Heliothis virescens* and *Trichoplusia ni*. By treating *Artemisia annua* extracts on *Eurygaster integriceps* adults, Zibaee and Bandani (2010) also reported that activity level of GST in 24 h post-treatment increased significantly.

CONCLUSIONS

The development of natural insecticides would help to decrease the negative impact of synthetic insecticides, such as residues, resistance, and environmental pollution. In the present study, *R. chalepensis* essential oils showed strong contact toxicity against *T. confusum*. Based on these findings, it can be suggested that essential oils of plant origin could have greater potential in future on the basis of their efficacy, economic value and use in large-scale storage.

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