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Article

Effects of commercial oils on the camel tick, *Hyalomma dromedarii* (Acari: Ixodidae) and their enzyme activities

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ABSTRACT

Hyalomma dromedarii is the predominant tick species parasitizing camels in Egypt. This study aimed to evaluate the acaricidal activity of selected oils against *H. dromedarii* and determine their effects on enzyme activities. A screening test was performed against unfed adults using 30 oils at a concentration of 20%. Rosemary, garlic, neem, and Cyperus oils were evaluated against semi-engorged females in a bioassay experiment. Semi-engorged females were exposed to LC_{50} that was calculated after two days to estimate enzyme activities of Glutathione S-transferase (GST), Acetylcholinesterase (AChE), and Catalase (CAT). Results of the screening test showed that the most effective oils against unfed adults were rosemary, neem, Cyperus, and garlic. In the bioassay experiment, rosemary oil exhibited the highest toxicity on the 2^{nd} day (LC_{50}: 12.3%) followed by garlic oil (LC_{50}: 17.4%). Neem and Cyperus oils revealed approximately the same toxicity recording an LC_{50} of around 29% on the 2^{nd} day. The four selected oils exhibited a significant increase or decrease in the activity of GST, AChE, and CAT enzymes ($P < 0.001$) during 48 h post-treatment. In conclusion, rosemary and garlic oils have strong acaricidal effects against *H. dromedarii*. Therefore, these two oils could be recommended in the control program of ticks as safe alternatives to chemical acaricides.

KEYWORDS: Acaricide activity, Cyperus, garlic, neem, rosemary.

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INTRODUCTION

Ticks are obligate blood-sucking ectoparasites worldwide on mammals and reptiles. Ticks are mainly controlled by synthetic acaricides such as organophosphates, carbamates, organochlorines, and pyrethroids, which are mostly used all over the world (Singh *et al.* 2016). Although these

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chemical acaricides are highly effective and less expensive, the use of several synthetic acaricides has been restricted in the global market (Selles *et al.* 2021) The misuse, overuse, and inappropriate application of chemical acaricides led to the appearance of several problems like the development of resistant tick populations around the world, negative effects on the non-target organisms including human, environmental pollution, toxic residues in milk and meat (Showler 2017). Consequently, it is necessary to search for alternative options incorporated in strategic and integrated control programs for ticks (Khater *et al.* 2016; Aboelhadid *et al.* 2018; Radwan *et al.* 2022a, b).

Plant-derived materials, including essential oils, are promising alternative pesticides (Massoud *et al.* 2005, Abdel-Shafy *et al.* 2007; Abdel-Meguid *et al.* 2022; Baz *et al.* 2022a, b) because they have low toxicity to non-target organisms and rapid biodegradability (Khater 2012; Iqbal *et al.* 2021). In previous studies, essential oils of different plants revealed acaricidal activity against different tick species; such as *Artemisia annua*, *Artemisia herba-alba*, *Eucalyptus globulus*, *Lavandula angustifolia*, *Mentha piperita*, and *Thymus vulgaris* oils against *Rhipicephalus microplus* and *Hyalomma* spp. (Charlie-Silva *et al.* 2018; Djebir *et al.* 2019; Valcarcel *et al.* 2021).

Hyalomma dromedarii C.L. Koch, 1844 is the most common tick species parasitizing camels in Egypt. It mainly behaves as a two host life cycle (Abdel-Ghany *et al.* 2021). In Egypt, only two previous studies tested essential oils extracted from local plants against Egyptian ticks. The first study revealed that the essential oil of *Citrus sinensis* exhibited an ovicidal effect against *H. dromedarii* ticks (Habeeb *et al.* 2007). In the second study, five essential oils extracted from the plants *Mentha piperita*, *Mentha viridis*, *Marjorana hortensis*, *Lavandula officinalis*, and *Ocimum basilicum* showed acaricidal activity against *Rhipicephalus* (formerly *Boophilus*) *annulatus* tick (Abdel-Shafy and Soliman 2004).

Therefore, this study was designed to screen the acaricidal activity of 30 oils in the Egyptian local market against the camel tick *H. dromedarii* and to determine the lethal values and enzymatic activities of the most promising ones.

MATERIALS AND METHODS

Ticks

Fully engorged females of *H. dromedarii* were collected from camels at Toukh (30° 21′ 11.6″ N, 31° 11′ 31.5″ E), Qalyubia, Egypt. These ticks were examined under a stereomicroscope (LEICA DM 750, Russia) and identified according to the key of Walker *et al.* (2003).

For oviposition, fully engorged females were placed separately in plastic cups (one female/cup) and tightly closed with a muslin cloth then incubated at 25 ± 1 °C with 75–80% relative humidity (RH) in an incubator (Friocell, MMM, Germany). The female ticks were observed daily till oviposition and eggs hatching occurred. To obtain fully engorged nymphs, the hatched larvae were fed on healthy rabbits using the capsule technique (Abdel-Shafy 2008). After that, these engorged nymphs were incubated at the same conditions until molting to unfed adults that were used in the screening test of the tested oils.

The semi-engorged females of *H. dromedarii* used for the bioassay experiment via adult immersion test and estimation of enzymes activity were obtained from naturally infested camels from the same locality.

Oils

Thirty oils were purchased from EL CAPTAIN Company for extracting natural oils, plants, and cosmetics "Cap Pharm," El Obor, Cairo, Egypt, and Harraz for Food Industry & Natrual products, Cairo, Egypt (Table 1). The selected oils were rosemary, neem, Cyperus, avocado, garlic, gooseberries, lavender, coconuts, jojoba, apricot, chamomile, anis, ginger, pumpkin, parsley, Tilia, flaxseed, lupin, Citrullus, wheat germ, camphor, cactus, mustard, basil, lettuce, sesame, carrot, pepper, mint, and fenugreek. These oils were stored at 4 °C.

Oil immersion screening test on unfed adults

This test aimed to select the most effective oils out of 30 commercial oils to be used in the bioassay test against semi-engorged females. A concentration of 20% (v/v) was prepared for each tested oil using Tween 80 (5%) to emulsify the oils in water with the aid of a two-minute vortex. *Hyalomma dromedarii* unfed adults (7~9-day-old) were used in this test using the adult immersion test (AIT) as described by Abdel-Ghany *et al.* (2021).

Bioassay of the effective oils on semi-engorged females

According to the results of the screening test, the most effective four oils (rosemary, garlic, neem, and Cyperus) were selected to be used in this experiment. A preliminary test was made to consider the used range of concentrations. The used concentrations were 20, 15, 10, 5, and 1% for rosemary; 20, 17.5, 15, 12.5, 10% for garlic, and 30, 25, 20, 15 and 10% for Cyperus and neem. Thirty active semi-engorged females were used for each concentration divided into three replicates (10 adults/replicate). These adults were immersed in 10 ml of the tested concentration for 1 min. after that, the solution was decanted, and the adults were dried using filter paper then transferred into plastic cups to be incubated at 25 ± 1 °C with 75–80% RH. The control group was immersed in Tween 80 (5%) for 1 min. Treated ticks were checked daily for five days to calculate the mortality percentage and the lethal concentrations of 50% (LC_{50}) and 90% (LC_{90}) of the treated ticks.

Determination of enzymatic activity

The effect of four oils, rosemary (*Salvia rosmarinus*), garlic (*Allium sativum*), neem (*Azadiracta indica*), and Cyperus (*Cyperus rotundus*), on the enzyme activities of *H. dromedarii* semi-engorged females were evaluated after immersion for 1 min., in the calculated LC_{50} values (12.3% 17.4%, 29.5%, 29.6%, respectively) after 48 h in the bioassay experiment. A total of 75 ticks were divided into five groups of 15 ticks, one control group, and four treated groups representing the four oils. Each group was divided into three sub-groups (after 1 h, after 24 h, and after 48 h) with three replications in each. The treated groups were immersed for 1 min. in the nominated oil. Whereas, the control group was immersed for 1 min. in Tween 80 (5%). After immersion, the ticks of 1h groups were stored at −20 ℃ for the next step, and the ticks of the 24 h and 48 h groups were incubated at 27 ± 1.5 ℃ with 70–80% RH. After incubation, the ticks were homogenized and kept at −20 °C until further analysis.

Preparation of tick homogenates

Ticks (weighing 0.1–0.15 g each) were homogenized in 20% (w/v) of either 20 mM Tris-HCl buffer (pH 8.0) containing 5 mMβ-mercaptoethanol for the estimation of total protein and glutathione S- transferase (GST) activity. Furthermore, ticks were homogenized in 50 mM potassium phosphate buffer, pH 7.0 for the estimation of catalase and acetylcholinesterase. The homogenates were centrifuged at 11000 ×*g* for 15 min. The supernatants were filtered through a plug of glass wool to remove floating lipids; the cytosolic fractions were termed as crude homogenates and stored at −20 ℃ for further analysis (Guneidy *et al.* 2021).

Glutathione S-transferase

The glutathione S-transferase (GST) activity was determined spectrophotometrically with the aromatic substrate [1-chloro-2, 4-dinitrobenzene, (CDNB)] and reduced glutathione (GSH), by monitoring the change in absorbance (increase in absorbance at 340 nm) because of thioether formation at 25 ℃ as described by Habig *et al.* (1974). The assay mixture was in a total volume of 1 ml, 100 mM potassium phosphate buffer, pH 6.5, 1 mM CDNB in ethanol (final concentration of ethanol less than

4%), 1 mM GSH, and the enzyme solution. The increase in absorbance at 340 nm of the complete assay reaction mixture was monitored against a control containing buffer instead of the enzyme and treated similarly. The molar extinction coefficient of the product was taken to be 9.6 mM^{-1} cm^{-1}. One unit of GST activity is defined as the amount of enzyme which catalyzes the formation of 1 µmol of thioether per min.

Acetylcholinesterase

The activity of AChE was estimated using AcSChI as a substrate, according to Ellman *et al.* (1961). The reaction mixture contained in 1 ml: 60 mM Tris-HCl buffer, pH 8.5, 1 mM AcSChI, 1 mM DTNB. The reaction mixtures were incubated at 37 °C for 1h and the absorbance was measured at 412 nm. One unit of AChE activity was defined as the amount of enzyme that catalyzes the hydrolysis of 1µmol of substrate per hour under standard assay conditions.

Catalase

Catalase (CAT) activity was assayed according to the method described by Aebi (1984). The method is based on monitoring the rate of decomposition of H_2O_2 at 25 °C. For CAT activity determination, a suitable volume of the enzyme was added to 3 ml of substrate mixture, which comprised 20 mM H_2O_2 in 50 mM phosphate buffer, pH 7.0. The decomposition of H_2O_2 was followed as a decline in absorbance at 240 nm for 1 min. One unit of CAT activity was defined as the calculated consumption of 1 µmol of H_2O_2/min at 25 °C. The extension coefficient of H_2O_2 was taken to be 43.6 M^{-1} cm^{-1}.

Protein determination

Protein concentration in the tick homogenates was determined by the method of Bradford (1976) using BSA as a standard with a series of BSA standard solution. BSA (10%) was dissolved in 10 ml distilled water, and then serial dilutions with water were prepared for standard protein calibration covering the concentrations from 1 to 10 µg ml^{-1} of BSA. One hundred mg of coomassie brilliant blue (CBB, G-250) was dissolved in 50 ml of 95% ethanol. To this solution, 100 ml of ortho-phosphoric acid (85% w/v) was added and the final volume was adjusted to one liter with distilled water. The dye solution was filtered and kept for two weeks at 25 °C before use. Then, a known volume of the protein sample was adjusted to a total volume of 1 ml by distilled water. One ml of the dye solution was added to the sample and mixed. The absorbance of the blue color formed was measured within 1 h at 595 nm against blank prepared by mixing 1 ml of the distilled water with 1 ml of the dye solution. A standard protein covering the range of 1 to 10 µg in 1 ml using BSA was carried out.

Statistical analysis

Data were statistically analyzed by one-way ANOVA test followed by Tukey test using SPSS program version 20. The LC_{50} and LC_{90} values were calculated by applying regression equation analysis to the probit transformed data of mortality. The dose-response data were analyzed by probit method (Finney 1952) using Ehab software.

RESULTS

Effect of oils on H. dromedarii *unfed adult ticks*

Table 1 shows the mortality percentages of 30 oils against unfed adults of *H. dromedarii* at 1, 2, 3, 7, and 15 days post-treatment. In general, the most effective four oils were rosemary, neem, Cyperus, and garlic, which recorded mortalities as 40.00 ± 30.00%, 33.33 ± 3.33%, 30.00 ± 17.32%, and 23.33 ± 13.33%, respectively at 15th day post-treatment

Table 1. Mortality percentages (Mean ± SE) for *Hyalomma dromedarii* unfed adults exposed to 30 oils through 15th day after treatment.

No.	Oil	Days after treatment				
		1	2	3	7	15
1	**Rosemary**	6.67 ± 6.67	16.67 ± 16.67	33.33 ± 28.48	33.33 ± 28.48	40.00 ± 30.00
2	**Neem**	0.00	0.00	0.00	0.00	33.33 ± 3.33
3	**Cyperus**	10.00 ± 10.00	16.67 ± 16.67	16.67 ± 16.67	16.67 ± 16.67	30.00 ± 17.32
4	**Avocado**	0.00	0.00	0.00	0.00	30.00 ± 11.55
5	**Garlic**	16.67 ± 16.67	16.67 ± 16.67	20.00 ± 15.28	20.00 ± 15.28	23.33 ± 13.33
6	**Gooseberries**	0.00	0.00	0.00	0.00	20.00 ± 5.77
7	**Lavender**	0.00	0.00	0.00	0.00	20.00 ± 10.00
8	**Coconuts**	10.00 ± 10.00	10.00 ± 10.00	10.00 ± 10.00	13.33 ± 13.33	16.67 ± 16.67
9	**Jojoba**	0.00	0.00	0.00	0.00	13.33 ± 23.09
10	**Apricot**	0.00	0.00	0.00	0.00	10.00 ± 10.00
11	**Chamomile**	0.00	0.00	0.00	0.00	6.67 ± 6.67
12	**Anis**	0.00	0.00	0.00	0.00	6.67 ± 6.67
13	**Ginger**	0.00	0.00	0.00	0.00	6.67 ± 6.67
14	**Pumpkin**	0.00	0.00	0.00	0.00	3.33 ± 3.33
15	**Parsley**	0.00	0.00	0.00	0.00	3.33 ± 3.33
16	**Tilia**	0.00	0.00	0.00	0.00	3.33 ± 3.33
17	**Flaxseed**	0.00	0.00	0.00	3.33 ± 3.33	3.33 ± 3.33
18	**Lupin**	0.00	0.00	0.00	3.33 ± 3.33	3.33 ± 3.33
19	**Citrullus**	0.00	0.00	0.00	0.00	3.33 ± 3.33
20	**Wheat germ**	0.00	0.00	0.00	0.00	3.33 ± 3.33
21	**Camphor**	0.00	0.00	0.00	0.00	3.33 ± 3.33
22	**Cactus**	0.00	0.00	0.00	0.00	3.33 ± 3.33
23	**Mustard**	0.00	0.00	0.00	0.00	3.33 ± 3.33
24	**Basil**	3.33 ± 3.33	3.33 ± 3.33	3.33 ± 3.33	3.33 ± 3.33	3.33 ± 3.33
25	**Lettuce**	0.00	0.00	0.00	0.00	0.00
26	**Sesame**	0.00	0.00	0.00	0.00	0.00
27	**Carrot**	0.00	0.00	0.00	0.00	0.00
28	**Pepper**	0.00	0.00	0.00	0.00	0.00
29	**Mint**	0.00	0.00	0.00	0.00	0.00
30	**Fenugreek**	0.00	0.00	0.00	0.00	0.00
Control		0.00	0.00	0.00	0.00	3.33±3.33
F values		0865	0.892	1.208	1.158	1.601
P value		0.661	0.627	0.261	0.307	0.059

Effect of oils on H. dromedarii *semi-engorged female ticks*

Four oils, rosemary, garlic, neem, and Cyperus which revealed the highest mortality against unfed adults were tested against semi-engorged female ticks. All oils caused mortality that increased gradually through days post-treatment to reach the maximum on the 5th day. No mortality was observed in the control group during the period of the experiment (Fig. 1). In general, more than 10% of rosemary oil achieved a significant acaricidal effect ($P < 0.001$) against semi-engorged females on the 5th day (Fig. 1a). Regarding garlic oil, the highest concentration (20%) recorded significant mortalities ($P < 0.001$), with the range 83.3% to 96.7% during all post-treatment days (Fig. 1b). Concerning neem oil, all concentrations revealed slight significance ($P < 0.05$) during five days after treatment. The highest tested concentration of neem oil (30%) recorded 63.3% mortality on the 5th day (Fig. 1c). The 30% Cyperus oil recorded 13.4% mortality higher than neem oil on the 5th day (Fig. 1d). Table 2 shows the LC_{50} and LC_{90} values of *H. dromedarii* semi-engorged females treated with rosemary, garlic, neem, and Cyperus oils at 2nd and 5th days after treatment. These values confirmed that the toxicity of all the tested oils increased with the increase of time after application. Rosemary oil exhibited the highest toxicity, followed by garlic, neem, and Cyperus.

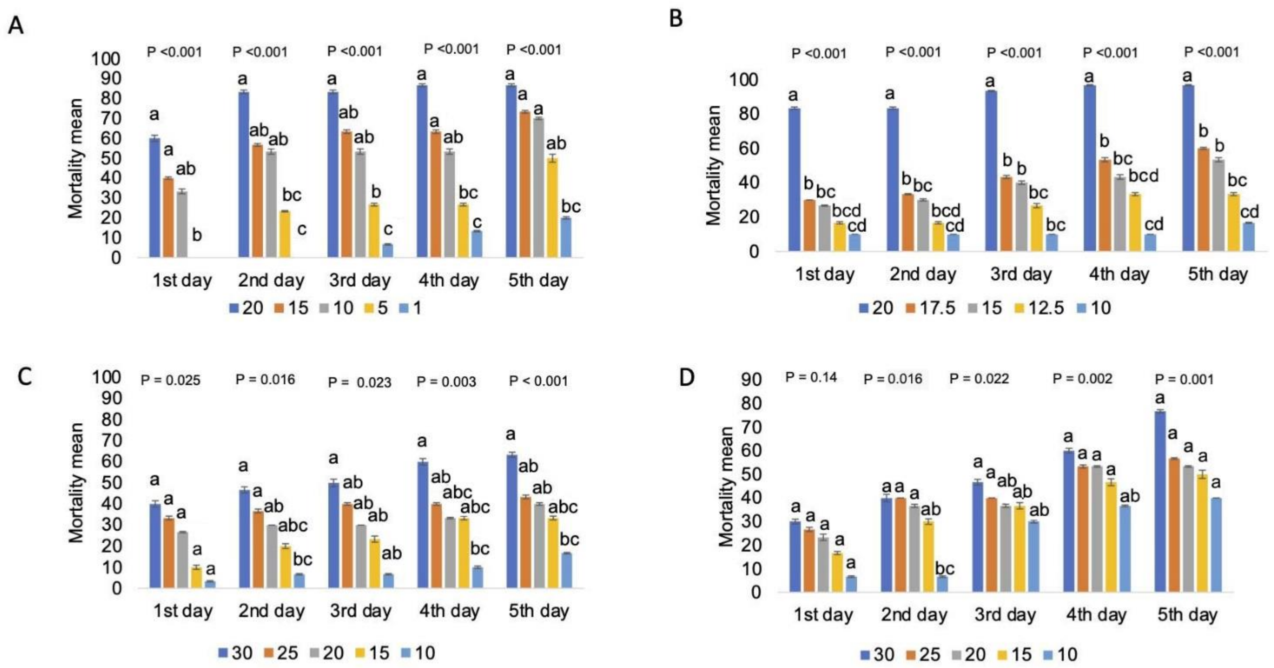

Figure 1. Mortality percentages of *Hyalomma dromedarii* semi-engorged females treated with different concentrations of four oils at five successive days after treatment – **A.** Rosemary; **B.** Garlic; **C.** Neem; **D.** Cyperus. a, b, … etc. indicate significant differences between concentrations (%) of each oil for each day according to Tukey test ($P < 0.001$).

Table 2. LC_{50} and LC_{90} values, together with their confidence limits, for semi-engorged females of *Hyalomma dromedarii* treated with rosemary, garlic, neem, and Cyperus oils at 2nd and 5th-day after treatment.

Oil	Day after treatment	LC_{50} (%)	LC_{90} (%)	Confidence limits (%)				Chi square	Heterogeneity
				LC_{50}		LC_{90}			
				Lower	Upper	Lower	Upper		
Rosemary	2nd	12.256	21.306	10.660	14.077	18.716	25.338	2.1872	1.0936
	5th	8.40	19.17	2.76	14.27	13.59	41.27	1.3837	0.6918
Garlic	2nd	17.391	23.669	16.265	21.434	21.434	27.959	7.3032	2.6499
	5th	14.67	20.41	13.61	15.71	18.82	23.15	6.3581	2.1193
Neem	2nd	29.494	47.598	25.802	36.537	39.475	66.082	0.2218	7.3940
	5th	24.73	42.93	21.71	29.27	36.27	56.49	1.1372	0.3790
Cyprus	2nd	29.613	50.828	25.429	38.119	41.129	74.472	1.7228	0.5742
	5th	18.78	37.58	15.79	21.98	32.04	48.11	1.4543	0.7271

Effect of oils on tick enzyme activities

Tables 3–6 show the activity of GST, AChE, and CAT enzymes in *H. dromedarii* semi-engorged female ticks exposed to 12.3%, 17.4%, 29.5%, 29.6% rosemary, garlic, neem, Cyperus oils, respectively, during 48 h post-treatment. The rosemary oil inhibited the GST and CAT activity of the crude *H. dromedarii* homogenates during the 1st h of incubation (Table 3). This oil increased

GST activity relative to control at 24 h and 48 h. However, it increased CAT activity at 24 h and decreased this enzyme's activity at 48 h. AChE remained to keep its elevated activity levels throughout all incubation periods. Garlic oil inhibited the GST activity of the crude *H. dromedarii* homogenates during the 1st h of incubation. When the incubation time was increased to 24 and 48 h, GST restored its activity (Table 4). AChE activity increased at 1 h, then it kept its increased rates during all the incubation periods. The activity of CAT increased during 1 and 24 h and decreased at 48 h. The activity of GST and CAT at 1 h was decreased (Table 5). After 24 h of incubation, the activity of both enzymes was recovered. By increasing the incubation time to 48 h, the activity of the two enzymes decreased once again. AChE exhibited elevated levels of activity during all the incubation periods. GST values were reduced in all incubation periods (Table 6). AChE activity increased at 1 and 24 h, and dropped to a very low level at 48 h. CAT lost about 30% of its activity at 1 h. The activity recovered after 24 h to 34% over the control value, and then it reduced again at 48 h as it lost about 45% of its activity.

Table 3. Detoxification enzymes and acetylcholinesterase activity in *Hyalomma dromedarii* semi-engorged females exposed to 12.3% rosemary oil during 48 h after treatment.

Incubation time (h)		Catalytic activity (μmole. min.$^{-1}$mg^{-1})		
		GST	**AChE**	**CAT**
1	Control	1.67 ± 0.2^{a}	334.7 ± 17.7^{a}	128.9 ± 6.2^{a}
	Rosemary oil (12.3%)	1.14 ± 0.004^{a} (68.6%)	542.5 ± 6.3^{b} (168%)	104.5 ± 3.6^{a} (81%)
24	Control	5.17 ± 0.2^{b}	789.6 ± 11.3^{c}	351.8 ± 3.9^{b}
	Rosemary oil (12.3%)	6.44 ± 0.18^{c} (124.4%)	2390.1 ± 5^{e} (302.6%)	975.4 ± 10.7^{d} (277.3%)
48	Control	6.9 ± 0.4 ^{c}	1440.9 ± 4.5^{d}	1852.8 ± 3.7^{e}
	Rosemary oil (12.3%)	7.16 ± 0.22^{c} (138.4%)	2354.3 ± 13.7^{e} (163.4%)	734.6 ± 6.6^{c} (39.6%)
	F value	130.275	6815.618	11040.487
	P value	< 0.001	< 0.001	< 0.001

Different letters show significant differences between incubation time in control and treatment together for each enzyme according to Tukey test (P < 0.001), h: hour, GST: Glutathione S-transferase, AChE: Acetylcholinesterase, CAT: Catalase. The percentage between parentheses indicates to the degree of increasing or decreasing in the enzyme activity.

Table 4. Detoxification enzymes and acetylcholinesterase activity in *Hyalomma dromedarii* semi-engorged females exposed to 17.4% garlic oil during 48 h after treatment.

Incubation time (h)		Catalytic activity (μmole. min.$^{-1}$mg^{-1})		
		GST	**AChE**	**CAT**
0	Control	1.67 ± 0.2^{a}	334.7 ± 17.7^{a}	128.9 ± 6.2^{a}
	Garlic oil (17.4%)	1.14 ± 0.004^{a} (68.6%)	633.8 ± 1.5^{b} (189.4%)	136.7 ± 3.3^{a} (106%)
24	Control	5.17 ± 0.2^{b}	789.6 ± 11.3^{c}	351.8 ± 3.9^{b}
	Garlic oil (17.4%)	6.4 ± 0.18^{c} (124.4%)	1819.7 ± 4.9^{e} (230.5%)	1064.7 ± 11.2^{d} (302.6%)
48	Control	6.9 ± 0.4^{c}	1440.9 ± 4.5^{d}	1852.8 ± 3.7^{e}
	Garlic oil (17.4%)	7.17 ± 0.22^{c} (104%)	1997 ± 4.8^{f} (138%)	458.7 ± 3.7^{c} (24.75%)
	F value	130.275	5384.769	12535.498
	P value	< 0.001	< 0.001	< 0.001

Different letters show significant differences between incubation time in control and treatment together for each enzyme according to Tukey test (P < 0.001), h: hour, GST: Glutathione S-transferase, AChE: Acetylcholinesterase, CAT: Catalase. The percentage between parentheses indicates to the degree of increasing or decreasing in the enzyme activity.

Table 5. Detoxification enzymes and acetylcholinesterase activity in *Hyalomma dromedarii* semi-engorged females exposed to 29.5% neem oil during 48 h after treatment.

Incubation time (h)		Catalytic activity (µmole. min.$^{-1}$mg^{-1})		
		GST	AChE	CAT
0	Control	1.67 ± 0.2^{a}	334.7 ± 17.7^{a}	128.9 ± 6.2^{b}
	Neem oil (29.5%)	0.73 ± 0.02^{a} (43.7%)	428.47 ± 4.6^{b} (128%)	78.96 ± 2.1^{a} (61.2%)
24	Control	5.17 ± 0.2^{c}	789.6± 11.3^{c}	351.8 ± 3.9^{c}
	Neem oil (29.5%)	6.8 ± 0.11^{d} (131.8%)	1466 ± 14^{d} (185.7%)	1316.4 ± 3.67^{e} (374.2%)
48	Control	6.9 ± 0.4^{d}	1440.9 ± 4.5^{d}	1852.8 ± 3.7^{f}
	Neem oil (29.5%)	6.8 ± 0.27^{d} (9%)	1746 ± 8^{e} (121%)	759.85 ± 3.6^{d} (41%)
	F value	138.935	2841.701	30467.130
	P value	< 0.001	< 0.001	< 0.001

Different letters show significant differences between incubation time in control and treatment together for each enzyme according to Tukey test (P < 0.001), h: hour, GST: Glutathione S-transferase, AChE: Acetylcholinesterase, CAT: Catalase. The percentage between parentheses indicates to the degree of increasing or decreasing in the enzyme activity.

Table 6. Detoxification enzymes and acetylcholinesterase activity in *Hyalomma dromedarii* semi-engorged females exposed to 29.6% Cyperus oil during 48 h after treatment.

Incubation time (h)		Catalytic activity (µmole. min.$^{-1}$mg^{-1})		
		GST	AChE	CAT
1	Control	1.67 ± 0.2^{a}	334.7 ± 17.7^{a}	128.9 ± 6.2^{b}
	Cyperus oil (29.6%)	1.17 ± 0.004^{a} (70%)	693.4 ± 2.8^{c} (207.2%)	87.2 ± 2.9^{a} (67.6%)
24	Control	5.17 ± 0.2^{c}	789.6 ± 11.3^{d}	351.8 ± 3.9^{c}
	Cyperus oil (29.6%)	3.67 ± 0.2^{b} (71%)	2129.7 ± 10^{f} (270%)	473.6 ± 1.8^{d} (134.6%)
48	Control	6.9 ± 0.4^{d}	1440.9 ± 4.5^{e}	1852.8 ± 3.7^{f}
	Cyperus oil (29.6%)	4.5 ± 0.14^{bc} (65%)	614.6 ± 3.6^{b} (42.65%)	1027.25 ± 5.7^{e} (55.4%)
	F value	90.088	4484.871	24470.027
	P value	< 0.001	< 0.001	< 0.001

Different letters show significant differences between incubation time in control and treatment together for each enzyme according to Tukey test (P < 0.001), h: hour, GST: Glutathione S-transferase, AChE: Acetylcholinesterase, CAT: Catalase. The percentage between parentheses indicates to the degree of increasing or decreasing in the enzyme activity.

DISCUSSION

There are many oils produced from plants in local markets in Egypt. These oils are affordable and available to poor farmers who possess few animals and can use these oils to control tick infestation instead of applying chemical acaricides. However, the acaricidal activity of the oils available in the local market of Egypt is unknown. Therefore, the first objective of this study was to evaluate 30 oils (20% v/v for each) produced by a local company in Egypt against *H. dromedarii* unfed adult ticks to detect the oils that possess acaricidal activity. The second aim of the current study was to determine the bioassay of *H. dromedarii* semi-engorged females tick treated by the most effective oils against unfed adults (rosemary, garlic, neem, and Cyperus). The third objective was to evaluate the enzymatic activities of GST, AChE, and CAT of semi-engorged female ticks that were treated with LC_{50} of oils calculated after two days in the bioassay experiment.

In the present study, 4 out of 30 oils exhibited the highest mortalities against unfed adults of *H. dromedarii*. These oils were rosemary, garlic, Cyperus, and neem recording a mortality range of 23.33 ± 13.33% to 40.00 ± 30.00% at 15 days after treatment. Despite applying a relatively high concentration of these oils (20%), the mortality% was not over 40% which may attribute to the chitinized structure of the unfed adults that may protect the ticks and reduce the penetration of oils inside the body of the ticks. Confirming this explanation, the semi-engorged females of *H. dromedarii* revealed faster and higher susceptibility to these oils. The LC_{50} range of these oils against semi-engorged females was 8.4% to 24.73% at five days after treatment. Higher and more rapid toxicity in the semi-engorged females may be attributed to the wide and soft surface of their integuments, which allows oils to penetrate inside the tick bodies. These findings indicated that these four oils have acaricidal activity in agreement with the previous publications that recorded these oils had either pesticide or repellent activities against acarines (Camarda *et al.* 2018; Nasreen *et al.* 2020; Wong *et al.* 2021). In this study, ≥ 10% rosemary oil and ≥ 20% garlic oil revealed a strong acaricidal effect against semi-engorged females of *H. dromearii*. The LC_{50} of these two oils on the 5^{th} day was 8.4% and 14.6%, respectively. However, the LC_{50} of Cyperus and neem oils on the 5^{th} day was 18.8% and 24.7%, respectively. In line with these findings, previous publications showed acaricidal effects of these oils against different tick species (Djebir *et al.* 2019; Elmhalli *et al.* 2019).

In the present study, both rosemary and garlic oil inhibited the GST activity during the 1 h incubation period. However, GST activity was significantly increased during the incubation period of 24 h and 48 h, respectively. These results were similar to the result of Shahriari *et al.* (2018) who found increased GST activity in *Ephestia kuehniella* larvae artificially fed on a diet containing α-pinene, trans-anethole, and thymol. On the other hand, neem and Cyperus oil significantly decreased GST activity during all incubation periods, except neem oil significantly increased GST activity during 24 h incubation period. These results were in agreement with a previous study by Aboelhadid *et al.* (2021) that revealed a significant decrease in GST activity after treatment of *R. annulatus* larvae with fennel essential oil. Regarding AChE, the four essential oils in the current study induced a significant increase in AChE activity at all incubation periods except for Cyperus oil, which induced a significant decrease in Cyperus oil during 48 h incubation. Our results were contrary to the results obtained by Aboelhadid *et al.* (2021) where AchE activity was significantly inhibited in larvae after treatment with fennel oil and its main constituents, trans-anethole, and fenchone. The current results agreed with the study evaluated by Jemec *et al.* (2007), who showed that imidacloprid can significantly increase AChE, GST activities per protein content of the water flea *Daphnia magna* (Daphniidae: Anomopoda) after 21 d exposure. The results of catalase activity in the present work showed the same effect in all treatments where it was significantly decreased at 1 h and 48 h and increased at 24 h incubation period except garlic oil which caused a significant increase at 1 h and 24 h and decreased at 48 h incubation period. The inhibited catalase activity was previously detected in a study conducted by Gupta *et al.* (2017) who recorded the decrease in catalase activity after-treatment of the *Spodoptera litura* larvae (Noctuidae: Lipedoptera) with hot hexane extract of *Brassica juncea* (Czern.) indicating that these enzymes had no defensive role against glucosinolates rich extracts of *B. juncea*.

CONCLUSION

The acaricidal effect of oils against semi-engorged females of *H. dromedarii* ticks can be ordered as follows: rosemary > garlic > Cyperus > neem. Rosemary and garlic oils are recommended in the control program of ticks as safe alternatives to chemical acaricides.

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اثرهای روغن‌های تجاری بر کنهٔ شتر، *Hyalomma dromedarii* (Acari: Ixodidae) و فعالیت‌های آنزیمی آنها

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چکیده

کنهٔ *Hyalomma dromedarii* گونه غالب کنه‌های انگل شتر در مصر است. این مطالعه با هدف بررسی فعالیت کنه‌کشی روغن‌های منتخب علیه *H. dromedarii* و تعیین اثرهای آن‌ها بر فعالیت آنزیم‌ها انجام شد. آزمایش غربالگری روی بزرگسالان تغذیه نشده با استفاده از ۳۰ روغن در غلظت ۲۰٪ انجام شد. روغن‌های رزماری، سیر، چریش و ساپیروس در یک آزمایش زیست‌سنجی در برابر ماده‌های کمی تغذیه کرده مورد ارزیابی قرار گرفتند. ماده‌های کمی تغذیه کرده در معرض LC50 قرار گرفتند که پس از دو روز تخمین فعالیت آنزیمی گلوتاتیون اس ترانسفراز (GST)، استیل کولین استراز (AChE) و کاتالاز (CAT) محاسبه شد. نتایج آزمایش غربالگری نشان داد که مؤثرترین روغن‌ها در برابر بالغ‌های تغذیه نشده رزماری، چریش، ساپیروس و سیر بودند. در آزمایش زیست‌سنجی، روغن رزماری بیشترین سمیت را در روز دوم (LC50: 12.3٪) و سپس روغن سیر (LC50: 17.4٪) نشان دادند. روغن‌های چریش و ساپیروس کم و بیش سمیت مشابهی را نشان دادند که برای هر یک LC50 حدود ۲۹ درصد در روز دوم ثبت شد. چهار روغن انتخاب شده افزایش یا کاهش زیادی در فعالیت آنزیم‌های GST، AChE و CAT (P < 0.001) در طول ٤٨ ساعت پس از درمان نشان دادند. در نتیجه، روغن رزماری و سیر اثرهای کنه‌کشی قوی علیه *H. dromedarii* دارند. بنابراین، این دو روغن را می توان در برنامه کنترل کنه به عنوان جایگزین ایمن برای کنه‌کش‌های شیمیایی توصیه کرد.

واژگان کلیدی: فعالیت کنه‌کش، ساپیروس، سیر، چریش، رزماری.

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