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Novel Acaricidal and Insect Growth Regulating Activity of Olive oil against *Hyalomma dromedarii* (Acari: Ixodida)

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Abstract

Ticks (Acari: Ixodida) are important blood- feeding ectoparasites acting as vectors of serious diseases of humans and animals. The camel tick, *Hyalomma dromedarii* is infesting camels and its control is very important for prevention of tick-borne diseases. This study evaluated the adulticidal toxicity of olive oil against *H. dromedarii* males and engorged females using the adult immersion test. Mortalities, the number of hatched eggs, hatchability%, weight of engorged females, and egg weight were recorded. The morality% of olive oils against male *H. dormidarii* 12 and 15 days post treatments (PT) reached 50 and 83.33%, respectively. Its lethal concentrations (LC) values were calculated (LC_{50} and LC_{95} values PT for 12 days were 12.715 and 46.386%, respectively). The lethal time values expressed as LT_{50} and LT_{99} after male treatment with 25% were 5.161 and 22.007 days, respectively. Olive oil adversely affected the reproductive potential of *H. dromedarii* engorged females as 25% PT, the number of hatched eggs, hatchability%, weight of engorged females, and egg weight were 2.83±2.31, 32.7, 52.50±2.88 g, and 0.27±0.27 g, respectively. It is recommended to apply olive oil as a safe control tool against *H. dormidarii* and it could be implemented in integrated- tick control strategies.

Keywords: *Olea europaea* L., adulticides, reproductive potential, hatchability, lethal time.

1. Introduction

Ticks **(**Acari: Ixodoidea) are ectoparasites transmit serious diseases [1-8]. The camel tick, *Hyalomma dromedarii* led to severe production losses plus being a vector of infectious diseases [9].

It has a wide distribution throughout North Africa, Asia, and the Middle East infesting mainly camels, but could infest a wide range of domestic animals and wildlife [10]. *H. dromedarii* is a vector of the Crimean–Congo haemorrhagic fever virus, *Theileria annulata*, *Theileria camelensis*, and *Coxiella burnetii* [11].

Chemical acaricides mainly control ticks as well as some other biorational pesticides [12-17]. Because of the development of resistance against acaricides, searching for natural alternative control strategies is very important. Plant- based parasiticides are highly effective [18-21]

Essential oils and plant extracts were used since ancient Egyptian civilizations [18,22] because of their high efficacy and low toxicity, safety to non-target organisms [15,19,23-29]. Development of resistance against oils is less likely to occur due to various mechanisms of actions [30-32]. Botanicals induce ovicidal, larvicidal, adulticidal, and insect growth regulating (IGRs) effects [26,28,33-45].

The olive tree, *Olea europaea* L., is a small tree found mainly in the Mediterranean countries. Consumption of the extra-virgin olive oil rich in phenolic compounds has valuable antioxidant effects on healthy human adults. It has experienced an increased popularity because of its organoleptic characteristics and its associated beneficial health effects [46,47].

Olive oil contains major compounds as unsaturated fatty acids (mainly oleic acid) and minor components like tocopherols or phenolic compounds (antioxidants); therefore, the daily consumption of olive oil promotes health benefits like antioxidant, cardioprotective, anti-inflammatory, anti-tumor properties, and act as a regulator of the intestinal microbiota [48].

There are no studies about using fixed oils against *H. dromedarii* ticks; consonequently, this study evaluated the adulticidal and insect growth regulating effects of olive oil against semifed males and engorged females of *H. dromedarii* and calculating their lethal time and concentration values.

2. Materials and Methods

2.1. Plant source

Extra virgin olive oil, *Olea europaea* L., Mano, Badr City– Egypt was used as acaricides.

2.2. Tick

Adult males and engorged females of *H. dromedarii* were manually collected from the environment around camels in Toukh (35 km north of Cairo: 30° 21′ 11.6″ N, 31° 11′ 31.5″ E), Qalyubia Governorate, Egypt. Morphological identification of ticks was done at the laboratory [49].

2.3. Adult immersion tests

2.3.1. Adulticidal effect

The bioassay, In-vitro adult immersion test (AIT)**,** was used to estimate the toxicity of olive oil against *H. dromedarii* [12]. Ten active males were immersed for 60s in 100 ml solution at each concentration dissolved in a solvent (5% tween 20 dissolved in distilled water).

Thirty ticks/ concentration were used in three replicates for each concentration group. The control group was treated with the solvent. The ticks were placed in a Petri dish with a filter paper (Whatman N. 1) after immersion. Tick mortalities were checked up to 15 days post-treatment (PT) and recorded as dead if no reaction was shown after stimulation with a needle. Mortalities were corrected (Abbott, 1925).

2.3.2. Insect growth regulating effect

With a slight modification to a previously described protocols [12,50], olive oil was tested against engorged females of *H. dromedarii*. Five concentrations were freshly prepared in the solvent. For each concentration, thirty ticks were tested, three replicates each. Each tick was weighted and treated individually. Immersed ticks were kept uprightly in a labeled vertical test tube covered with a cotton plug (27 ± 2°C and 80± 5% RH). Each female and egg mass was weighted and the number of hatched eggs was counted.

8. Data analysis

For data analyses, the-Way Analysis of Variance (followed by the Duncan test) and the probit analysis were used to compute lethal concentrations (LC) and lethal time (LT). Chi-square (X^2) and regression squared (R^2) were calculated and a p-value <0.05 was considered as the threshold of significance. SPSS V23 (IBM, USA) was used for data analyses.

3. Results

Table (1) The Efficacy of Olive Oil against male *Hyalomma dromedarii*.

Days PT	Mortality% ± SD					
	25%	**12%**	**6%**	**3%**	**1.5%**	**Control**
3	46.67 ± 0.58 b,A		23.33±1.15 a,AB	20.00±1.00 a,AB	13.33±1.15 a,B	0.00±0.00
7	66.67±0.58 a,A	36.67±1.15 a,B	30.00±1.00 a,B	23.33±0.58 a,BC	20.00±0.00 a, BC	0.00±0.00
12	80.00± 1.00 a,A	43.33± 1.52 a,B	36.67± 3.66 a,B	33.33± 1.00 a, BC	26.67± 0.57a, BC	3.33±0.58
15	83.33±0.58 a,A	50.00±1.00 a,B	43.33±1.15 a,B	40.00±1.00 a, B	30.00±0.00 a,B	6.66±0.58

PT: days post treatments

SD: standard deviation

Small letters indicated that there is no significant difference (P>0.05) between any two means, within the same column have the same letter

Capital letters indicated that there is no significant difference (P>0.05) between any two means, within the same raw have the same letter

Table (2) Lethal Concentration (LC) values of Olive Oil against *Hyalomma dromedarii* males, 12 days Post treatments.

	Values (%)	**Upper limit**	**Lower Limit**		
LC_{50}	12.715	9.771	16.988	**Chi square**	4.983
LC_{90}	31.264	24.722	44.351	**significance**	0.289
LC_{95}	36.522	28.701	52.367	**Degree of freedom (df)**	4
LC_{99}	46.386	36.104	67.466	**R squared**	0.832
				Regression equation	Y=0.95+0.07*X

Table (3) Lethal time (LT) values (per days) of Olive Oil against *Hyalomma dromedarii* males after treatment with 25%

	Values	**Upper limit**	**Lower Limit**		
LT_{50}	5.161	3.914	6.351	**Chi square**	10.912
LT_{90}	14.441	12.316	17.878	**significance**	0.143
LT_{95}	17.072	14.460	21.385	**Degree of freedom (df)**	7
LT_{99}	22.007	18.433	28.010	**R squared**	0.817
				Regression equation	Y=0.2+-2*X

Table (4) The Efficacy of Olive Oil against *Hyalomma dromedarii* engorged females.

Conc.%	**Number of hatched eggs**	**hatchability %**	**Weight of Engorged females(g)**	**Egg Weight (g)**
Control	1203.33± 323.04a	96.7	77.33±12.87a	38.43±9.10a
0.75	354.33± 144.39b	90.5	53.17±3.76b	5.58±3.46b
1.5	229.33±133.79bc	86.0	53.17±3.76b	2.24±1.39b
3	90.67±48.36bc	62.3	52.33±2.94b	2.24±1.39b
12	12.67±11.87c	45.5	48.00±10.80b	0.34±0.27b
25	2.83±2.31c	32.7	52.50±2.88b	0.27±0.27b

The data represent a dose and time dependent relationship which was significantly different from those of the control groups. The mortality% of olive oil against male *H. dromedarii* reached 50.00 and 83.33% post-treatment with 12 and 25%, respectively (Table 1). The LC_{50}, LC_{90}, LC_{95}, and LC_{99} values of olive oil against *H. dromedarii* males, 12 days Post treatments were 12.715, 31.264, 36.522, and 46.386%, respectively (Table 2).

The LT_{50}, LT_{90}, LT_{95}, and LT_{99} values of olive oil against *H. dromedarii* males after treatment with 25% were 5.161, 14.441, 17.072, and 22.007 days, respectively (Table 3).

The Efficacy of olive oil against *H. dromedarii* engorged females indicated marked and significant reduction of the number of hatched eggs, hatchability%, and engorged females and egg weights reaching 12.67g, 45.5%, 48.00g, and 0.34g, respectively, PT with 12%. The corresponding values were 2.83, 32.7%, 52.50g, and 0.27g, respectively, PT with 25% (Table 4).

4. Discussion

Plant extracts and oils are available and offer affordable alternatives [51,52]for poor farmers who owned a few animals with tick infestation. *H. dromedarii* is a serious vector infesting camels and the other livestock in Egypt. This study indicated the efficacy of olive oil against male and engorged females of *H. dromedarii* as a concentration and time dependent relationship. The mortality% of olive oil against male *H. dromedarii* was 83.33% PT with 25% and its LC_{50}value was 12.715%, 12 days PT.

In Egypt, there are only a few studies evaluated essential oils against ticks; *Citrus sinensis* exhibited an ovicidal effect against *H. dromedarii* [53]. Essential oils of *Mentha piperita*, *Mentha viridis*, *Marjorana hortensis*, *Lavandula officinalis*, and *Ocimum basilicum* showed acaricidal activity against *Rhipicephalus* (formerly *Boophilus*) *annulatus* [54].

Similar findings recorded that *Araucaria heterophylla* and *Commiphora molmol* extracts effectively controlled camel and cattle ectoparasites as 100% mortality was reached 7 days PT with 25 mg/mL of *H. dromedarii* and *R. (Boophilus) annulatus*. LC50 against *H. dromedarii* were1.13 and 1.04 mg/mL for methanol extracts and 1.47 and 1.38 mg/mL for hexane extracts, respectively. On the other hand, the LC_{50} values were 1.09 and 1.41 plus 1.55 and 1.08 mg/mL, respectively, PT with the methanol and hexane extracts against *R. annulatus* [55].

A field study about the limitation of Karoo paralysis ticks through vegetation management revealed that tick density at modified wild olive trees (*Olea europaea africana*) (0.027 ticks/m^2) differed significantly ($P < 0.05$) from that at control trees (0.088 ticks/m^2) [56]. Furthermore, methanolic extract of *Olea europaea* Inhibited growth of *Babesia* and *Theileria* spp. Transmitted by ticks [57]. Bulbs mixed with olive leaves were crushed to make paste was used traditionally used for the control of ticks and tick-borne diseases in South Africa [58].

Some other oils have acaricidal effects as hemp (*Cannabis sativa* L.) essential oil which induced larvicidal and ovicidal effects against *H. dromedarii* [59].

A similar study used olive as a carrier solvent for the acaricidal activity of essential oils extracted from cumin seeds (*Cuminum cyminum*), allspice berries (*Pimenta dioica*), and basil leaves (*Ocimum basilicum*) against 10-day-old *Rhipicephalus* (*Boophilus*) *microplus* tick larvae using the larval packet test. Cumin has a high toxicological effect (100% mortality) in all tested concentrations and allspice produced 100% mortality at all concentrations with the exception of a dramatic decrease at 1.25% concentration; conversely, basil was not shown to be toxic against *R. microplus* larvae [60].

This study indicated that the efficacy of olive oil against *H. dromedarii* engorged females and significantly reduced hatchability% and weight of engorged females and egg masses. A parallel study indicated that 100% acaricidal effects against *H. dromedarii* were reached 8 h PT with 2% rose Bengal (RB) and 24 h PT with 2.5% IVR. The LC_{50} values eight hours PT with RB and IVR were 0.08 and 0.35%, respectively; on the meanwhile, LC_{95} were 1.45 and 30.07%, respectively. LT50, values of 2% RB and 2.5% IVR were 0.92 and 2.63 h, respectively. Sublethal concentrations of RB reduced the number of survived and ovipositing females, eggs per female, ticks laid hatched eggs, and hatched eggs were 48.98, 93.33, 1854.53±45, 97.5, and 93.64%; whereas the related values PT with IVR were 26.53%, 86.67%, 7661.27±377, 87.80%, and 89.40%, respectively [61].

The efficacy of safranin (SF) and tetramethrin (TM) against engorged females of *H. dromedarii* through *in vitro* immersion bioassays pointed out that 100% acaricidal effect was reached PT with 4% SF and TM, for 8 and 48 h, respectively. LC_{50} values 8 and 24 h PT were 0.08, 0.03 and 0.78, 0.20%, respectively. Comparing LC_{50} and LC_{90} 2 h PT, SF was 33 and 22 times more potent than TM. LT_{50} PT with 4% SF and TM were 0.80 and 2.17 h, respectively. Sublethal concentrations of SF and TM reduced the number of ovipositing females, eggs per female, ticks laying viable eggs, and hatched eggs [12].

In general, the pesticidal effect of essential oils was widely studied, but such effect of fixed oils as olive oil, used in this study, was barely studied. Similar study indicated different biological effects of sunflower oil alone or as a carrier oil against the sheep blowfly, *Lucilia sericata* [33,62] and *Musca domestica* [63].

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Conflicts of Interest: the authors declare that there is no conflict of interest

Authors' contribution

HA, HK: Helped with lab work and writing; MY: Helped with writing; AS: Helped with statistical analyses.

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