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ORIGINAL ARTICLE

In vitro acaricidal effect of *Melia azedarach* and *Artemisia herba-alba* extracts on *Hyalomma dromedarii* (Acari: Ixodidae): embryonated eggs and engorged nymphs

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Abstract The present study aimed to evaluate the in vitro efficacy of four medicinal plant extracts: petroleum ether and ethyl alcohol extracts of the ripen fruits of *Melia azedarach* and whole aerial parts of *Artemisia herba-alba* against the two inactive stages of the camel tick *Hyalomma dromedarii*, embryonated eggs and engorged nymphs in comparison to reference acaricide Butox®5.0 (Deltamethrin). Egg and nymphal immersion tests at four concentrations with three replicates were used. The deformity in larvae hatched from treated eggs and adults moulted from treated nymphs were observed and photographed by light microscope (LM) and scanning electron microscope (SEM). The results showed that *M. azedarach* and *A. herba-alba* extracts revealed higher significant toxic effects on embryonated eggs and engorged nymphs comparing with the reference acaricide (Butox®5.0) and control. In egg emmersion test, the LC_{50} of petroleum ether extracts of *M. azedarach* and *A. herba-alba* was 3.14 and 3.91%, respectively and LC_{50} of the respective ethyl alcohol extracts was 1.77 and 2.45%. In nymphal immersion test, LC_{50} of petroleum ether extracts of *M. azedarach* and *A. herba-alba* was 0.26 and 1%, respectively, and LC_{50} of the respective ethyl alcohol extracts was 4.17 and 8.7%. Abnormalities were observed by LM and SEM in the larvae hatched from the treated eggs as incomplete development of legs and mouth parts as well as shrinkage mainly in legs and mouthparts of adults emerged from treated nymphs. In conclusion, all extracts and petroleum ether extracts of the two plants have great potential to be developed as a novel acaricidal for controlling eggs and nymphs of *H. dromedarii*, respectively.

Keywords *Hyalomma dromedarii* · Acaricidal activity · Tick · Control · *Melia azedarach* · *Artemisia herba-alba*

Introduction

Ectoparasites cause serious problem to animal health and economy all over the world. They can cause annoyance, skin infection, irritation, anaemia, fever and also act as a vector for various destructive diseases to animals (Fahmy 1980, 1982; Liebisch et al. 1984; Abbas et al. 2014; Yadav et al. 2017). Ticks are very important ectoparasites of domestic and wild animals (Roberts and Janovy 2005). Ticks can cause harm to animals through blood loss, depression of immune function, general stress and irritation and depreciation of the skin and hide value up to 20–30% (Ghosh et al. 2007). Ticks also act as vectors of a variety of infectious diseases harmful to humans and animals, including babesiosis, anaplasmosis, borreliosis, ehrlichiosis and rickettsoises (Abdel-Shafy et al. 2012; Guglielmone et al. 2014; Abdullah et al. 2016). The camel tick, *Hyalomma* (*H.*) *dromedarii* (Acari: Ixodidae), is the predominant tick species infesting camels, 95.6% in Sinai (Van Straten and Jongejan 1993) as well as 89% in Sudan (Elghali and Hassan 2009) and 57.13% in Benha and Belbis, Egypt (Ramadan 1997). *H. dromedarii* behaved as a three

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or two host species, although the most common is the two host life cycle.

Ectoparasites infecting various species of animals are controlled by using chemical acaricides which is mostly the method used throughout the world in spite of several problems like resistance development and negative effects on the non target organisms including humans (Maxwell et al. 2002; El-Seedi et al. 2017; Showler 2017). Control of ticks should be a priority, especially in developing countries due to the significant economic impact of ticks and tick borne diseases on the national economics (Bansal 2005). Due to resistance problems, alternative options are being incorporated in strategic and integrated control programs of the parasites (Masood et al. 2013; Abbas et al. 2017; Idris et al. 2017; Khan et al. 2017; Aboelhadid et al. 2018). Products derived from plants have been extensively used as an alternative way to control parasites, aiming to decrease the resistance development and obtain low-cost biodegradable parasiticides (Chagas et al. 2012). There were many previous reports on the acaricidal activity of plant extracts against *H. dromedarii*, Ascardiac glycosidal extract from *Calotropis procera*, azadirachtin and neem oil from *Azadirachta indica* against larvae and adults of *H. dromedarii* (Al-Rajhy et al. 2003), *Artemisia herba-alba*, *Artemisia monosperma*, *Euphorbia aegyptiaca*, *Francoeuria crispa*, *Mesembryanthemum forskalii* and *Reaumuria hirtella* extracted with successive solvents; hexan, diethyl ether, ethyl acetate and ethanol against the larvae of *H. dromedarii* (Abdel-Shafy et al. 2007) and essential oil from *Citrus sinensis* against *H. dromedarii* eggs (Habeeb et al. 2007).

Melia azedarach Linn. is wide spread and naturalized in most of the tropics and subtropical countries. It was introduced and naturalized in United States of America, Philippines, Argentine, Brazil, many African countries and many Arab countries (Rubae 2009). The effect of extracts from different parts of *M. azedarach* on many pests has been already evaluated. Also the efficacy of hexane, chloroform and ethanolic extracts of *M. azedarach* Linn. ripen fruit was reported against the tick *Rhipicephalus* (*Boophilus*) *microplus* (Borges et al. 2003) and *Dermanyssus gallinae* (Sariosseiri et al. 2018). *Artemisia herba-alba* is one of the plants that grown in Sinai, Egypt. It is a greyish-white perennial dwarf shrub, with small flowers. It is commonly grown in the central and southern wadis (Mohamed et al. 2010). Essential oils from *A. herba-alba* against *Ixodes ricinus* ticks (El-Seedi et al. 2017) and hexan, diethyl ether, ethyl acetate and ethanol extracts of *A. herba-alba* against larvae of *H. dromedarii* (Abdel-Shafy et al. 2007) have been evaluated.

The aim of the present study was to evaluate in vitro acaricidal activity of petroleum ether and ethyl alcohol extracts of *M. azedarach* Linn. and *A. herba-alba* in controlling the inactive stages; emeryonated eggs and engorged nymphs of *H. dromedarii*.

Materials and methods

Collection of plant materials

The ripen fruits of *M. azedarach* (Family: Meliaceae) and whole aerial parts of *A. herba-alba* (Family: Asteraceae) were obtained from Genetics and Cytology Department, Biotechnology Division, National Research Centre.

Preparation of petroleum ether and ethyl alcohol extracts

Melia azedarach ripen fruits and *A. herba-alba* aerial parts were grinded using stainless steel knife mill. Petroleum ether and ethyl alcohol extracts were prepared by adding 150 g powder for each plant to one litter of petroleum ether (grade 40–60 °C) or 70% ethanol and the mixtures were kept for 72 h in Soxhlet extractor. Then, the obtained extracts were filtrated using Whatman filter paper No. 1. Extracts were then concentrated using vacuum rotary evaporator at 40 °C, and a rotation speed of 20 rpm. The plant extracts were transferred to dark glass vials and kept under 4 °C until use.

Ticks

Engorged females of *H. dromedarii* (Acari: Ixodidae) were collected from naturally infested camels at Birqash camel market (30°09′58.4″N 31°02′13.2″E), Giza province, Egypt. The ticks were identified according to (Walker 2003; Estrada-Pena et al. 2004). Females were kept in an incubator in plastic cups (one female/cup) at 25 ± 1 °C and 75–80% RH to provide optimum conditions for oviposition. The eggs laid by each female were collected daily then kept in the incubator. Lots of eggs were used for the immersion test and the remaining eggs were put in labeled plastic tubes, closed by nylon gauze and kept at the same condition to hatch. Hatched larvae were allowed to fed on healthy pathogen free rabbits at room temperature using a capsule technique (Madbouly 1965; El-Kammah 1969) to obtain nymphs which used for nymph immersion test (Osman et al.2014).

Bioassay of plant extracts on ticks

Egg immersion test

For evaluation the acaricidal activities of petroleum ether and ethyl alcohol extract of *M. azedarach* and *A. herba-*

alba on the eggs approximately, 300 embryonated eggs of *H. dromedrii* were divided into three replicates contained 100 eggs in each. These eggs were placed in plastic cups and immersed for 1 min in 500 µl of the tested concentration. The concentrations of petroleum ether extract were 19.1, 9.5, 4.7, 2.3% for *M. azedarach* and 16, 8, 4, 2% for *A. herba-alba.* The concentrations of ethyl extract were 14.3, 7.1, 3.5, 1.7% for *M. azedarach* and 9.6, 4.8, 2.4, 1.2% for *A. herba-alba*. Eggs immersed in ethyl alcohol and petroleum ether for 1 min were considered as solvent control while eggs immersed for 1 min in Butox®5.0 (Deltamethrin) 1 cm/L were considered as reference drug. Subsequently, the solutions were decanted and after evaporation of solvent, the tubes closed with muslin clothes and were incubated at 25 ± 1 °C and relative humidity of 75–80% for 14 days to examine the effect of the extracts by counting the hatched larvae and dead eggs under Carl Zeiss stereo microscope, calculating the mortality percentages in eggs and monitoring the abnormality in the eggs and hatched larvae that photographed by light microscope (LM) and scanning electron microscope (SEM).

Nymphal immersion test

To evaluate the effect of petroleum ether and ethyl alcohol extract of *M. azedarach* and *A. herba-alba* extracts on nymphs, 30 engorged nymphs were divided into three replicates (10/replicate). These nymphs were immersed in 5 mL of the tested concentration. The concentrations of petroleum ether extract were 1.1, 0.59, 0.29, 0.14% for *M. azedarach* and 4, 2, 1.0, 0.5% for *A. herba-alba*. The concentrations of ethyl alcohol extract were 14.3, 7.1, 3.5, 1.7% for *M. azedarach* and 19.3, 9.6, 4.8, 2.4% for *A. herba-alba*. Nymphs immersed in petroleum ether and ethyl alcohol for 1 min were considered as solvent control while nymphs immersed for 1 min in Butox®5.0 (1 cm/L) were considered as reference drug. The tested nymphs were incubated at a temperature of 25 ± 1 °C and 75–80% RH. The number of nymphs failed to moult was counted and their mortality percentages were calculated. The abnormal adults moulted from the treated nymphs were estimated and photographed by LM and SEM.

Preparation of tick specimens for scanning electron microscopy

The tick specimens prepared for scanning electron microscopy (SEM) were; abnormal larvae hatched from eggs treated with the plant extracts, normal larvae hatched from untreated eggs, deformed adults emerged from treated nymphs with the plant extracts and normal adults emerged from untreated nymphs. The tick specimens were

Table 1 Mortality percentages (mean ± SE) of *Hyalomma dromedarii* eggs treated with petroleum ether and ethylalcohol extracts of *Melia azedarach* and *Artemisia herba-alba*

Melia azedarach						*Artemisia herba-alba*					
Petroleum ether			Ethyl alcohol			Petroleum ether			Ethyl alcohol		
Conc. (%)	Mortality (%)	Deformed larvae (%)	Conc. (%)	Mortality (%)	Deformed larvae (%)	Conc. (%)	Mortality (%)	Deformed larvae (%)	Conc. (%)	Mortality (%)	Deformed larvae (%)
19.1	100 ± 0.00d	0.00 ± 0.00^{a}	14.3	100 ± 0.00^{c}	0.00 ± 0.00^{a}	16	100 ± 0.00^{d}	0.00 ± 0.00^{a}	9.6	93.77 ± 1.04^{d}	0.00 ± 0.00^{a}
9.5	92.46 ± 4.17 cd	0.00 ± 0.00^{a}	7.1	90.19 ± 9.80^{bc}	0.00 ± 0.00^{a}	8	81.65 ± 6.16 ^{cd}	0.00 ± 0.00^{a}	4.8	65.53 ± 4.61^{c}	12.52 ± 3.43^{b}
4.7	66.35 ± 10.44c	6.65 ± 2.10^{b}	3.5	73.24 ± 21.40^{bc}	4.06 ± 2.03^{a}	4	52.71 ± 21.8^{bc}	0.00 ± 0.00^{b}	2.4	58.82 ± 4.43^{c}	7.89 ± 0.55^{ab}
2.3	36.65 ± 7.12b	14.06 ± 1.57^{c}	1.7	50.82 ± 3.800^{ab}	3.75 ± 1.87^{a}	2	16.57 ± 2.00^{ab}	2.83 ± 1.42^{a}	1.2	26.35 ± 4.05^{b}	7.37 ± 1.67^{ab}
Butox®5.0 (1 cm/L)	71.66 ± 4.40c	0.00	Butox®5.0 (1 cm/L)	71.66 ± 4.406^{c}	0.00	Butox®5.0 (1 cm/L)	71.66 ± 4.40 ^{cd}	0.00 ± 0.00	Butox®5.0 (1 cm/L)	71.66 ± 4.40^{c}	0.00 ± 0.00
Control	7.09 ± 1.63a	0.00 ± 0.00^{a}		8.10 ± 3.03^{a}	0.00 ± 0.00^{a}		7.09 ± 1.63^{a}	0.00 ± 0.00^{a}		8.10 ± 3.03^{a}	0.00 ± 0.00^{a}
F value	36.93	28.24		10.86	3.00		15.08	3.99		67.75	9.98
P value	< 0.001	< 0.001		< 0.001	NS		< 0.001	0.034		< 0.001	0.002

a, b… etc., indicate the significant difference between the means of mortality percentages according to Tukey test

Conc. concentration, *NS* non significant

well cleaned by overnight immersion in water–glycerol–KCl solution (Homsher and Sonenshine1977; Brody and Wharton,1971). Then, ticks were washed in distilled water several times and immersed one hour in each concentration of 25%, 50%, 70%, 80% ethanol followed by 10 min in each 90% and 100% ethyl alcohol (Keirans et al.1976). Following this, ticks were glued by their dorsal and ventral surfaces to the SEM stub, and were dried by the dryer (Blazer Union, F1-9496 Blazer/Fürstentun Liechtenstein), using liquid carbon dioxide. Ticks mounted on SEM stubs were coated with gold by using a S15OA Sputter Coater. The coated larvae and adults were examined by SEM (QUANTA FEG 250) in National Research Centre.

Statistical analyses

The data was statistically analyzed by one-way ANOVA test following by Tukey test using SPSS program version 20. The lethal concentrations (LC_{50} and LC_{99}) and their respective 95% confidence intervals (CI) were determined by applying regression equation analysis to the probit transformed data of mortality. The concentration response data were analysed by probit method (Finney 1962) using Ehabsoft.

Results

Effect of plant extracts on embryonated eggs

The results indicated that there was a significant effect for *M. azedarach* and *A. herba-alba* extracts on embryonated eggs comparing with the reference acaricide (Butox®5.0) and control (Table 1). The mortality percentage of eggs increased with the increase of the concentration in all plant extracts. The two plants showed strong ovicidal effects comparing with Butox®5.0, especially, in concentrations higher than 9.5% and 8% petroleum ether extracts of *M. azedarach* and *A. herba-alba*, respectively. Furthermore, the concentrations 7.1 and 14.3% ethyl extract of *M. azedarach* and 9.6% ethyl extract of *A. herba-alba* revealed higher significant mortalities than Butox®5.0. At the highest concentration of petroleum ether and ethyl extract of *M. azedarach* the mortality rate reached 100%, while at the highest concentration of petroleum ether and ethyl extract of *A. herba-alba* the mortality rate reached 100% and 93.77% respectively. At concentration of 9.5%, 4.7%, 2.3% and 7.1%, 3.5%, 1.7% the mortality rate reached 92.46%, 66.35%, 36.65% and 90.19%, 73.24%, 50.82% for petroleum ether and ethyl extract of *M. azedarach*, respectively. The percent mortality caused by the petroleum ether extract of *A. herba-alba* varied from 16.57 to 100%, when the eggs tested at concentrations ranging from 2 to 16%, while percent mortality caused by ethyl alcohol extract of *A. herba-alba* varied from 26.35 to 93.77% when the eggs tested at concentration ranging from 1.2 to 9.6%. Lower concentrations of both plant extracts didn't cause 100% mortality but incomplete hatching and deformed larvae were occurred. The percentage of deformed larvae for petroleum ether extracts of *M. azedarach* and *A. herba-alba* was 6.6, 14 and 2.8% at concentrations of 4.7, 2.3 and 2%, respectively. While the percentage of deformed larvae for ethyl alcohol extract of *M. azedarach* and *A. herba-alba* was 4, 3.7, 12.5, 7.8 and 7.3% at concentrations of 3.5, 1.7, 4.8, 2.4 and 1.2%, respectively.

The calculated LC_{50} was 3.14, 3.91% and LC_{99} was 18.64, 18.84% for petroleum ether of *M. azedarach* and *A. herba-alba* while LC_{50} and LC_{99} for ethyl alcohol extracts of *M. azedarach* and *A. herba alba* were 1.77, 2.45% and 17.42, 29.71%, respectively, as shown in Table 2. Furthermore, the LC_{50} and LC_{99} confirmed that the two plants had strong ovicidal effects and *M. azedarach* had slightly higher toxicity on eggs than *A. herba-alba*.

Morphological abnormalities of treated eggs were noticed by light microscope (LM) for the two applied plant extracts (Fig. 1a–g), where eggs appeared dark in color shrinked and dull in appearance (Fig. 1c, d). Also there were several abnormalities appeared in the larvae hatched

Table 2 LC_{50} and LC_{99} values with their confidence limits for *Hyalomma dromedarii* eggs treated with petroleum ether and ethyl alcohol extracts of *Melia azedarach* and *Artemisia herba-alba*

Solvent	Plant	LC_{50} (%)	LC_{99} (%)	Confidence limits				Slope ± SE
				LC_{50} (%)		LC_{99} (%)		
				Lower	Upper	Lower	Upper	
Petroleum ether	*Melia azedarach*	3.14	18.64	2.69	3.57	14.27	27.46	3.00 ± 0.30
	Artemisia herba-alba	3.91	18.84	3.50	4.345	14.94	25.87	3.40 ± 0.29
Ethyl alcohol	*Melia azedarach*	1.77	17.42	1.371	2.12	12.34	29.78	2.34 ± 0.27
	Artemisia herba-alba	2.45	29.71	2.08	2.84	19.78	54.25	2.14 ± 0.22

LC_{50}: lethal concentration for 50% of individuals, LC_{99}: lethal concentration for 99% of individuals

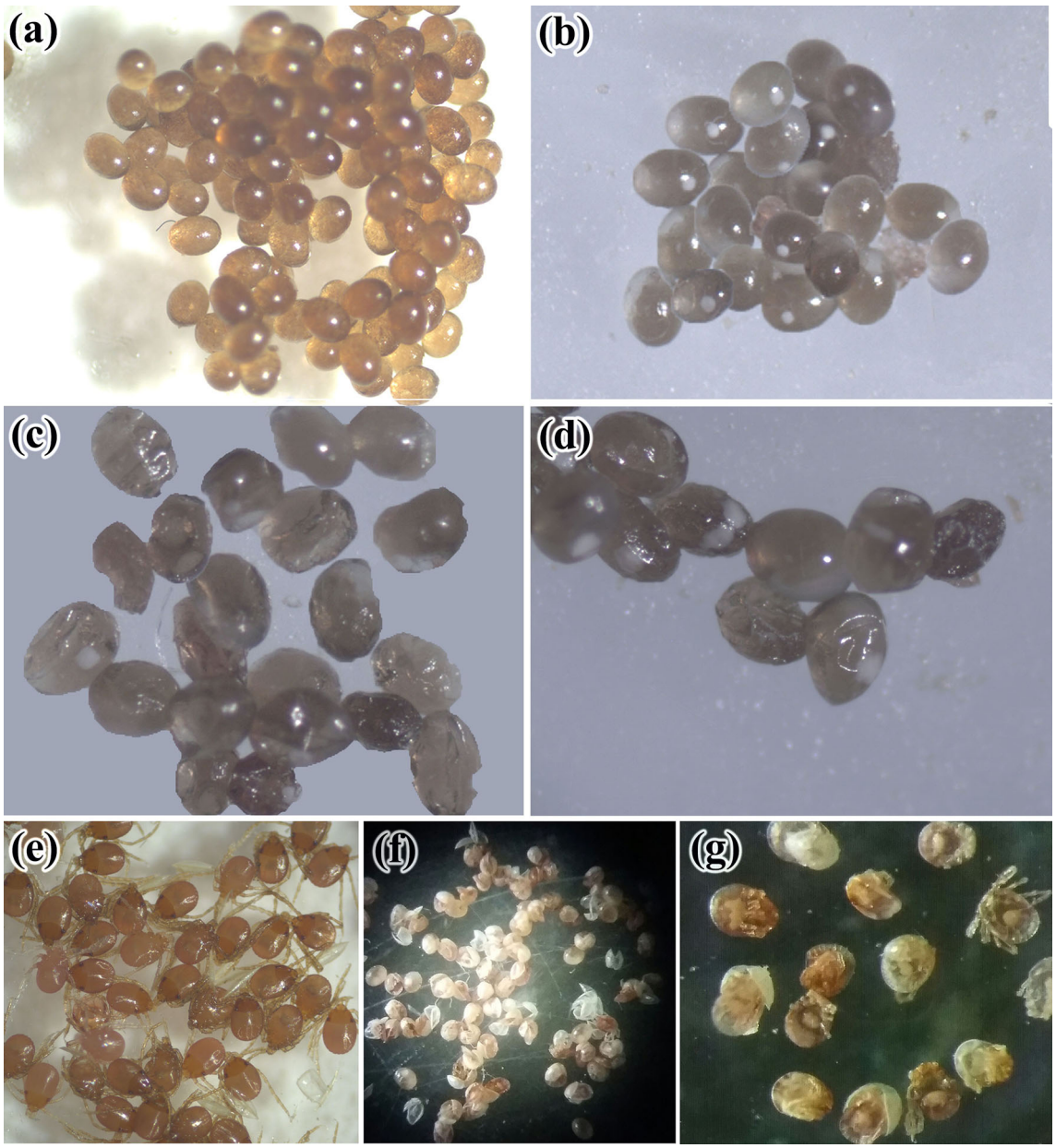


Fig. 1 Light micrograph of normal and treated *H. dromedarii* eggs with plant extracts. **a** Freshly normal laid eggs (× 2), **b** Embryonated normal eggs (× 2.5), **c, d** Shrunken eggs exposed to plant extracts (× 5), **e** Normal larvae hatched from untreated eggs (× 2), **f** Incompletely hatched larvae from treated eggs (× 1.5), **g** Deformed larvae hatched from treated eggs with incomplete development of their legs and mouth parts (× 2.5)

from the treated eggs as incomplete development of legs and mouth parts. Some of larvae partially hatched from treated eggs and maintained inside the egg shell (Fig. 1f, g).

Scanning electron microscopy (SEM) showed normal features of the larva as dorsal surface (Fig. 2a), ventral surface (Fig. 2b), three pairs of legs (Fig. 2c) and mouth parts of larva (Fig. 2d). SEM of abnormal larvae hatched from eggs after treatment with petroleum ether and ethyl extracts of *M. azedarach* and *A. herba-alba* showed some alterations on the dorsal and ventral surfaces. At the dorsal surface there was degeneration of legs and mouth parts (Fig. 2e), also distortion of mouth parts with severely wrinkled cuticle (Fig. 2f). At the ventral surface legs were severely degenerated and wrinkled with complete degeneration of mouth parts (Fig. 2g, h).

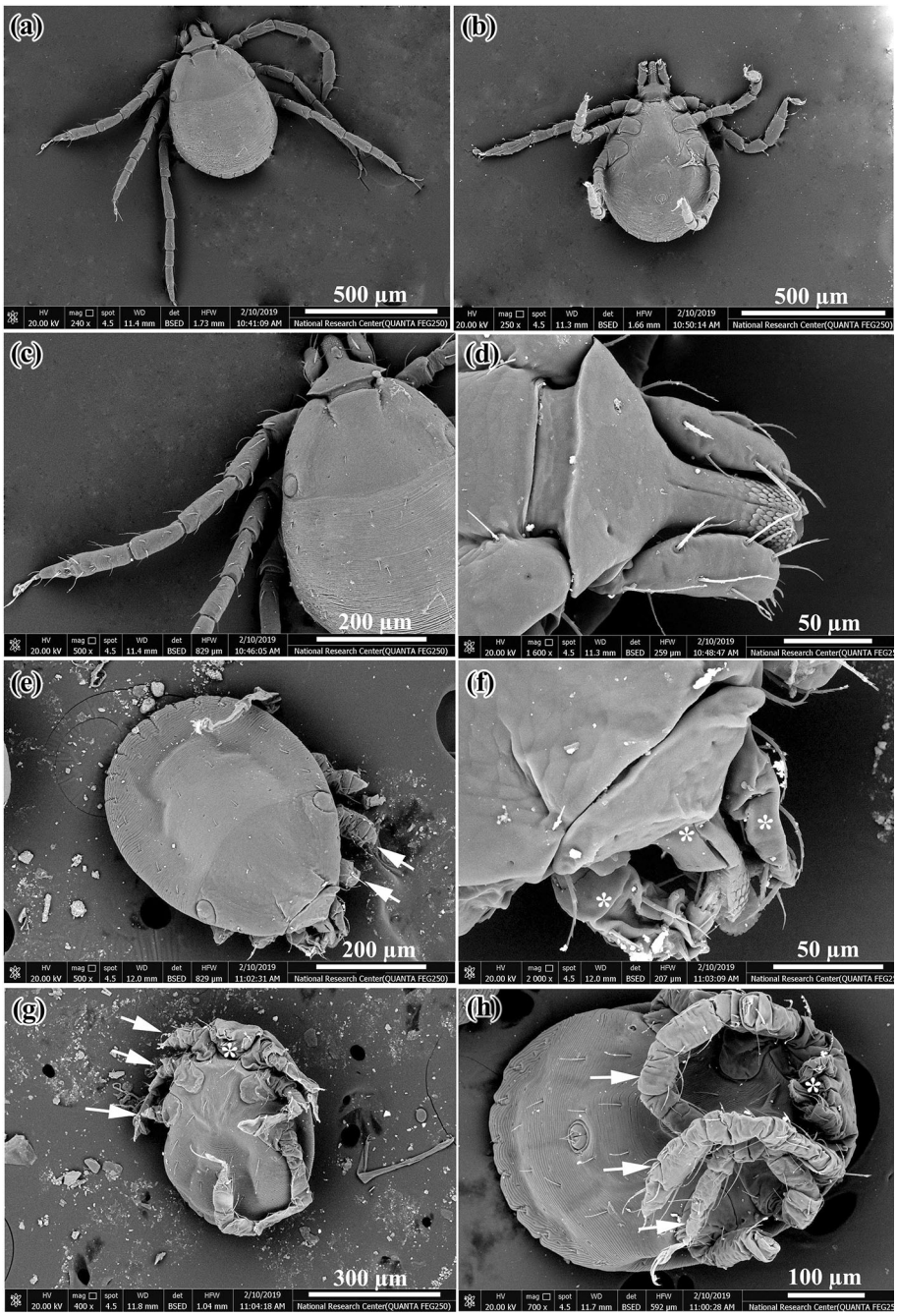


Fig. 2 SEM of normal larvae and deformed larvae hatched from *H. dromedarii* eggs exposed to plant extracts. **a** Dorsal surface of normal larva, **b** Ventral surface of normal larva, **c** Three pair of legs in normal larva, **d** Mouth parts of normal larva, **e** Shrinkage with degeneration of legs in deformed larva (white arrows), **f** Shrunken and distorted mouth parts in deformed larva (white stars), **g** Complete degeneration of legs (white arrows) and mouth parts (white star) in deformed larva and **h** Severely wrinkled legs (white arrows) with degenerated mouth parts (white star) in deformed larva

Effect of plant extracts on engorged nymphs

The results demonstrated that there was a significant effect of petroleum ether and ethyl extract of *M. azedarach* and *A. herba-alba* on the moulting of engorged nymphs of *H. dromedarii* comparing with Butox®5.0 and control (Table 3). The effect of *M. azedarach* extracts was significantly higher than that of *A. herba-alba* where the petroleum ether and ethyl extracts of *M. azedarach* at concentrations of 1.1% and 14.3% caused 100% and 86.66% mortality, respectively. While the petroleum ether and ethyl extracts of *A.herba-alba* at concentration of 4% and 19.3% caused 100% and 70% mortality. However, some deformities were observed in 23.33% and 16.66% of moulted adults which emerged from the nymphs treated with 1% and 0.5% of petroleum ether extracts of *A. herba-alba*, repcetively. LC_{50} values for petroleum ether extracts of *M. azedarach* and *A. herba-alba* were 0.26 and 1%, respectively, while their LC_{99} values were 1.3, and 5.29%, respectively. For ethyl extracts of *M. azedarach* and *A. herba-alba*, LC_{50} and LC_{99} values were (4.17 and 8.7%) and (51.56 and 89.75%), respectively as shown in Table 4.

Table 3 Mortality percentages (mean ± SE) of *Hyalomma dromedarii* nymphs treated with petroleum ether and ethyl alcohol extracts of *Melia azedarach* and *Artemisia herba-alba*

Melia azedarach						*Artemisia herba-alba*					
Petroleum ether			Ethyl alcohol			Petroleum ether			Ethyl alcohol		
Conc. (%)	Mortality (%)	Deformed adults (%)	Conc. (%)	Mortality (%)	Deformed adults (%)	Conc. (%)	Mortality (%)	Deformed adults (%)	Conc. (%)	Mortality (%)	Deformed adults (%)
1.1	100 ± 0.00^{d}	00 ± 0.00	14.3	86.66 ± 3.33^{d}	00 ± 0.00	4	100 ± 0.00^{c}	0.00 ± 0.00^{a}	19.3	70 ± 0.00^{d}	00 ± 0.00
0.59	86.66 ± 3.33 ^{cd}	00 ± 0.00	7.1	70 ± 10.00 ^{cd}	00 ± 0.00	2	76.6 ± 8.81^{c}	0.00 ± 0.00^{a}	9.6	46.66 ± 3.33^{c}	00 ± 0.00
0.29	46.66 ± 17.6^{bc}	00 ± 0.00	3.5	43.33 ± 6.66^{bc}	00 ± 0.00	1	53.33 ± 3.33^{b}	23.33 ± 3.33^{b}	4.8	33.33 ± 3.33bc	00 ± 0.00
0.14	23.33 ± 8.81^{ab}	00 ± 0.00	1.7	20 ± 5.77^{ab}	00 ± 0.00	0.5	16.66 ± 6.66^{a}	16.66 ± 3.33^{b}	2.4	16.66 ± 3.33^{ab}	00 ± 0.00
Butox®5.0 (1 cm/L)	66.66 ± 6.66 ^{cd}	00 ± 0.00	Butox®5.0 (1 cm/L)	66.66 ± 6.66 ^{cd}	00 ± 0.00	Butox®5.0 (1 cm/L)	66.66 ± 6.66^{b}	0.00 ± 0.00	Butox®5.0 (1 cm/L)	66.66 ± 6.66^{d}	00 ± 0.00
Control	0.00 ± 0.00^{a}	00 ± 0.00		3.33 ± 3.33^{a}	00 ± 0.00		0.00 ± 0.00^{a}	0.00 ± 0.00^{a}		3.33 ± 3.33^{a}	00 ± 0.00
F value	19.585	–		25.173	–		47.80	28.25		48.42	–
P value	< 0.001	–		< 0.001	–		< 0.001	< 0.001		< 0.001	–

a, b… etc., indicate the significant difference between the means of mortality percentages according to Tukey test

Conc. concentration

Table 4 LC_{50} and LC_{99} values with their confidence limits for *Hyalomma dromedarii* nymphs treated with petroleum ether and ethyl alcohol extracts of *Melia azedarach* and *Artemisia herba-alba*

Solvent	Plant	LC_{50} (%)	LC_{99} (%)	Confidence limits				Slope ± SE
				LC_{50} (%)		LC_{99} (%)		
				Lower	Upper	Lower	Upper	
Petroleum ether	*Melia azedarach*	0.26	1.3	0.12	0.42	1.47	15.46	3.24 ± 0.28
	Artemisia herba-alba	1.00	5.29	0.52	1.60	5.33	52.35	3.21 ± 0.27
Ethyl alcohol	*Melia azedarach*	4.17	51.56	3.57	4.83	33.93	95.24	2.13 ± 0.21
	Artemisia herba-alba	8.70	89.75	4.16	17.17	132.26	1407.56	2.29 ± 0.17

LC_{50}: lethal concentration for 50% of individuals, LC_{99}: lethal concentration for 99% of individuals

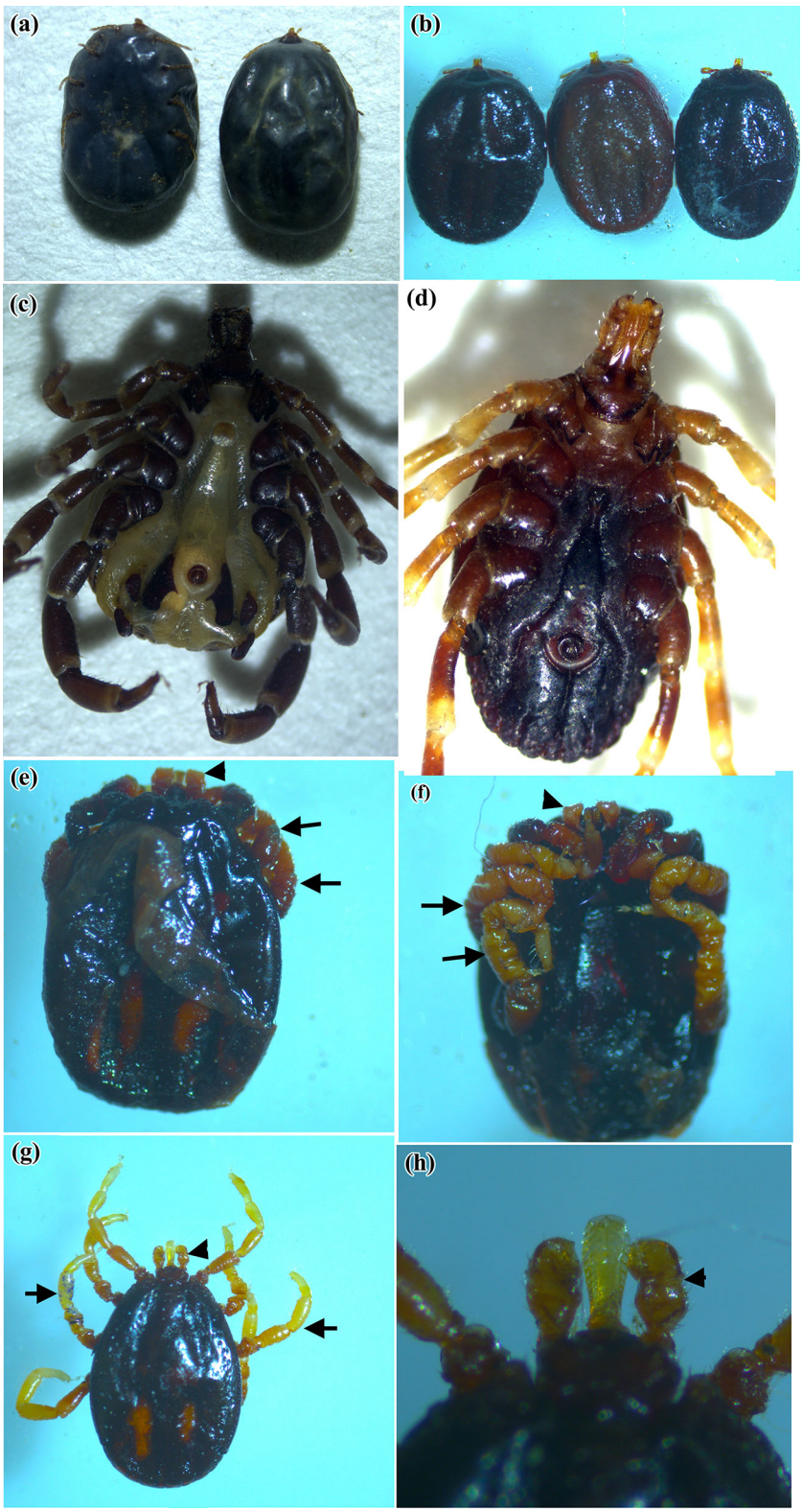
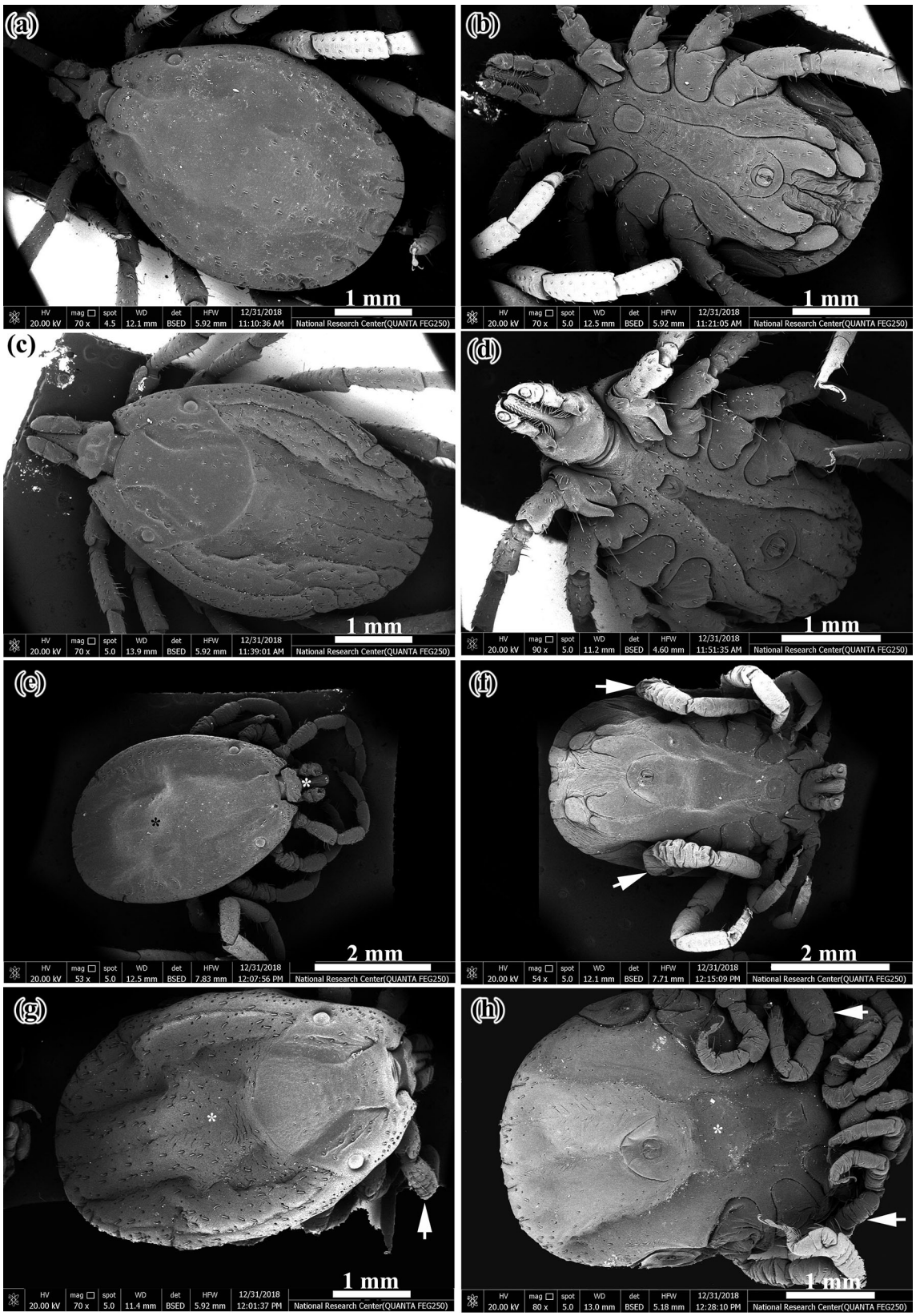


Fig. 3 Light micrographs of *H. dromedarii* nymphs treated by plant extracts and the adults moulted from normal and treated nymphs. **a** Normal nymphs (× 0.8), **b** Dead nymphs with dark colored cuticle (× 0.7), **c** Ventral surface of male moulted from normal nymph (× 1.25), **d** Ventral surface of female moulted from normal nymph (× 2), **e, f** Shrunken legs (black arrows) and degenerated mouth parts (black head arrow) of adult moulted from treated nymph (× 1.5), **g** Swollen leg (black arrows) and mouth parts (black head arrow) of adult moulted from treated nymph (× 1.2) and **h** Magnification of swollen mouth parts (black head arrow) of the deformed adult (× 5)

According to LC_{50} and LC_{99} values, petroleum ether extract of *M. azedarach* was the most toxic one to the engorged nymphs followed by petroleum ether extract of *A. herba-alab*, ethyl extract of *M. azedarach* and ethyl extract of *A. herba-alba*.

The normal nymphs (control), treated nymphs and adult ticks moulted from both untreated and treated nymphs were


(a)
1 mm
HV 20.00 kV | mag 70 x | spot 4.5 | WD 12.1 mm | det BSED | HFW 5.92 mm | 12/31/2018 11:10:36 AM | National Research Center(QUANTA FEG250)
(b)
1 mm
HV 20.00 kV | mag 70 x | spot 5.0 | WD 12.5 mm | det BSED | HFW 5.92 mm | 12/31/2018 11:21:05 AM | National Research Center(QUANTA FEG250)
(c)
1 mm
HV 20.00 kV | mag 70 x | spot 5.0 | WD 13.9 mm | det BSED | HFW 5.92 mm | 12/31/2018 11:39:01 AM | National Research Center(QUANTA FEG250)
(d)
1 mm
HV 20.00 kV | mag 90 x | spot 5.0 | WD 11.2 mm | det BSED | HFW 4.60 mm | 12/31/2018 11:51:35 AM | National Research Center(QUANTA FEG250)
(e)
2 mm
HV 20.00 kV | mag 53 x | spot 5.0 | WD 12.5 mm | det BSED | HFW 7.83 mm | 12/31/2018 12:07:56 PM | National Research Center(QUANTA FEG250)
(f)
2 mm
HV 20.00 kV | mag 54 x | spot 5.0 | WD 12.1 mm | det BSED | HFW 7.71 mm | 12/31/2018 12:15:09 PM | National Research Center(QUANTA FEG250)
(g)
1 mm
HV 20.00 kV | mag 70 x | spot 5.0 | WD 11.4 mm | det BSED | HFW 5.92 mm | 12/31/2018 12:01:37 PM | National Research Center(QUANTA FEG250)
(h)
1 mm
HV 20.00 kV | mag 80 x | spot 5.0 | WD 13.0 mm | det BSED | HFW 5.18 mm | 12/31/2018 12:28:10 PM | National Research Center(QUANTA FEG250)

◀**Fig. 4** SEM of normal adults emerged from untreated *H. dromedarii* nymphs (control) and abnormal adults emerged from treated *H. dromedarii* nymphs. **a** Dorsal surface of normal adult male, **b** Ventral surface of normal adult male, **c** Dorsal surface of normal adult female, **d** Ventral surface of normal adult female, **e** Dorsal surface of male appeared corrugated (black star) and the mouth parts appeared shrunken (white star), **f** At the ventral surface of male, there was swelling and severe corrugation of legs (white arrows). **g, h** Swelling in whole body (white star) and shrinkage in legs (white arrows) were apparent in dorsal and ventral surfaces of female

photographed by LM (Fig. 3a–h). Normal and dead nymphs were shown in Fig. 3a, b. Dead nymphs appeared with dark colored cuticle (Fig. 3b). Normal features of ventral surface of both male and female were shown in Fig. 3c, d. Emerged adults showed some alterations on appendages such as swollen legs and degenerated mouth parts (Fig. 3e–h).

SEM of adults moulted form untreated nymphs (control) showed normal features of dorsal and ventral surfaces of both male and female (Fig. 4a–d). The abnormalities in

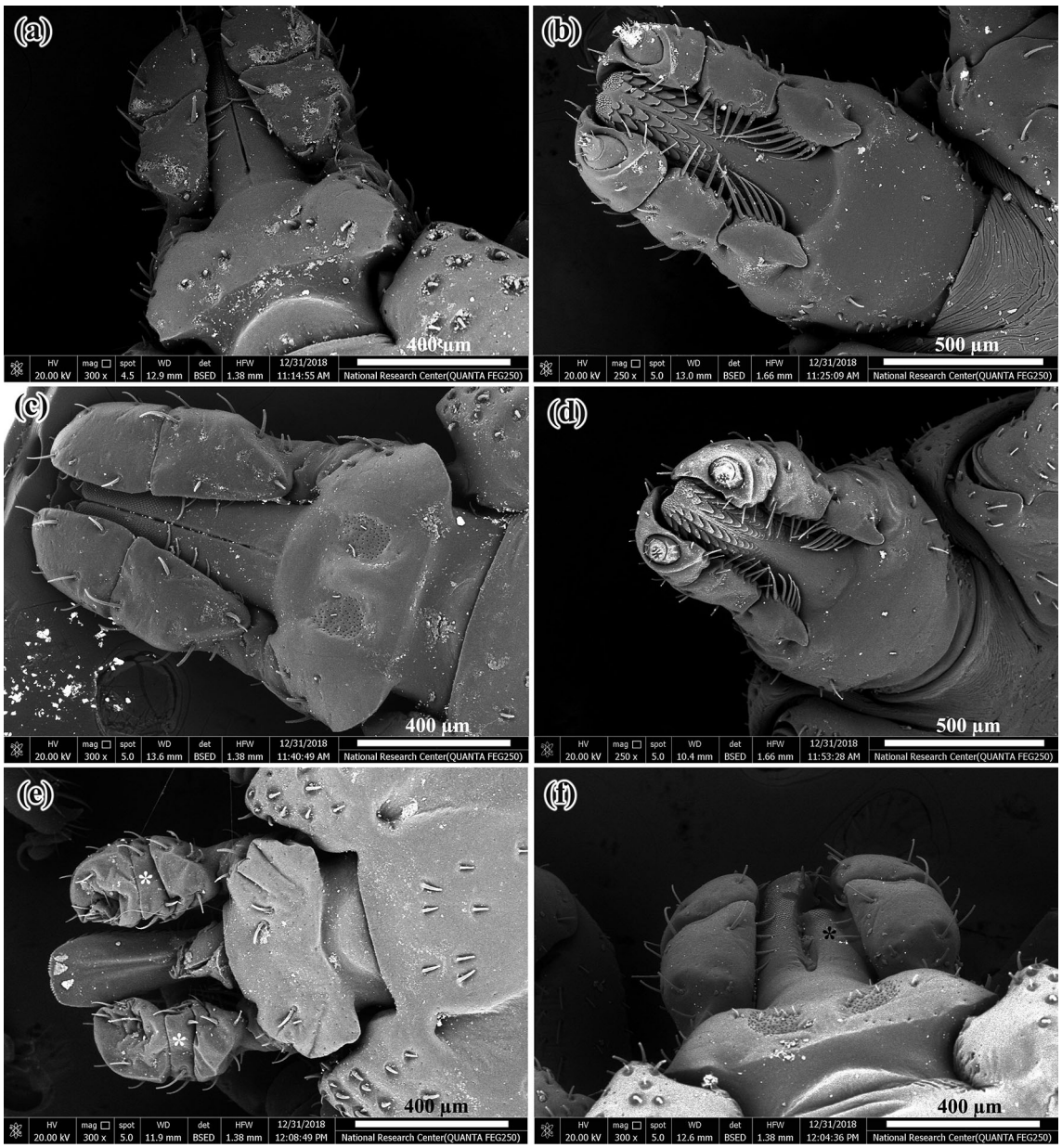
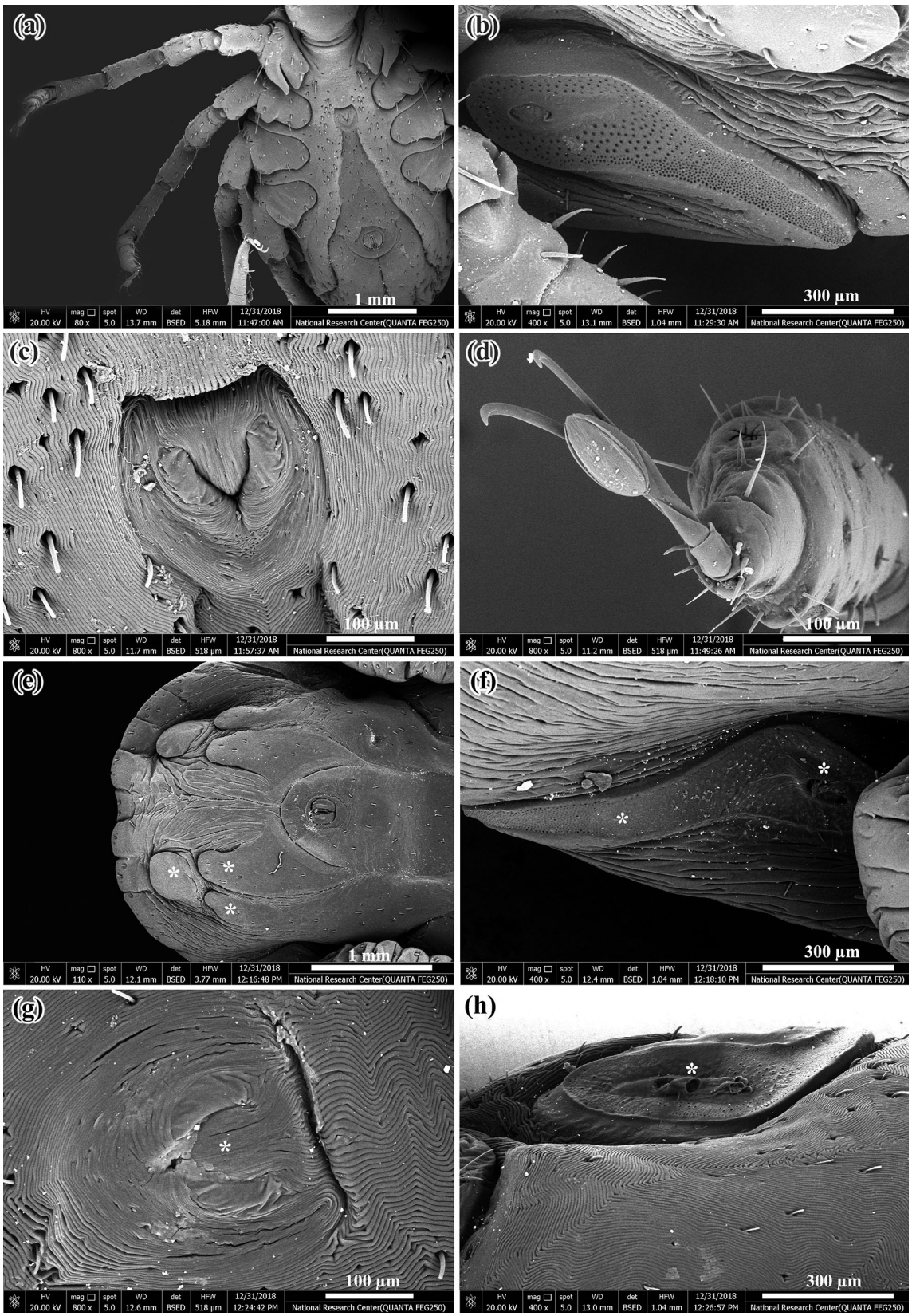


Fig. 5 SEM of male and female mouth parts of *H. dromedarii*. **a** Dorsal surface of normal male mouth parts, **b** Ventral surface of normal male mouth parts, **c** Dorsal surface of normal female mouth parts, **d** Ventral surface of normal female mouth parts, **e** Shrinkage of the two palpi which made hypostome appeared longer than the palpi (white stars) and **f** Incomplete development of cheliceral sheath (black star)


(a)
1 mm
HV 20.00 kV | mag 80 x | spot 5.0 | WD 13.7 mm | det BSED | HFW 5.18 mm | 12/31/2018 11:47:00 AM | National Research Center(QUANTA FEG250)
(b)
300 µm
HV 20.00 kV | mag 400 x | spot 5.0 | WD 13.1 mm | det BSED | HFW 1.04 mm | 12/31/2018 11:29:30 AM | National Research Center(QUANTA FEG250)
(c)
100 µm
HV 20.00 kV | mag 800 x | spot 5.0 | WD 11.7 mm | det BSED | HFW 518 µm | 12/31/2018 11:57:37 AM | National Research Center(QUANTA FEG250)
(d)
100 µm
HV 20.00 kV | mag 800 x | spot 5.0 | WD 11.2 mm | det BSED | HFW 518 µm | 12/31/2018 11:49:26 AM | National Research Center(QUANTA FEG250)
(e)
1 mm
HV 20.00 kV | mag 110 x | spot 5.0 | WD 12.1 mm | det BSED | HFW 3.77 mm | 12/31/2018 12:16:48 PM | National Research Center(QUANTA FEG250)
(f)
300 µm
HV 20.00 kV | mag 400 x | spot 5.0 | WD 12.4 mm | det BSED | HFW 1.04 mm | 12/31/2018 12:18:10 PM | National Research Center(QUANTA FEG250)
(g)
100 µm
HV 20.00 kV | mag 800 x | spot 5.0 | WD 12.6 mm | det BSED | HFW 518 µm | 12/31/2018 12:24:42 PM | National Research Center(QUANTA FEG250)
(h)
300 µm
HV 20.00 kV | mag 400 x | spot 5.0 | WD 13.0 mm | det BSED | HFW 1.04 mm | 12/31/2018 12:26:57 PM | National Research Center(QUANTA FEG250)

◀**Fig. 6** SEM of normal adults emerged from untreated *H. dromedarii* nymphs (control) and abnormal *H. dromedarii* adults emerged from treated nymphs. **a** Ventral surface showed normal coxae, **b** Spiracle plate of normal male, **c** Genital aperture of normal female with posterior lips that form a narrow V shape. **d** Tarsus with normal pulvillus and two claws. **e** Swollen ventral plates of male (white stars), **f** Incomplete development of pores in spiracle plate of male (white stars), **g** Swelling and distortion of the genital aperture of female (whit star) and **h** Degeneration of spiracle plate with its structure not clear in female (white star)

whole body of the adults moulted from the treated nymphs were observed by SEM as shown in Fig. 4e–h. The dorsal surface of male was corrugated with shrunken mouth parts (Fig. 4e). The ventral surface of male was swollen with severe corrugation of the legs (Fig. 4f). Swelling in whole body and shrinkage in legs were apparent in dorsal and ventral surfaces of female (Fig. 4g, h). Normal characteristics of mouth parts in male and female was shown by SEM (Fig. 5a–d). SEM of mouth parts of the abnormal adults appeared with sever shrinkage of the two palpi which made hypostome to be longer than the palpi, also incomplete development of hypostome and cheliceral sheath was occurred (Fig. 5e, f). Furthermore, SEM of normal adults showed the following structures; ventral surface with normal coxae (Fig. 6a), spiracle plate of male (Fig. 6b), genital aperture of female with posterior lips that form a narrow V shape (Fig. 6c) and tarsus with pulvillus and two claws (Fig. 6d). SEM of abnormal adults emerged from treated nymph appeared swollen ventral plates (Fig. 6e), incomplete development of pores in spiracle plate of male (Fig. 6f), swelling and distortion of the genital aperture of female (Fig. 6g) and degeneration of spiracle plate to such extent that no recognizable structure remained in female (Fig. 6h).

Discussion

In previous studies, the extracts of *M. azedarach* and *Artemisia* spp plants revealed strong acaricidal effects against different Ixodidae tick species. The successive extracts of hexan, $CHCl_3$ and 96% aqueous ethanol from *M. azedarach* ripen fruits revealed acaricidal effect against larvae and engorged females of the tick *Rhipicephalus microplus* (Borges et al. 2003). The hexan extract of *M. azedarach* unripe fruits had effects on engorged females of *R. microplus* and their egg production and hatching (De Sousa et al. 2014). The ethanolic *Artemisia annua* extract had acaricidal effect on engorged females of *R. microplus* (Chagas et al. 2011). The chloroform extract of the plant *Artemisia absinthium* had acaricidal properties on engorged females, eggs and unfed larvae of the tick *R. sanguineus* (Godara et al. 2014). The ethanolic extract of the plant *A. absinthium* had acaricidal properties on adults, eggs and larvae of the tick *Hyalomma anatolicum* (Godara et al. 2015). A repellent effect of a toluene extract of *Artemisia abrotanum* and essential oil of *A. herba-alba* against the nymphs of the tick *Ixodes ricinus* was occurred (Tunon et al. 2006; El-Seedi et al. 2017). The above mentioned studies indicated that the acaricidal potency of the two plants *M. azedarach* and *A. herba-alba* were not evaluated on the camel tick *H. dromedarii*, excepting a unique study conducted by Abdel-Shafy et al. (2007) showed acaricidal activity of *A. herba-alba* extracts on the larval stage of *H. dromedarii*. Furthermore, there is no study evaluated plant extracts on all stages of any tick species. Therefore, we try to evaluate the acaricidal efficacy of the two plant extracts *M. azedarach* and *A. herba-alba* on all stages of *H. dromedarii*. In the current study, the petroleum ether and ethanolic extracts of both *M. azedarach* and *A. herba-alba* were evaluated on embryonated eggs and engorged nymphs of *H. dromedarii*. In forthcoming research, these extracts will be evaluated against larvae and adults of *H. dromedarii*.

In the present study, the petroleum ether and ethanol extracts of *M. azedarach* and *A. herba-alba* revealed ovicidal effect on *H. dromedarii* eggs. In all extracts, the highest ovicidal activity recorded at concentrations less than 20%. At the lowest concentrations in all extracts, the eggs succeeded to hatch with 2.83 to 14.06% deformities in larvae. By both LM and SEM examination, the malformation in larvae occurred mainly on mouthparts and legs those prevent larvae from feeding and moving to find their hosts. According to the LC_{50} values, the toxicity of the two plants extracts on *H. dromedarii* eggs seems to be close to each other. The toxicity of the extracts on *H. dromedarii* eggs can be ordered as follows; *M. azedarach* ethanol > *A. herba-alba* ethanol > *M. azedarach* petroleum ether > *A. herba-alba* petroleum ether. This finding means that the polar solvent (ethanol) is better than the less polar solvent (petroleum ether) in the extracting of the tested two plants purposely to control eggs of ticks. In contrary, Borges et al. (2003) stated that the less polar hexan and CHCl3 extracts of *M. azedarach* were more effective on larvae and engorged females of *R. microplus*. They found that the ethanolic extract of *M. azedarach* caused mortality (46–50%) on engorged females and larvae of *R. microplus* lower than hexan and CHCl3 those caused mortality 98–100%. This may attribute to the differences in the susceptibility of tick stage or the active components of the extracts.

The engorged nymph of *H. dromedarii* was less susceptible than egg stage to the ethanol extracts of *M. azedarach* and *A. herba-alba*. However, the petroleum ether extracts of the two plants were more effective on engorged nymphs. This finding agreed with Laghzaoui et al. (2018) who found that the nymphs of *Hyalomma aegyptium* was

more susceptible than eggs and larvae to the essential oil of *A. herba-alba*, wherever, the LD_{50} was 1.105 µL/mL, 0.755 µL/mL and 0.0079 mL/cm^2 for eggs, larvae and nymph, respectively. The two lowest concentrations of petroleum ether extract of *A. herba-alba* only elucidated deformity in adults emerged from treated nymphs. The deformity in adults was not only in appendages like mouthparts and legs but also in the fine structures those photographed by SEM such as spiracular plates, genital opening and anal shields. These abnormalities in the emerged adults can constrain many tick activities such as feeding, moving, mating and finally led to death. LC_{50} values confirmed that the toxicity of the petroleum ether extracts in the two plants on *H. dromedarii* nymphs was higher than the ethanol extracts. However, the toxicity of the extracts on *H. dromedarii* nymphs can be ordered as follows; *M. azedarach* petroleum ether > *A. herba-alba* petroleum ether > *M. azedarach* ethanol > *A. herba-alba* ethanol. This study is considered the first in evaluation of *A. herba-alba* and *M. azedarach* against *H. dromedarii* nymphs. However, a few studies evaluated *Artemisia* spp. and *M. azedarach* as repellents against nymphs of the tick *Ixodes ricinus* and *Amblyomma cajennense*. The essential oil of *A. herba-alba* revealed 84.2% repellency against *I. ricinus* nymphs (El-Seedi et al. 2017). The repellent effect of toluene *A. abrotanum* extract against *I. ricinus* nymphs may be attributed to coumarin and thujyl alcohol those were found in the extract (Tunon et al. 2006). The hexan extract of *M. azedarach* in concentrations varied from 2.200 to 0.275 mg/cm^2 was considered repellent for *A. cajennense* nymphs (Soares et al. 2010).

The mode of action of plant extracts almost attribute to their major active constituents. The major active constituent of the Meliaceous trees that including the genera *Azadirachta* and *Melia* is azadirachtin that causes negative effects on the development of insects (Mulla and Su 1999). Furthermore, the phytochemical analyses of ethanolic *M. azedarach* extract revealed the presence of triterpenoids, steroids, alkaloids and tannins. These compounds have ovicidal, feeding inhibition and insecticidal effects on insects (Mulla and Su 1999). Moreover, the active component of the genus *Artemisia* is artemisinin that revealed toxic effects on many parasites (Pillay et al. 2008; Chagas et al. 2011). This compound reacts with the heme molecules, alters the cell structure, thus affecting the growth and reproduction (Ferreira and Gonzalez 2008).

Conclusion

The ethanolic and petroleum ether extracts of the ripen fruits of *M. azedarach* and aerial parts of *A. herbal alaba* revealed ovicidal efficacy against the camel tick *H. dromedarii*. The petroleum ether extracts of the two plants exhibited higher toxicity than ethanolic extracts on *H. dromedarii* nymphs. Malformation was observed in larvae hatched from eggs treated with the lowest concentrations of all the tested extracts. Deformed adults were only emerged from the two lowest concentrations of the petroleum ether *A. herba-alba* extract. It is recommended to use all the tested extracts in controlling *H. dromedarii* eggs, and petroleum ether extracts of the two plants against *H. dromedarii* nymphs.

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Authors' contributions MMF, SA, MMA, RME, and HSMA designed the experiments. EMH and HSMA participated in the preparation of the plant extracts. MMF, SA, MMA, RME, and HSMA shared in the following protocols: bioassay of plant extracts against both embryonated eggs and engorged nymphs, detection of abnormalities in treated tick specimens by light microscope and scanning electron microscopy. SA and HSMA analysed and tabulated the data. MMF, SA, MMA, and HSMA wrote the draft of the manuscript. All authors revised and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical standard This study was approved by Ethical Committee for Medical Research (MREC) at the National Research Centre (NRC), Egypt in accordance with local laws and regulations (Approval Protocol No. 19085).

Informed consent Consent was obtained from the owners of camels included in this study.

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