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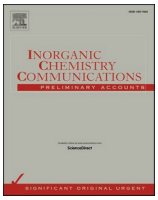

Acaricidal efficacy of silver nanoformulations of *Commiphora molmol* and *Zingiber officinale* against the camel Tick, *Hyalomma dromedarii* (Ixodida: Ixodidae)

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ABSTRACT

Ticks are blood- feeding ectoparasites transmitting many dangerous pathogens to humans and animals, leading to great economic losses. Emerging acaricide resistance has urged the use of eco-friendly acaricides. The novel *In vitro* application of the aqueous extracts of myrrh, *Commiphora molmol* (AME) and ginger, *Zingiber officinale* (AGE) and their corresponding silver nanoparticles (AgNPs), synthesized by subjecting myrrh and ginger extracts to laser ablation (NME and NGE, respectively), was evaluated against the camel tick, *Hyalomma dromedarii* by adult immersion bioassays. Moreover, spot- on treatments of infested cattle was done. AgNPs were characterized by Transmission Electron Microscopy and UV–visible spectrophotometry. The phytochemical profile showed that the used extracts contained phenols, gallic acid, flavonoids, and tannins. Fifteen days post- treatment (PT), 96 and 84.01% mortalities were observed PT with AME and AGE, respectively; 100% mortality was reached 7 and 9 days PT with 12% of NME and NGE, respectively. Three days PT, the LC_{50} values of AME, AGE, NME, and NGE were 10.37, 12.81, 2.38, and 4.12%, respectively. The corresponding LT_{50} values PT with 4% were 5.6 and 6.73, 2.25, and 3.56 days, respectively. Three days PT, AME, AGE, NME, and NGE reduced cattle- tick infestations by 54.45, 45.73, 100, and 100%, respectively. Ticks showed resistance against Deltamethrin (Butox®). This study demonstrated the novel acaricidal effect of myrrh and ginger and their silver nanoformulations through laser ablation, which increased the speed and efficacy of the aqueous extracts against *H. dromedarii*. Consequently, they could be produced as efficient eco-friendly pesticides after revealing their ecotoxocological profile.

1. Introduction

Ticks are important ectoparasites that transmit pathogens of medical and veterinary importance, which cause serious health issues to humans and domestic animals [1,2] leading to great economic losses [3]. Ticks are vectors of severe viral, bacterial, and parasitic diseases [4–9]. In addition to spreading diseases, ticks have direct effects on the health of livestock through their bites, which can be painful, cause blood loss, damage the skin, and make animals lose their appetite, which slows their growth [10,11]. Therefore, it is necessary to fight these pests to reduce the damage caused by them and limit their spread through the application or use of the appropriate control strategies. Using of chemical pesticides in the control of ticks led contaminated meat and dairy products, polluted environment, and development of acaricide- resistant strains of ticks. Consequently, using eco-friendly acaricides is a potential solution to the resistance problem, which is what we seek in this current work [12,13].

Biorational pesticides are safe, cost-effective, and biodegradable [12–14]. Besides their antiparasitic effect [15,16], botanicals have ovicidal, adulticidal [17]; larvicidal [18,19], repellent, and deterrent effects [20]. Pesticidal activity of *Commiphora molmol* and *Araucaria heterophylla* methanol and hexane extracts against ectoparasites

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effectively controlled the camel and cattle ticks, *Hyalomma dromedarii* and *Rhipicephalus annulatus*, respectively [21].

Nanomaterials such as metallic nanoparticles and nano-emulsions had a great interest in biomedical and pharmaceutical fields and have anticancer and antibacterial properties [22–27] and under extensive research in various areas and gained a lot of attention because they are suitable for human and veterinary use [28]. The green synthesis of plant-mediated nano-emulsion is worthwhile over the other chemical approaches since they have relatively small droplet sizes, and mean radii up to 200 nm. So, the green synthesized nanoparticles (NPs) are novel pesticides against arthropod pests with several advantages regarding chemical NPs, which usually produce undesirable toxic residues [29–32].

In the strategy for controlling disease vectors, aqueous nano-emulsions with plant extracts or oils are a cheaper, more effective alternative to chemical pesticides. The novel NP production by pulsed laser ablation in liquid medium is characterized by high stability, simple production, and the absence of chemical reagents in the final preparation [33,34]. In particular, noble metal NP increased recently because of their optical, catalytic, electronic, and magnetic features. Silver nanoparticles (Ag NPs) are commonly used nanostructures for noble metals [35]. As a result, we hypothesize that *C. molmol* and *Z. officinale* extracts, containing a variety of active bioactive components, loaded in silver nanoparticles could be used to control pests without contaminating the environment and could be used as alternatives to synthetic pesticides, which could be reflected on animals and human health and improving the national ecomomy.

This study aimed to evaluate for the first time the *in vitro* and *in vivo* acaricidal effects of locally available and affordable plants such as myrrh and ginger against the camel tick, *H. dromedarii* before and after loaded in silver NPs using laser ablation, for the first time, and revealing the size distribution of NPs via the High-Resolution Transmission Electron Microscopy (HRTEM).

2. Materials and methods

2.1. Ticks

Hyalomma dromedarii (adult) were collected from sites close to camels (5–15 years old) in Toukh, 35 km north of Cairo, Qalyubiya Governorate, Egypt (30° 21′ 11.6″ N and 31° 11′ 31.5″ E). Ticks were identified as in Apanaskevich et al., [33] and only male ticks were used for the bioassays.

2.2. Preparation of plant extracts

Plants were identified at the National Research Center, Egypt. The aqueous extracts (AEs) were made using resins of myrrh (*Commiphora molmol:* Burseraceae), AME, and ginger rhizomes (*Zingiber officinale:* Zingiberaceae), AGE. Extracts were prepared as follows: 50 g of each plant (*C. molmol* resin and *Z. officinale* roots) was added to 300 mL of heated water (17%) to around 60 °C, and then add the plant material, and let it cool down to room temperature 27 ± 2 °C, RH of 75 ± 5%, and kept for 6 h in a dark place. The adult camel ticks were treated with the following concentrations: 0.2, 0.4, 0.8, 1.5, 3.0, 6.0, and 12.0%. After soaking, the extract was sieved then filtered and used as a stock solution [36].

2.3. Laser ablation

Silver NPs were produced by subjecting the previously described AE of myrrh and ginger (AME and AGE) to laser ablation of a silver plate (purity: 99.99) in each extract for 20 min (Fig. 1).

The plate surface was initially treated with an emery paper sheet (with a 500 grit size) to remove surface imperfections. The plate was then cleaned with ethanol, placed with an ablation vessel in an ultrasonic bath, and rinsed with deionized water before each experiment, in order to eliminate any contaminants present.

A metal plate of 0.5 mm thickness and of 2.5x2.5 cm^2 size was placed in a glass vessel containing 20 mL of *C. molmol extract* and *Z. officinale* extracts, respectively, and irradiated with the focused output of a Q-S witched Nd:YAG (Quanta-Ray) laser (wavelength = 1064 nm; pulse duration = 8 ns; repetition rate = 10 Hz), and focused on the silver plate at a focal length lens (f) of 20 cm. During ablation, the Ag colloidal solution was magnetically stirred to remove the Ag NPs from the laser's path.

The spot size of the 1064 nm laser beam output ranged between 2 and 7 mm in diameter according to the distance between the metal plate and the focused lens. I performed ablation at room temperature, and the color change confirmed NP formation in the extracts during ablation.

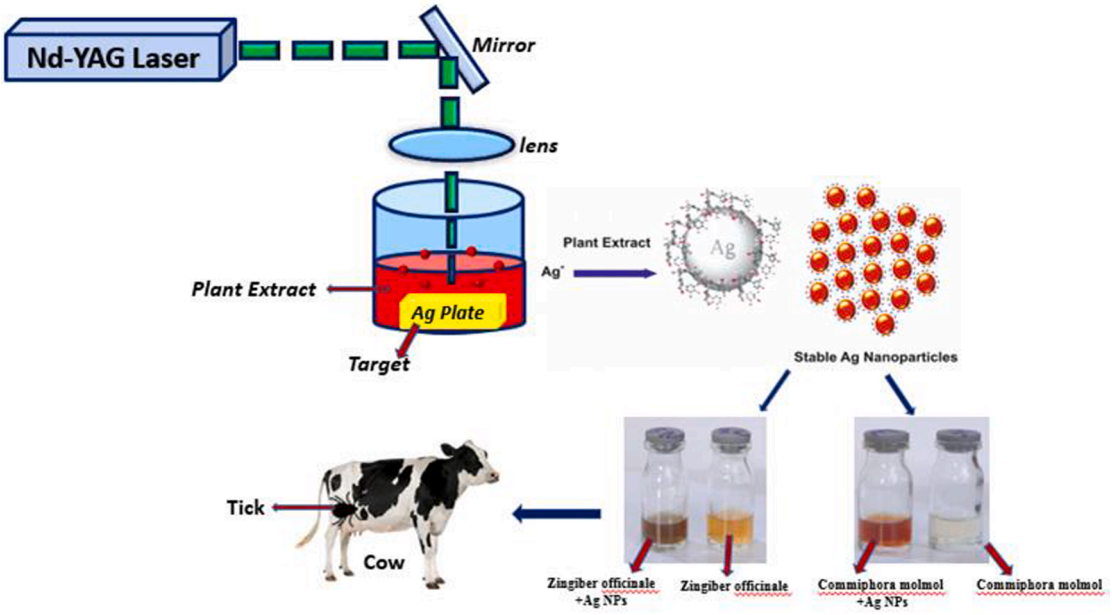

Fig. 1. Schematic diagram of the experimental laser ablation setup for commiphora molmol extract and Zingiber officinale extract with and without Ag NPs respectively by laser ablation.

2.4. Adult immersion tests before nanoformulation

The efficacy of myrrh and ginger extracts against *H. dromedarii* was determined through adult immersion tests (AITs) (Khater et al., 2016). Ten semi-fed males per replicate were used in each test. Each group of ticks was immersed for 60 s in 100 mL solution at all concentrations. Seven dilutions in distilled water were used for this purpose.

Six replicates were included to assay each concentration. The immersed ticks were placed in a Petri dish containing a filter paper (Whatman no. 1) at 27 ± 2 °C and 80 ± 5% relative humidity, whereas the control groups were treated with distilled water. Tick mortalities were recorded up to 15 days post- treatment (PT) and corrected for the control mortality [37]. The Corrected Mortality (%) was calculated as (MT%-MC%) / (100- MC%) *100.

2.5. Adult immersion tests after nanoformulation

AITs were used to determine the effect of myrrh and ginger nanoformulations (NME and NGE, respectively) obtained after 20 min of laser ablation at the same condition against *H. dromedarii*, according to the previously described AITs. Distilled water was used to treat the negative (-ve) control group; the positive (+ve) control groups were immersed in distilled water, which was subjected to laser ablation.

2.6. Chemical analysis

2.6.1. Total polyphenol

The total polyphenol content of AME and AGE before and after nanoformulation was determined [38] by adding aliquots of each extract to a test tube filled up with distilled water up to 1 mL. The following reagents were added to each tube, 0.5 mL of Folin–Ciocalteu reagent, 1:1 diluted with water, and 2.5 mL of sodium carbonate solution at 20%. The tubes were maintained for 40 min in the dark after vortexing. The absorbance against the reagent blank was recorded at 725 nm. Total polyphenol contents were calculated from a standard curve prepared with different gallic acid concentrations and expressed as mg gallic acid equivalents (GAE)/g dry matter (DW).

2.6.2. Total flavonoid content

The total flavonoid content was calculated according to the previously described protocol [39]. The estimation of the total flavonoid content in mg quercetin equivalents (QE)/g D. W. resulted from a standard curve prepared with 0–500 μg of flavonoid.

2.6.3. Total tannin

Before and after formulation, the total tannin content in each AME and AGE was measured by a modified Folin–Ciocalteu method using polyvinyl polypyrrolidone to free tannin from non-tannin phenols. The difference between total and simple phenol values represented the total tannin content (expressed as mg GAE g D. W) [40].

2.7. Field application

The spot-on treatment method was selected as it is an easy and practical method for treating a limited number of animals and does not require specialized equipment or dip tanks. The diagnostic (therapeutic) dose was 2x of the LC_{95} values, 9 days PT [17].

Seemingly healthy cattle with an average bodyweight of 400 kg were selected, except for their natural tick infestations with the common tick species in Egypt, *Rhipicephalus* (*Boophilus*) *microplus*. Cattle were categorized into five groups containing five animals each. Infested cattle areas were treated with C. *molmol* (AME), 37.6% (Group 1); *Z. officinale* (AGE), 50.5% (Group 2); *C. molmol* (NME), 8.3% (Group 3); *Z. officinale* (NGE), 4.3% (Group 4); 0.1% solution of Butox® EC 50%, Deltamethrin (EL Shorok company for importation and Pharmaceuticals, Cairo, Egypt) (Group 5).

The tick infestations of the treated areas (30 × 30 cm) were counted daily by visual inspection for four days PT to calculate the percentage of reduction. Animals and spot-on operators were examined daily for any abnormal symptoms.

2.8. Data analysis.

For data analyses, the-Way Analysis of Variance (followed by the Duncan test) and Probit analyses were used to compute lethal concentrations (LC) and lethal time (LT) values, 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. The percentage of reduction (%) was calculated according to Khater et al., [17] using the following formula: Reduction (Pre-treatment count - post treatment count/ pre- treatment count) X 100.

3. Results

3.1. Characterization of silver NPs

The absorption spectrum of Ag NPs decorated by laser ablation in *C. molmol* and *Z. officinale* extracts were measured using a V-670 Jasco double-beam spectrophotometer, which utilizes a unique, single monochromator design covering a wavelength range from 190 to 2700 nm (Fig. 2 (a),(b), respectively). After mixing, the solutions were kept under UV light for 2 h and the UV absorption of the solution was measured. The spectrum exhibits a characteristic peak at 418 nm, with a broad plasmon resonance band tail extending toward the UV–vis for *C. molmol* extract and *Z. officinale* extract, respectively, with and without Ag NPs.

Transmission electron microscopy (TEM) images were obtained to get information about the particles size of the metal nanocatalysts on a JEOL 200 kV TEM instrument. More than 300 particles were measured to get the integrated information about the catalyst. For TEM analysis, the samples were prepared by ultrasonically suspending the catalyst powder in ethanol. The drops of the nanoparticle solutions were cast on the 400-mesh copper grid covered with a continuous carbon film. The excess solution was then removed with an absorbent paper, and then the samples were dried under a vacuum at room temperature before the analysis.

TEM analysis was used to determine morphology, structure, and average particle size of Ag NPs as can be seen in Fig. 3 (a-c) and Fig. 3 (e-g), Ag NPs were spherical in shape for *C. molmol* extract and *Z. officinale* extract respectively. Approximately 100 particles were counted for the average particle size calculation. The particle size histogram (Fig. 3c and 3g) shows that the average particle size of Ag NPs was found to be 14 nm and 15.3 nm for *C. molmol* extract and *Z. officinale* extract, respectively. The Selected Area Electron Diffraction (SAED) pattern for the Ag NPs in the HR-TEM image was shown and the SAED pattern of the Ag NPs indicated ring patterns with intense spots showing that the Ag NPs are polycrystalline *C. molmol extract* and *Z. officinale* extract, respectively (Fig. 3 (d) and 3 (h).

3.2. Efficacy of aqueous extracts

Fifteen days PT with 17% of AE of myrrh, AME, ginger, AGE, caused 96 and 84.01% mortality, respectively (Fig. 4 and Fig. 5). With AME, the LC_{50} values PT for 3 and 9 days PT were 10.37 and 3.40%; while the LC_{99} was 45.65 and 25.15%, respectively. The corresponding LC_{50} values of AGE were 12.81 and 3.96%, and the LC_{99} values were 55.23 and 34.06%, respectively (Table 1).

All values represented the mean of the corrected mortalities of three independent experiments ± SE.
Bars represent the slandered error.

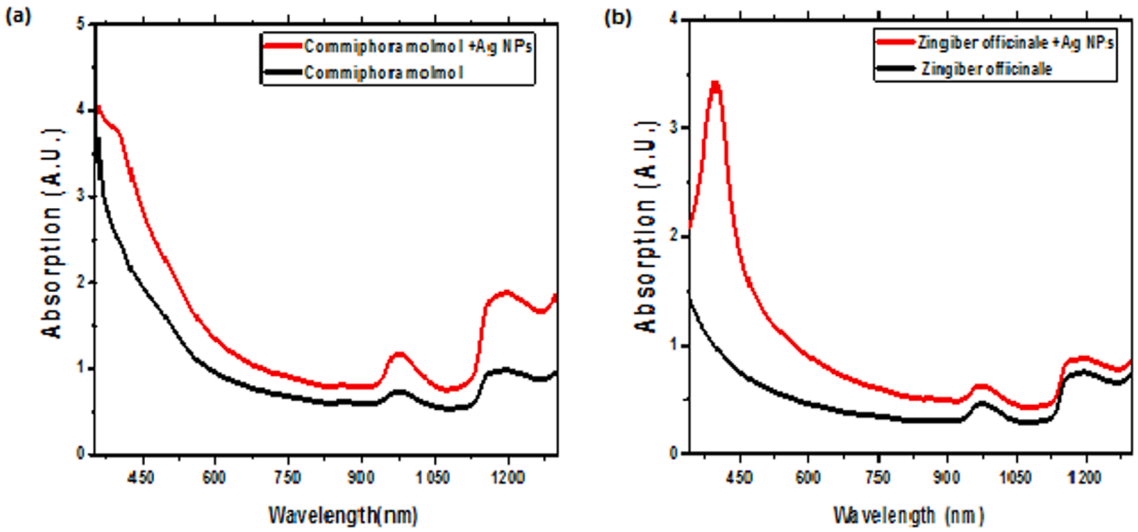

Fig. 2. UV–vis absorption for the aqueous extract before and after Ag NPs of **(a)** Commiphora molmol **(b)** Zingiber officinale.

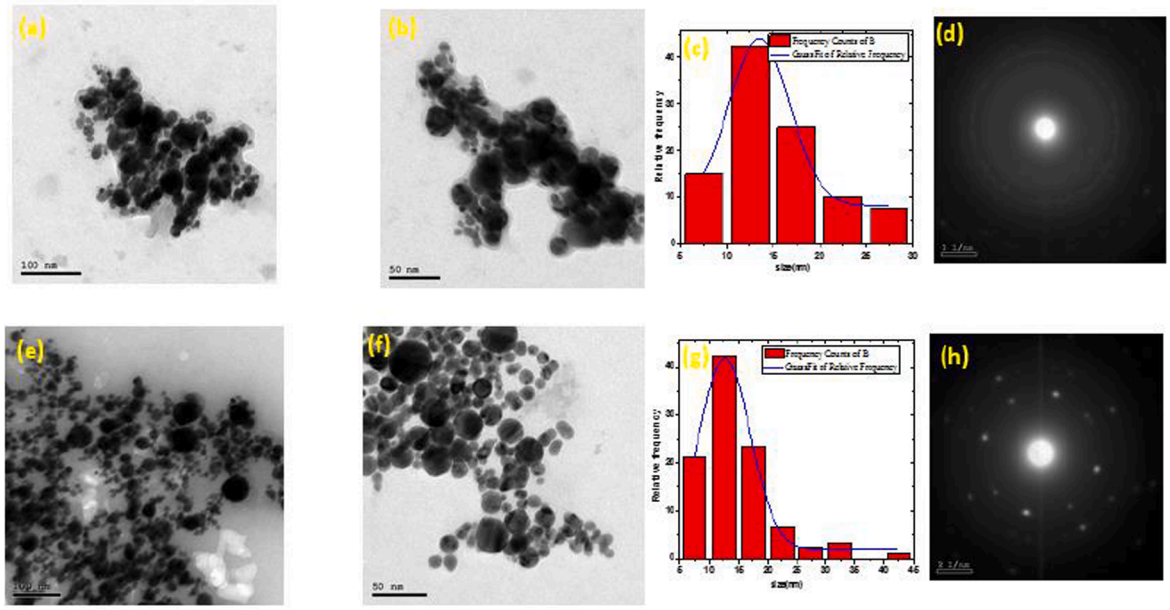

Fig. 3. (a-c) TEM analysis and histogram distributions of particle size for Ag NPs in Commiphora molmol extract and **(e-g)** for Zingiber officinale extract. (d) and (f) SAED pattern of Ag NPs in Commiphora molmol extract and Zingiber officinale extract.

All values represented the mean of the corrected mortalities of three independent experiments ± SE.

3.3. Efficacy of the nanoformulations

After treatment with 12%, 7 and 9 days PT, 100% mortality was achieved PT with NME and NGE, respectively. There was no significant difference regarding mortalities between the + ve and –ve control groups (Fig. 6 and Fig. 7). The LC_{50} values of NME, 3 and 9 days PT, were 2.38 and 1.08% and LC_{99} values were 18.03 and 5.40%, respectively. Regarding NGE, the corresponding LC_{50} and LC_{99} values were 4.12, 0.66, 21.33, and 2.73%, respectively (Table 2).

All values represented the mean of the corrected mortalities of three independent experiments ± SE. C + ve: the positive control group, treated with distilled water subjected to laser ablation

All values represented the mean of the corrected mortalities of three independent experiments ± SE. C + ve: the positive control group, treated with distilled water subjected to laser ablation.

The relative effect of the LC values between treatments was calculated (Table 3). Three days PT, the LC_{50} and LC_{99} values of AME were 1.2 folds more effective than those of AGE.

Nine days PT, the relative efficacy according to LC_{50} and LC_{99} values indicated that NME was 1.2 and 1.4 times more effective than AGE. On the other hand, NGE was 1.6 and 2 times more effective than NME, respectively. Moreover, NGE was 6 and 12.5 times more effective than its aqueous extract, AGE; whereas NME was 3.1 and 4.7 times more potent than its aqueous extract, AME (Table 3).

3.4. Lethal time

After treatment with AME (4%) and NME (3%), LT_{50} values were 5.60 and 2.25 days, respectively, and the LT_{99} were 21.57 and 8.62 days, respectively. The corresponding values of ginger were 6.73 and 3.56

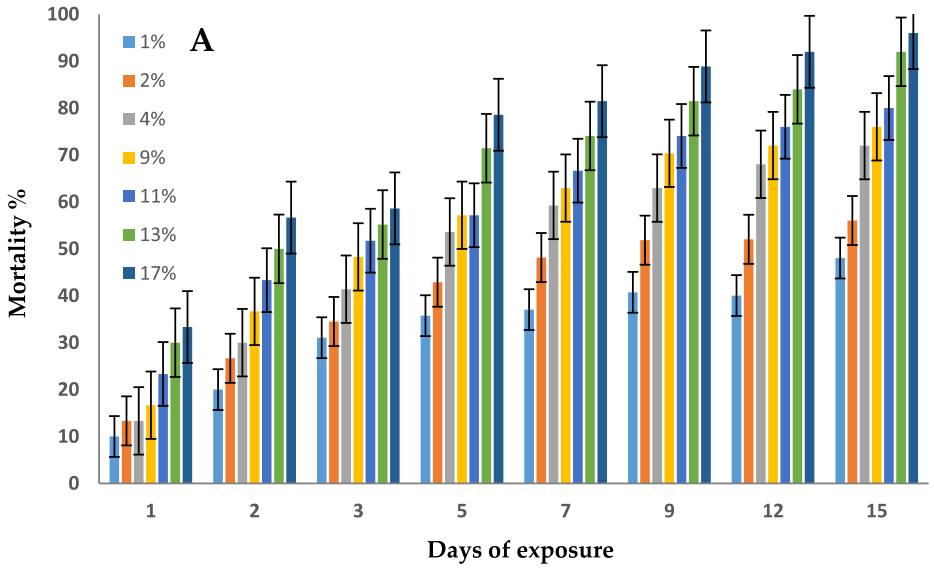

Fig. 4. Mortalities of the camel tick (Hyalomma dromedarii) post-treatment with the aqueous extract of Commiphora molmol.

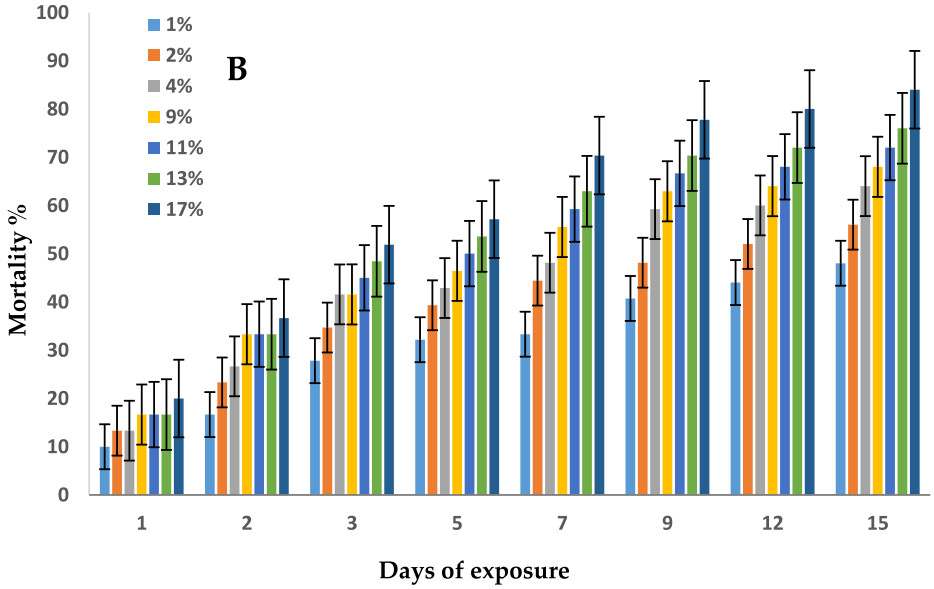

Fig. 5. Mortalities of camel ticks (Hyalomma dromedarii) post-treatment with the aqueous extract of Zingiber officinale.

Table 1
The lethal concentration values of aqueous plant extracts against tick camels, Hyalomma dromedarii.

Days	Plant extracts	LC_{50} (Low.– Up.)	LC_{90} (Low.– Up.)	LC_{95} (Low.– Up.)	LC_{99} (Low.– Up.)	X^2 (Sig.)	R^2
3	Commiphora molmol (AME)	10.37 (7.88 – 14.24)	29.81 (22.72 – 47.34)	35.32 (26.62–57.03)	45.65 (33.89 – 75.25)	10.78 (0.09a)	0.553
	Zingiber officinale (AGE)	12.81 (9.62 – 19.93)	36.18 (26.07 – 67.61)	42.80 (30.48–81.38)	55.23 (38.71 – 107.24)	10.75 (0.09a)	0.485
7	Commiphora molmol	4.41 (1.84 – 7.95)	18.81 (13.36 – 39.75)	22.89 (16.06–50.37)	30.55 (20.96 – 70.46)	12.79 (0.04a)	0.697
	Zingiber officinale	6.32 (3.66 – 8.71)	24.23 (18.90 – 36.31)	29.31 (22.60–44.76)	38.84 (29.47 – 60.67)	10.57 (0.10a)	0.561
9	Commiphora molmol	3.40 (2.24 – 6.45)	15.38 (11.17 – 28.64)	18.78 (13.54–36.37)	25.15 (17.79 – 51.04)	13.58 (0.03a)	0.747
	Zingiber officinale	3.96 (−8.85–8.55)	20.54 (13.61 – 70.03)	25.24 (16.46–90.97)	34.06 (21.63 – 130.44)	15.77 (0.01a)	0.593

LC: lethal concentrations; X^2: chi square; R2: R squared values; Low. – Up.: Lower –Upper limits.
n = 10, three replicates were used for each concentration.

days and 22.98 and 10.23 days, respectively. The nanoformulations reduced (almost halved) the time needed to remove ticks. Regarding LT_{99}, an almost similar speed of tick killing was recorded for AME and AGE as well as with NME and NGE (Table 4).

3.5. Chemical analyses

The chemical profile of the extracts AME and NME indicated a phenol content of 44.68 ± 0.31 and 19.93 ± 0.07 mg gallic acid /g DW, and flavonoid content of 30.20 ± 0.19 and 8.45 ± 0.10 mg quercetin/g

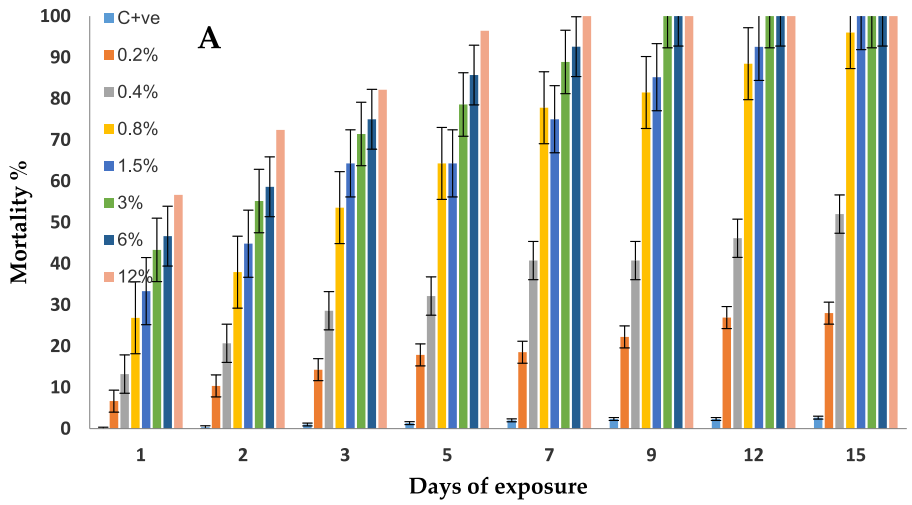

Fig. 6. Mortalities induced by Commiphora molmol NPs (20 min of laser ablation) against the camel tick (Hyalomma dromedarii).

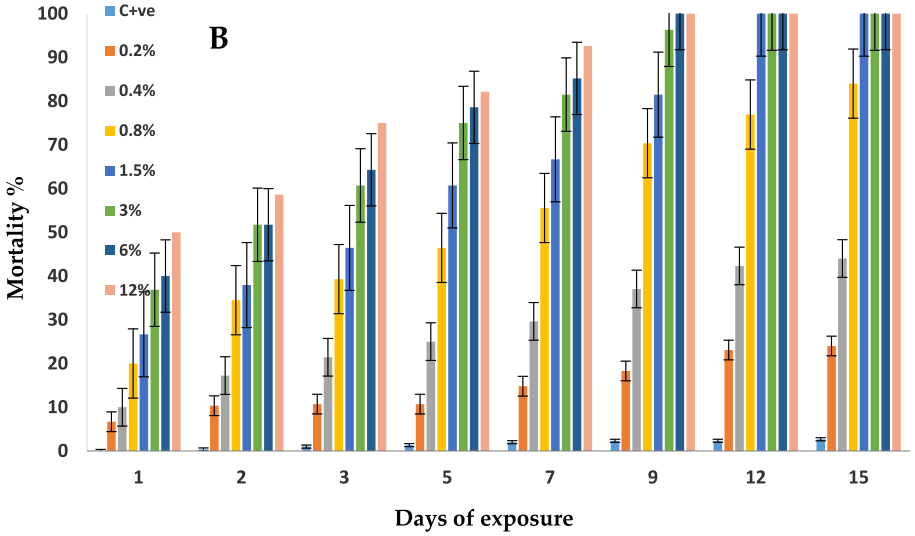

Fig. 7. Mortalities induced by Zingiber officinale NPs (20 min of laser ablation) against the camel tick (Hyalomma dromedarii).

Table 2
Lethal concentration values of the plant extracts after 20 min of laser ablation.

Days	Plant extracts	LC_{50} (Low.– Up.)	LC_{90} (Low.– Up.)	LC_{95} (Low– Up.)	LC_{99} (Low.– Up.)	X^2 (Sig.)	R^2
3	Commiphora molmol	2.38 (0.84.- 8.76)	11.00 (6.09–56.81)	13.45 (7.45–72.16)	18.03 (9.89–10.14)	33.13 (0.000[a])	0.507
	Zingiber officinale	4.12 (2.83–12.16)	13.60 (8.32–57.75)	16.29 (9.90–71.21)	21.33 (12.81–96.54)	21.04 (0.002[a])	0.593
7	Commiphora molmol	0.94 (0.28–3.55)	3.59 (2.07–36.53)	4.34 (2.53–46.83)	5.75 (3.33–66.20)	34.16 (0.000[a])	0.678
	Zingiber officinale	1.43 (0.14–7.04)	7.27 (4.03–16.52)	8.92 (5.00–21.46)	12.02 (6.71–30.39	35.43 (0.000[a])	0.629
9	Commiphora molmol	1.08 (0.02–2.27)	3.46 (2.26–9.31)	4.13 (2.70–11.51)	5.40 (3.48–15.66)	19.32 (0.004[a])	0.812
	Zingiber officinale	0.66 (0.260–1.10)	1.80 (1.28–3.44)	2.12 (1.51–4.18)	2.73 (1.91–5.56)	12.78 (0.047[a])	0.868

LC: lethal concentrations; X^2: chi square; R2: R-squared values; Low.– Up.: Lower–Upper limits.
n = 10, three replicates were used for each concentration.

DW, respectively. Tannins were found only in AME (6.50 ± 0.33 mg gallic acid/g DW). On the other hand, *Z. officinale* silver NPs had higher contents of plant extracts (phenols and flavonoids). They recorded 13.59 ± 0.15, 17.98 ± 0.16 mg gallic acid / g DW, and 4.24 ± 0.12, 6.44 ± 0.12 mg quercetin/g DW, respectively (Fig. 8).

3.6. Field application

Field experiments indicated that AME, AGE, NME, NGE, and Butox® reduced natural tick infestation by 54.45, 45.73, 100, 100, and 3.65%, respectively, 3 days PT (Table 5). No symptoms of skin irritation or abnormal health observation were observed among treated animals and spot-on operators.

4. Discussion

Ticks still carry a wide range of pathogens, including viruses, bacteria, and parasites that can infect domestic and wild animals and humans [41,42]. These pathogens can cause bacterial diseases such as Q fever, rickettsiosis, erythrocytosis, and anaplasmosis, as well as protozoa

Table 3
The relative effect of the lethal concentrations of the applied materials against Hyalomma dromedarii.

Days PT	Plant extracts	Before formulations (AME and AGE)				After formulations (NME and NGE)				Aqueous extracts vs Nanoformulations			
		LC_{50}	LC_{90}	LC_{95}	LC_{99}	LC_{50}	LC_{90}	LC_{95}	LC_{99}	LC_{50}	LC_{90}	LC_{95}	LC_{99}
3	Commiphora molmol	1.2	1.2	1.2	1.2	1.7	1.2	1.2	1.2	4.4	2.7	2.6	2.5
	Zingiber officinale	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	3.1	2.7	2.6	2.6
7	Commiphora molmol	1.4	1.3	1.3	1.3	1.5	2.0	2.1	2.1	4.7	5.2	5.3	5.3
	Zingiber officinale	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	4.4	3.3	3.3	3.2
9	Commiphora molmol	1.2	1.3	1.3	1.4	1.0	1.0	1.0	1.0	3.1	4.4	4.5	4.7
	Zingiber officinale	1.0	1.0	1.0	1.0	1.6	1.9	1.9	2.0	6.0	11.4	11.9	12.5

PT: post treatments; LC: lethal concentrations.
Zingiber officinale is the reference material in the case of before and after Nano formulations, with the exception of 9 days after formulation as Commiphora molmol was the reference substance.

Table 4
The lethal time values of the applied material against Hyalomma dromedarii.

Plant extracts	Conc.	Aqueous extracts				Conc.	Nanoformulations			
		LT_{50} (Low.– Up.)	LT_{90} (Low – Up.)	LT_{95} (Low. Up.)	LT_{99} (Low.– Up.)		LT_{50} (Low.– Up.)	LT_{90} (Low.– Up.)	LT_{95} (Low.– Up.)	LT_{99} (Low. – Up.)
Commiphora molmol	4%	5.6 (4.31–7.16)	13.52 (10.96–18.52)	16.32 (13.09–22.75)	21.57 (17.05–30.72)	3%	2.25 (0.93–3.31)	5.76 (4.44–8.85)	6.76 (5.19–10.67)	8.62 (6.53–14.13)
Zingiber officinale		6.73 (5.41–8.58)	14.80 (11.98–20.31)	17.64 (14.15–24.61)	22.99 (18.17–32.72)		3.56 (2.93–4.24)	6.87 (5.94–8.28)	8.04 (6.92–9.80)	10.24 (8.72–12.67)
RE*		1.20	1.09	1.08	1.07		1.6	1.2	1.2	1.2
Commiphora molmol	2%	8.86 (6.92–12.77)	19.79 (14.97–32.11)	23.66 (17.68–39.08)	30.90 (22.73–52.19)	1.5%	2.81 (2.16–3.43)	6.78 (5.88–8.12)	7.90 (6.82–9.57)	10.00 (8.56–12.30)
Zingiber officinale		9.22 (7.30–13.00)	19.55 (14.98–30.58)	23.20 (17.58–36.91)	30.05 (22.43–48.81)		4.03 (3.28–4.77)	9.10 (7.96–10.78)	10.53 (9.17–12.59)	13.23 (11.41–16.03)
RE*		1.0	1.0	1.0	1.0		1.4	1.3	1.3	1.3

LT: lethal time values Low.–Up.: Lower–Upper value.
n = 10, three replicates were used for each concentration.
*RE: Relative efficacy and ginger was used as a reference substance.

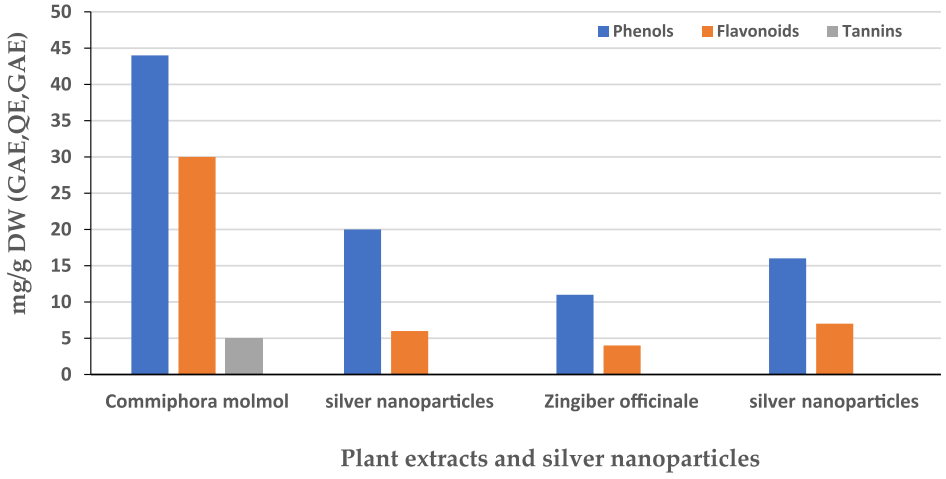

Fig. 8. Total content of phenols (mg/g DW Gallic acid equivalent), total flavonoids (mg/g DW Quercetin) and tannins (mg/g DW Gallic acid equivalent) of Zingiber officinale and Commiphora molmol) plant extracts and their silver nanoparticles.

such as babesiosis and theilerioses, and viral diseases such as Crimean-Congo hemorrhagic fever. Ticks of the genus *Hyalomma* are more closely related to Arabian camels and cattle and are well-known vectors of *Theleria*, *Babesia, Anaplasma, Rickettsia*, and *Erichia*, which negatively affect animal's productivity and the national economy [43,44].

Eco-friendly tick control is crucial [45,46]. This study evaluated for the first-time novel laser-ablated formulations of myrrh and ginger plant extracts against *H. dromedarii.*

Aqueous extraction was chosen for its simplicity and potential use by farmers. AIT was chosen to be used *in vitro,* as it is very close to the topical application (spot-on) used *in vivo*, which is more efficient and requires a small amount of the applied materials. Regarding the treated animals and spot-on operators, there were no abnormal health observations or skin irritations related to treatment.

Some plant resins, including myrrh or *Commiphora* species, show numerous biological effects against pests [47–49], but not much is known about their effect against *H. dromedarii.*

The present study evaluated *C. molmol's* effects against *H. dromedarii* and revealed that 17% AME effectively controlled ticks as shown by a mean mortality of 96%, 15 days PT; three and nine days PT, its LC_{50} values were 10.37 and 3.40% and LC_{99} values were 45.65 and 25.15%, respectively. Its LT_{50} and LT_{99} values PT with 4% AME were 5.60 and

Table 5
Treatment of cattle naturally infested with ticks.

Groups	Plant extracts	Days PT	Mean number+ SE	Reduction %
1	Commiphora molmol (AME)	0	48.67 ± 3.65a	0.00
		1	41.50 ± 2.96a	14.73
		2	32.50 ± 2.75b	33.22
		3	22.17 ± 1.72c	54.45
		4	14.67 ± 0.84c	69.86
2	Zingiber officinale (AGE)	0	48.83 ± 2.50a	0.00
		1	42.83 ± 2.29b	12.29
		2	22.17 ± 0.54c	54.61
		3	26.50 ± 0.72c	45.73
		4	24.83 ± 0.98c	49.15
3	Commiphora molmol (NME)	0	40.83 ± 3.26a	0.00
		1	28.67 ± 2.62b	29.80
		2	9.50 ± 0.76c	76.73
		3	0.00 ± 0.00d	100.0
		4	0.00 ± 0.00d	100.0
4	Zingiber officinale (NGE)	0	38.17 ± 1.90a	0.00
		1	33.50 ± 1.23b	12.23
		2	8.00 ± 0.37c	79.04
		3	0.00 ± 0.00d	100.0
		4	0.00 ± 0.00d	100.0
5	Butox® EC 50%	0	45.67 ± 1.56a	0.00
		1	45.67 ± 1.56a	0.73
		2	45.33 ± 1.38a	0.73
		3	45.33 ± 1.73a	3.65
		4	44.00 ± 1.46a	0.00

PT: post treatments; Day 0: pre-treatment; SE: standard error.

21.57 days, respectively.

Alike recent study indicated that *A. heterophylla* and *C. molmol* extracts induced 100% mortality PT of *H. dromedarii* and *R. (Boophilus) annulatus* with 25 mg/mL for 7 days. Against *H. dromedarii*, the LC_{50} values of both methanol and hexane extracts were 1.13 and 1.04 mg/mL and 1.47 and 1.38 mg/mL, respectively, whereas those against *R. annulatus* were 1.09 and 1.41 and 1.55 and 1.08 mg/mL, respectively [21].

Similarly, a stem bark petroleum ether extract of *Commiphora swynnertonii* (Burtt) induced 100% acaricidal activity (LC_{50} = 72.31 and 71.67 mg/mL) against *Amblyomma variegatum* and 87% against *Rhipicephalus appendiculate*, respectively, after 156 h [50].

Similarly, *C. swynertonii* extract induced acaricidal activity against ticks with LC_{50} values of 1.72 and 1.91 mg/mL for *R. appendiculate* and *A. variegatum*, respectively; LC_{99} values were 3.5 and 3.7 mg/mL, respectively. Moreover, it also prevented or reduced oviposition and egg hatching [51] and repelled R. appendiculatus larvae [52].

Comparable to our results, ethanol extracts of castus, *Vitex castus,* and ginger*, Z. officinale,* were effective 15 days PT of *H. dromedarii*, 80.8 and 84.7%, respectively. The LC_{50} values, three days PT, were 12.2 and 11.8%, respectively, whereas their LT_{50} values were 2.6 and 2.5 days, respectively, PT with 25% [53].

Moreover, some extracts, including crude *Commiphora* merkeri extracts, showed larvicidal and adulticidal effects against R. appendiculatus and Rhipicephalus averts and the surviving engorged adults laid unviable eggs [48]; *C. molmol* effectively controlled the fowl tick, *Argas persicus*, and the mortality at 12 days PT was 63, 67, 76, 87, and 94% at 0.62, 1.25, 2.5, 5, and 10%, respectively [54].

Five oil-resin plants were effective against the mosquito larvae *Culex pipiens*, including *C. molmol*. At 1 and 2 days PT, the highest mortality reported was that with acetone extract (1500 ppm) of *C. molmol* which induced 83.3 and 100% mortality (MO%); LC_{50} values were 23.52 and 300.63 ppm, respectively [47].

Seven days PT with 12.5 mg/mL, *A. heterophylla* and *C. molmol* extracts totally effective against the louse and fly infesting cattle, *Haematopinus eurysternus* and *Hippobosca maculate*. The LC_{50} values of methanol extracts hexane extracts against *Ha. eurysternus* were 0.56 and 0.62 mg/mL besides 0.55 and 1.00 mg/mL, respectively. On the other hand, such values against *Hi. maculata* were 0.67 and 0.78 mg/mL along with 0.68 and 0.32 mg/mL, respectively [21].

According to our results, the efficacy of AE of ginger was relatively lower than myrrh. The mortality percentage of AGE was 84.01%, 15 days PT with 17%. Its LC_{50} values three- and nine-days PT were 12.81 and 3.96% and the LC_{99} were 55.23 and 34.06%, respectively, and the LT_{50} and LT_{99} values PT with 4% were 6.73 and 22.98 days, respectively. Similar investigation indicated that the essential oil of Z. officinale induced 100% MO at 25 mg/mL against larvae of the cattle tick, *Rhipicephalus (Boophilus) microplus* with LC_{50} and LC_{90} values of 7.75 and 13.62 mg/mL, respectively [55].

In addition to its acaricidal effects, ginger essential oil adversely affected the reproductive potential of R. microplus [55,56]. In contrary to our observation and that of previous studies, neither ginger alcoholic nor its AE induced significant effects against *H. anatolicum* unfed larvae [57]. Differing findings might be attributed to different extracts, methodology, species, or developmental stages.

Some studies had recorded the effect of plant extracts and their nanoformulations, which improved efficacy and reduced repeated applications [29,30,32,36]. The nanoformulations in the present study have similar characteristics to those of previous studies [58] that reported increased and accelerated tick mortalities. The present study indicated that treatment with 12% of NME and NGE killed all ticks 7 and 9 days PT. NPs significantly ($p < 0.05$) reduced the concentrations and time needed for killing ticks compared to AE. The mortality was not significantly different between groups treated with distilled water subjected to laser ablation and negative control groups, treated with distilled water only.

Similar to our findings, several NPs are described as highly effective acaricides *in vitro*. Zn NPs had a potent acaricidal effect against *Hyalomma* spp. [59]. The acaricidal activity of nickel oxide nanoparticles (NiO NPs) using an aqueous extract of *Melia azedarach* effectively controlled *H. dromedarii* with LC_{50} values in case of treated embryonated eggs, larvae, and engorged nymphs of 5.00, 7.15, and 1.90 mg/mL, respectively. Some parameters, such as egg number, egg-productive index, and hatchability (%) decreased in females treated with NiO NPs compared to the control group [29]. Zinc oxide NPs prepared from the ethanolic extract of neem (*Azadirachta indica*) and lemongrass (*Cymbopogon citratus*) effectively controlled *Hyalomma* ticks with LC_{50} values of 4.76 and 4.92 mg/L, respectively; whereas their LC_{90} values were 8.87 and 9.1 mg/L, respectively [60]. In addition, synthesized zinc oxide NPs controlled the larvae of cattle tick, *R.* (*Boophilus*) *microplus*; head louse, *Pediculus humanus capitis*; mosquito larvae, *Culex quinquefasciatus* and *Anopheles subpictus* [61].

Three days PT, the reduction induced by AME, AGE, NME, NGE, and Butox® against cattle infested with *R. (Boophilus) microplus* was reported as 54.45, 45.73, 100, 100, and 3.65%, respectively; Butox® was ineffective, indicating resistance. A similar resistance had also been reported for *Rhipicephalus (Boophilus) microplus* [62,63]. Green silver NPs effectively controlled deltamethrin-resistant *R.* (*Boophilus*) *microplus in vitro* [64].

Similar studies also indicated that botanical extracts induced dose- and time-dependent responses and the efficacy of botanicals after field application. The AE of *Azadirachta indica* leaves, *Nicotiana tabacum* leaves, *Calotropis procera* flowers, and *Trachyspermum ammi* seeds were evaluated via a larval packet test, AIT, and ear bag method. Topical application of the extract exhibited adverse effects on the egg-laying index, hatchability (0.371404 ± 0.00435 and 22.35%, respectively); larval mortality, and tick intensity on infested calves at 50 mg/mL quantified as 18 detached ticks out of 35 with a 45% (w/w) suspension [65].

Essential oils induced *in vivo* lousicidal and repellent effects against the buffalo louse, *Haematopinus tuberculatus* [17]. *In vivo* application of NPs is extremely rare; a study indicating similar speed and potency of the formulations; encapsulated amitraz and fluazuron, as well as

nanostructures like chitosan-poly-Ɛ-caprolactone NPs, effectively treated tick infestations [66].

The phytochemical analysis in the present work indicated the existence of tannins, phenols, and flavonoids as major functional groups. Similar findings were recorded for different plants [67]. Tannins, phenols, and flavonoids may cause cellular and physiological disorders by inhibiting acetylcholinesterase and interfering with mitochondrial respiration, Malpighian tubules, midget epithelium, and gastric caecae [68]. Such bioactive ingredients could be used for pest management as secondary plant metabolites [45,67–69].

Our data indicated that myrrh is slightly more effective than ginger; this may be because of its high contents of flavonoids and phenols, which were relatively higher than double in AE or silver NPs. In contrast, Gas chromatography–Mass spectrometry analyses of chemical extracts of *C. molmol* and *A. heterophylla* revealed the presence of 4,4′-Dimethyl-2,2′-dimethylenebicyclohexyl-3,3′-diene (14.62%) and Copaene (13.64%) as major components, respectively [47]. *C. molmol* hexane and methanol extracts contained sesquiterpene, fatty acid esters and phenols, whereas those of *A. heterophylla* possessed monoterpene, sesquiterpene, terpene alcohols, and fatty acid [21].

Similar results reported that the major components in *Z. officinalis* oil were geranial (23.2%), neral (16.7%), 1,8-cineole (15.8%), and camphene (11.3%). Despite variations in oil composition, most of its components induced various biological activities against the larval stage of *R.* (*Boophilus*) *microplus* (100% MO at 25 mg/mL) [55].

This study showed reduced active compounds in NPs but with higher acaricidal effect, which may be due to silver NPs enhancing the efficacy of the AE. It could also be inferred that the phenolics present in myrrh and ginger extracts might be responsible for the observed acaricidal effects. A previous study revealed that *C. molmol* oil and oleoresin decreased the number of enzymes and affected cell proteins in *Cx. pipiens* larvae [70]. On the other hand, histopathological and Transmission Electron Microscopy (TEM) studies revealed that myrrh rapidly penetrates the cuticle to the body cavity of ticks, destroying the epithelial gut cells [54]. Analogous to our results with ginger extract, 5% AE of *Z. officinale* induced 100% MO against females, males, and larvae of the brown dog tick, *Rhipicephalus sanguineus* through cuticular damage, breaching, and homogeneity loss of epicuticle and endocuticle [71].

Similar study found that the methanol and hexane extracts of *Saussurea costus* extracts contained mainly sesquiterpene, fatty acid esters, phenols, and acyclic hydrocarbons and effectively controlled H. dromedarii (LC50 = 1.37 and 2.33 mg/ml, respectively) and *Rhipicephalus (Boophilus) annulatus* seven days after treatment (1.23 and 1.95 mg/ml, respectively) [72].

This study proved *in vivo* acaricide resistance to Deltamethrin (Butox®) against H. dromedarii. A very recent study recorded similar resistance to Phoxim, Volaxo® against the same species of tick, but indicated that some photosensitizers effectively contolled *H. dromedarii* [73].

5. Conclusions

Eco-friendly tick control is crucial; therefore, this study proved that tick showed (*in vivo*) resistance against Deltamethrin (Butox®) and validated the efficacy of the novel myrrh and ginger extracts and their silver nanoformulations against *H. dromedarii*. Subjecting myrrh and ginger to laser ablation for 20 min enhanced the speed and efficacy of killing ticks both *in vitro* and *in vivo* than those of the crude aqueous extracts. Myrrh and ginger extracts before and after nanoformulations (whichever available) are promising alternatives for an integrated approach in protection against tick bites and their associated diseases through a topical spot-on application. Future studies are warranted to investigate the ecotoxicological profile of the applied materials.

Ethical approval

Animal treatment in this study was done according to the guidelines of Benha University and approved by the Ethical Committee of the Faculty of Veterinary Medicine, Benha University (BUFVTM 02-10-22), Egypt.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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