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Toxicity, repellency, and anti-cholinesterase activities of bioactive molecules from clove buds *Syzygium aromaticum* L. as an ecological alternative in the search for control *Hyalomma scupense* (Acari: Ixodidae)

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ABSTRACT

Introduction: The goal of the current study is to evaluate the acaricidal and repellent properties of the ethanolic extract, essential oil, and primary component eugenol from *Syzygium aromaticum* against *Hyalomma scupense* cattle ticks. Their potential mechanisms of action were also examined, using an *in vitro* assay.

Methods: Clove essential oil was extracted using hydrodistillation technique. Gas chromatography-mass spectrometry (GC-MS) was performed to identify the chemical composition of clove. To evaluate the adulticidal, ovicidal, larvicidal and repellent proprieties of clove essential oil, eugenol and ethanolic extract on *H. scupense, in vitro* assays were performed using the adult immersion test (AIT), the ovicidal test, the larval packet test (LPT), the filter paper test and anti-acetylcholinesterase (AChE) activity.

Results: After treatment, eugenol, the primary phytoconstituent of clove oil, which accounts for 97.66% of the whole oil, had 99.22% acaricide activity and inhibited egg hatching at a concentration of 10 mg/mL. Eugenol and clove essential oil showed potent adulticidal effect at high concentrations (10 mg/mL), achieving 100 and 93.76% mortality, respectively. The ethanolic extract exhibited moderate activity. At high concentration, the larvicidal activity of *S. aromaticum* oil, eugenol, and ethanolic extract were 100, 100, and 77.18%, respectively. In filter paper experiments, when tested at the concentration 5 mg/mL; eugenol showed the longest repellent effect up to 6 h. We also found that eugenol was the most active AChE inhibitor ($IC_{50} = 0.178$ mg/mL). Nevertheless, additional investigations are required to confirm the accurate mechanism and the relevance of clove in practical application.

Conclusion: Overall, our research indicated that, because its effectiveness as acaricide, *S. aromaticum* essential oil and its phytoconstituent eugenol may offer an alternative source for the control of *H. scupense* cattle ticks.

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1. Introduction

Ticks are the most predominant ectoparasites of cattle capable of transmitting a wide range of pathogenic viruses, bacteria, protozoa, and helminthic parasites. Cattle herds cost a heavy price if infected with tropical bovine theileriosis transmitted by *Hyalomma scupense,* the most common tick species infesting cattle in Tunisia [1]. Economic losses due to *H. scupense* in Tunisia are estimated to amount to 15,115.058 TD (€9388.20) [2]. This arthropod is also directly responsible for production losses including damage in meat, milk, and leather production [1]. Many strategies have been employed for tick control including vaccination, biological control employing pathogens or predators, pheromone-assisted control, herbal pour-on or dip formulations, and green produced nanoparticles [3]. Although applications of chemical acaricides and repellents such as; organophosphates, carbamates, pyrethroids and amidines are still seen as the most way of control, they have a number of disadvantages, including expense, toxic effects, long waits, and chemoresistance of marketed acaricides [3].

The search of new substitutes and the need for safer products, with novel mode of action, less toxicity to both human and the environment are badly needed. Among these substitutes, botanical alternatives like essential oils are currently receiving particular interest. Due to their phytoconstituents' pharmacological activity, less side effects, and increased survivability compared to their synthetic counterparts, medicinal plants are currently receiving intention.

Syzygium aromaticum (English name "clove"), belonging to the family Myrtaceae, is an aromatic spice with the approximate height of 8–12 m and is indigenous to east Indonesia. Clove essential oil has wide global usage in cuisines as a food preservative, perfumes and also in traditional medicine as a common healing agent for wounds and burns and as treating tooth infections [4]. Similarly, in modern therapeutic applications, *S. aromaticum* has important biological proprieties such as; antioxidant [5], antibacterial [6], antifungal [7] , antiviral [8] and insecticidal [9].

The major bioactive constituent in the clove oil is eugenol that displayed a potential lethal efficacy against various parasites [10], mites [11] as well as ticks (including *Rhipicephalus microplus*) [12].

Although previous works focused on the chemical composition of *S. aromaticum* extracts using different solvents [13–15], there are no studies describing their use in the toxicity and repulsive properties. Thus, the aim of the present research was to establish the acaricidal and repellent activity of essential oil, eugenol and ethanolic extract from *S. aromaticum,* against *H. scupense.* as well as their putative mode of action.

2. Materials and methods

2.1. Plant collection

The flower buds of clove plant used in this study were purchased from local market in Tunisia. The plant was identified by a botanist at the herbarium of the laboratory of functional physiology and valorization of bio-resources at Higher Institute of Biotechnology of Beja, Tunisia. Certified voucher specimens of the plant of air-dried leaves were deposited at the laboratory under voucher number CEO 187 for *Syzygium aromaticum*. After air-drying in the shade for a week, the air-dried buds were powdered and used for the extraction.

2.2. Obtaining essential oil

150 g of dried clove flower buds was subjected to hydrodistillation for 3 h using a modified Clevenger extraction apparatus. Essential oil was collected and the residual water in oil was eliminated with anhydrous magnesium sulfate ($MgSO_4$). The oil was kept in glass vials covered with aluminum foil at a low temperature (5 to 2 °C). Eugenol was purchased commercially from Sigma Aldrich Chemicals (N°97-53-0; 0,98.5% purity).

2.3. Gas Chromatography–Mass spectrometry (GC-MS)

A gas chromatograph HP-7890 connected to a mass spectrometer (Agilent Technologies Wilmington, DE, USA) was used to identify the phytoconstituents in *S. aromaticum* oil. Initially, the equipment had a fused silica capillary column installed (30 m 0.25 mm, 0.25 m film thickness). The oven temperature was set to increase by 3 °C/min from 60 °C in 2 min to 240 °C in 20 min. The injector and detector had temperatures of 250 °C and 280 °C, respectively. A split ratio of 1:50 was used to inject a 0.2 μl oil sample into the GC. The carrier gas, helium, flowed at a rate of 2 mL/min. The MS was produced using an ion source at 230 °C and an ionization voltage of 70 eV. The scan mode was used to gather MS data in the 40–650 *m/z* range. Three replications were used for the entire experiment. The clove components were confirmed by using the retention times values. The characterization of volatile components was also achieved by comparing the mass spectra with those reported in standard libraries. Also, by comparing the mass spectra with those that have been reported in standard libraries (Wiley275.L - Wiley7n.L).

2.4. Preparation of plant extract

100 g of dried cloves were macerated in ethanol (1:1; v/v, 500 mL) at room temperature for 72 h while being shaken (40 cycles/min). Three times this process was carried out. The extracts were filtered, Rotavapor-evaporated, and kept at 4 °C.

2.5. Phytochemical analysis

In accordance with our earlier research [16], a quantitative examination of clove extract was carried out.

2.6. Tick collection

Hyalomma scupense ticks were randomly gathered (n ≅ 200) from naturally infested cattle, that receiving any treatment, belonging to a farm in the municipality of Soliman, Northwestern region of Nabeul. Tunisia. In the laboratory, on the basis of normal appearance and motility, an intact body, and maximum engorgement with uniform weight, only the engorged females were chosen and they were recognized based on Walker et al. (2003) protocols. After being cleaned with distilled water and 2% sodium hypochlorite solution, ticks were dried. Groupe of ticks were performed for adulticidal test. While the others were kept in an incubator at a temperature of 27 °C and a relative humidity (RH) range of 75 to 80%, until oviposition was complete, and they were utilized for the ovicidal test. To obtain eggs and later larvae, additional female ticks were incubated in the same manner. The hatched larvae were then utilized for the larvicidal test.

2.7. Adult immersion test (AIT)

The procedure outlined by Ref. [17] was used to perform the adult immersion test. In 2% dimethyl sulfoxide, clove essential oil, eugenol, and ethanolic extract were dissolved in DMSO (2%) at concentrations of 10, 5, 2.5, 1.25, and 0.625 mg/mL. Three repetitions of each concentration or control treatment were carried out, and each replication had five females. Ticks were immersed in each concentration for 5 min. Ticks were then collected, dried, and put in Petri dishes. Subsequently, the Petri dishes were maintained at 27 °C and 70–80% relative humidity conditions and mortality rate was recorded for 1, 5, 10 and 15 days.

2.8. Ovicidal activity

Hyalomma scupense eggs were collected, weighed, and then deposited on a filter paper at a standard weight of 50 mg [18]. Subsequently, test solutions (10, 5, 2.5, 1.25, and 0.625 mg/mL in DMSO (2%)) were sprayed into a volume of 1 mL. Amitraz 12.5%, was made as the positive control group in distilled water at a dilution of 1:1000, as instructed by the manufacturer. Eggs that had been treated with water and DMSO (2%) served as the negative control. To calculate the percentage of hatching, the eggs were incubated in the identical circumstances as above. For each sample, three repetitions were carried out.

2.9. Larvicidal activity

A modified larval packet technique (LPT) was adopted considering the procedure of [19]. Using a fine-tipped paintbrush, approximatively 100 larvae were deposited on filter sheets (7 × 7 cm) soaked with 100 μl of the sample solution at various doses (10, 5, 2.5, 1.25 and 0.625 mg/mL in DMSO (2%)). The treated filter sheets were then formed into packets and sealed. After 2, 4, 8, 12 and 24 h, the treated packets were examined to determine the death rates by counting both live and dead larvae. The control groups received treatment with distilled water. Each concentration was carried out four times.

2.10. Repellent activity

Using the preference zone method on filter paper adopted by Ref. [20], the repellent properties of clove essential oil, eugenol, and ethanolic extract against *H. scupense* were evaluated. Filter paper discs (diameter = 9 cm) were divided into two equal sections, and 0.5 mL of each sample solution was equitably applied at various doses (10, 5, 2.5, 1.25 and 0.625 mg/mL). Just acetone was used to impregnate the remaining filter paper. The two half-discs were then thoroughly dried for 10 min, put in a Petri dish, and recovered. Once ten adult mixed-sex ticks were placed in the center of the filter paper, the petri dishes were covered and kept in the dark at 26 1 °C with 80% humidity. Procedure was carried out four times. The number of ticks on the treated and untreated portions of the experimental paper were counted after 1, 2, 4, and 6 h of contact to each tested dose. following [21] percentage repellency (PR) was determined:

$$PR = [(Nc - Nt)/(Nc + Nt)] \times 100$$

where Nc represents the number of ticks on the untreated region after the exposure time, and Nt represents the number of ticks on the treated area after the exposure period.

2.11. Acetylcholinesterase activity (Ellman's assay)

The acetylcholinesterase activity (%) of treated engorged female ticks was examined. The substrate for acetylcholinesterase was acetyl-thiocholine iodide. Acetate and thiocholine are produced during the hydrolysis of this substrate. After being exposed to dithiobisnitrobenzoate (DTNB) for 10 min at 37 °C, the emitted thiocholine conducts a reaction to generate 5-thio-2-nitrobenzoate, a yellow compound whose absorbance at 405 nm can be used to measure its concentration [21]. Acetylcholinesterase activity was

assessed as follows: % acetylcholinesterase = 100 × (absorbance of sample – absorbance of blank]/absorbance of blank.

2.12. Statistical analysis

For all statistical analyses, Statview v.5.0.1 software (SAS Institute, Cary, NC) was used. The mean and standard error were used to express the data. Tukey's HSD tests were conducted after an analysis of variance (ANOVA) to compare groups. When $p > 0.05$, differences between the treatment and control groups were not deemed statistically significant. Using Probit analysis and GraphPad Prism 9.0 software, the lethal concentration of sample solution for 50% (LC_{50}) and 90% (LC_{90}) of the tick population with a 95% confidence interval was determined.

3. Results

3.1. GC-MS analysis of clove oil

Clove essential oil was extracted by hydrodistillation with a yield of 6.87% (9.6 mL). The composition of clove oil is listed in Table 1. Seven components have been detected, comprising 100% of the entire oil from clove essential oil (Fig. 1). The main active component was eugenol (97.66%) (Fig. 2).

3.2. Phytochemical analysis of clove extract

Total phenol (127.26 mg GAE/g), flavonoid (26.72 mg CE/g), and tannin amounts in clove buds, were found in ethanolic extract obtained by maceration technique (Table 2).

3.3. Toxicity activities

3.3.1. Adulticidal activity

The toxicity of *S. aromaticum* on *H. scupense* adults was shown in Table 3. At the highest measured concentration, clove essential oil caused 82.76% mortality in engorged females after five days, reaching 100% on the tenth day after treatment. At a concentration of 5 mg/mL, clove oil achieved 100% mortality rate at the end of the experiment (day 15). However, clove essential oil at 0.625 mg/mL led to a 28.36% mortality rate on day 15 after treatment.

Clove essential oil demonstrated lower efficacy than eugenol. In fact, after 24 h, the treatment with eugenol, at 5 mg/mL or 10 mg/mL, caused 31.39 and 38.27% mortality in engorged females, respectively. On day 15 following the treatment, the mortality rate reached 100%.

Anti-tick activity of clove ethanolic extract was also studied (Table 3); all tested concentrations produced relatively low adult mortality when compared to the action of the oil and eugenol. With doses of 10 mg/mL and 5 mg/mL, respectively, ethanol extract caused deaths of 77.01 and 51.15% on the 15th day after treatment. Ethanolic extract induced 77.01 and 51.15% mortality on the 15th day after treatment at concentrations of 10 mg/mL and 5 mg/mL, respectively. Our findings showed that *H. scupense* mortality was dose-dependent, and all treatment groups significantly differed from the negative control group. While amitraz-treated groups recorded 96.80% mortality of *H. scupense* engorged females (Table 3).

In overall, in terms of LC values, eugenol had better efficacy than oil and ethanolic extract from clove (Table 4). The highest efficacy was achieved by eugenol, with LC_{50} values of 0.48 mg/mL. Amitraz was shown to be much less efficacious than eugenol ($LC_{50} = 1.92$ mg/mL). followed by clove essential oil (1.17 mg/mL) and ethanolic extract (3.98 mg/mL).

3.3.2. Ovicidal activity

As detailed in Fig. 3, concerning tick egg hatching experiments on *H. scupense*, eugenol, at 10 mg/mL, totally inhibited egg hatching in *H. scupense* cattle tick. While, at the same concentration, *S. aromaticum* essential oil maintained significantly lower egg hatching rates close to 95%. The ethanolic extract did not reduce egg hatching in *H. scupense* even at the maximum tested concentration. The egg hatching rate in the negative control was 94.97%. Amitraz revealed a high inhibition of egg-hatching (96%).

Table 1
The output results of the GC/MS analysis of *Syzygium aromaticum* essential oil.

Peak	RT (min)	Area	% of total	Compounds
1	14.575	967376	0.110	Thymol
2	16.160	857548741	97.665	Eugenol
3	16.487	2891433	0.329	Isoeugenol
4	17.431	6140173	0.699	Caryophyllene
5	18.138	805841	0.092	Humulene
6	19.544	8540472	0.973	Acetyleugenol
7	20.747	1160893	0.132	Caryophyllene oxide

RT: Retention time.

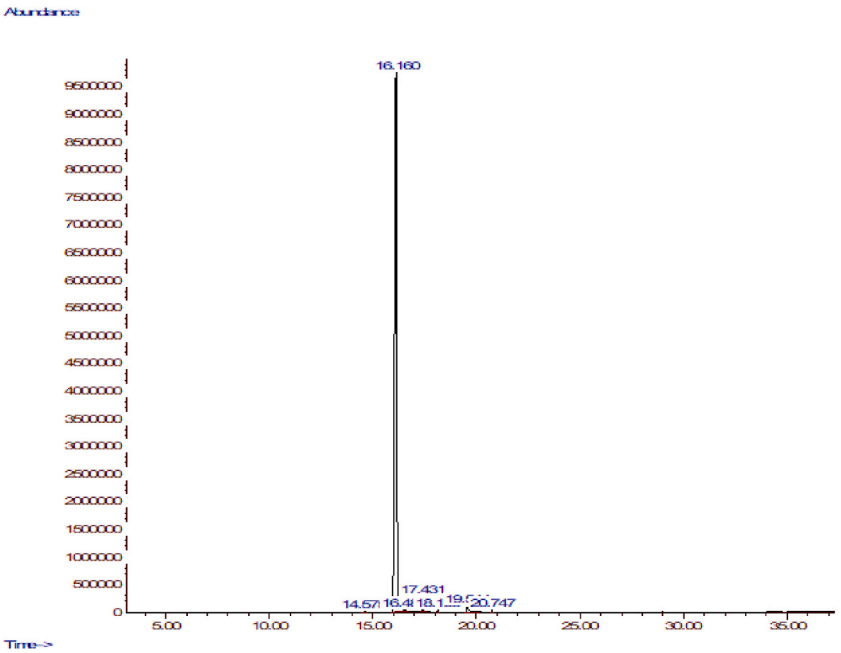

Fig. 1. Chromatogram of *Syzygium aromaticum* essential oil.

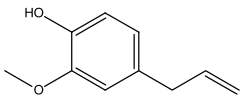

Fig. 2. Eugenol.

Table 2
Phytochemical content of ethanolic extract of clove buds *Syzygium aromaticum*.

Extract samples	Extract yield (%)	Total phenolic content (mg of GAE/g DW)	Total flavonoid content (mg of QE/g DW)	Condensed tannin contents (mg TA/g DW)
Ethanolic extract	11.30	2.10.45 ± 0.85	10.73 ± 0.29	1.32 ± 0.66

3.3.3. Larvicidal activity

Fig. 4 outlines in tick larvicidal experiments on *H. scupense,* that eugenol was the most effective component, achieving 100% of larvae mortality when tested at 2.5 mg/mL and it was higher ($p > 0.05$) than that of amitraz, when tested at the same concentration. In addition, at the same concentration, clove essential oil led to mortality rates close to 94%. While, ethanolic extract was the least effective compound, achieving 77% of mortality rates, even when tested at 10 mg/mL. No larval mortality was shown in the negative control. Table 5 lists the results of the probit analysis performed on the larvicidal data. Eugenol had the lowest LC_{50} value, reaching 0.51 mg/mL. Moreover, eugenol was more effective than amitraz ($LC_{50} = 0.60$ mg/mL). On other hand, the LC_{50} value for ethanolic extract was 3.89 mg/mL, while the LC_{50} value for clove essential oil was 1.67 mg/mL.

3.4. Repellent activity

Fig. 5 depicts the percentage repellency of the essential oil, eugenol and ethanolic extract from *S. aromaticum* against *H. scupense* ticks tested at various doses for different exposure times. Our experiment revealed that the percent repellency of the clove oil reached 100% and was active up to 2 h of post-treatment at the highest concentration (10 mg/mL). At the tested concentration 5 mg/mL, eugenol from clove exhibited highest repellent activity (100%) after 1 h and it showed the longest repellent effect up to 6 h when tested at the dose of 10 mg/mL. While, at the highest concentration, ethanolic extract exhibited repellent effect of 60.21% only for a few hours of application; after that it decreased with time and showed repellent effect of about 18% after 60 min post-exposure.

Table 3
Adulticidal activity of essential oil, eugenol and ethanolic extract of *Syzygium aromaticum* on *Hyalomma scupense*.

Treatment	Concentrations	Days ± SD			
		1-day	5-day	10-day	15-day
Essential oil	10	18.11 ± 6.33	82.76 ± 9.44	100.00 ± 0.00	100.00 ± 0.00
	5	13.84 ± 5.55	41.06 ± 8.15	58.33 ± 5.50	100.00 ± 0.00
	2.5	12.74 ± 8.00	36.31 ± 6.90	45.18 ± 7.33	66.37 ± 6.20
	1.25	9.34 ± 2.33	26.17 ± 3.77	38.77 ± 5.25	50.55 ± 6.50
	0.625	2.86 ± 3.55	8.31 ± 1.01	9.22 ± 2.00	26.32 ± 4.55
Eugenol	10	38.27 ± 6.33	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	5	31.39 ± 7.55	97.8 ± 4.23	100.00 ± 0.00	100.00 ± 0.00
	2.5	26.28 ± 4.66	42.33 ± 3.20	63.70 ± 3.77	89.76 ± 1.77
	1.25	20.80 ± 7.50	35.29 ± 6.45	42.11 ± 3.55	74.69 ± 2.55
	0.625	17.21 ± 3.66	21.96 ± 6.25	38.06 ± 7.81	61.44 ± 5.15
Ethanolic extract	10	8.37 ± 5.55	37.11 ± 4.00	57.01 ± 4.18	67.01 ± 1.5
	5	4.08 ± 1.55	22.03 ± 2.66	46.15 ± 5.15	51.11 ± 5.50
	2.5	2.55 ± 2.55	9.1 ± 1.00	13.89 ± 3.33	29.50 ± 7.15
	1.25	1.50 ± 3.00	2.50 ± 4.55	6.33 ± 4.00	8.69 ± 3.66
	0.625	0.00 ± 0.00	1.50 ± 1.00	2.00 ± 1.00	2.00 ± 1.00
Positive control Amitraz	10	36.80 ± 7.50	50.06 ± 4.15	78.11 ± 3.66	86.66 ± 1.00
	5	29.05 ± 8.66	35.99 ± 7.48	58.71 ± 6.80	71.00 ± 1.5
	2.5	20.74 ± 6.66	24.39 ± 6.50	40.14 ± 4.33	62.14 ± 4.24
	1.25	11.08 ± 3.30	18.93 ± 6.18	22.25 ± 7.05	32.15 ± 7.00
	0.625	4.66 ± 5.00	9.06 ± 5.50	13.74 ± 7.15	17.55 ± 7.00
Negative control		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 4
Lethal concentrations of *Syzygium aromaticum* essential oil, eugenol and ethanolic extract against adult *Hyalomma scupense*.

Tests	Tested compounds	LC_{50} [a]	LCL-UCL	LC_{90} [a]	LCL-UCL	Slope±SE	R^2	df
Adulticidal	Essential oil	1.17	1.08–2.33	7.61	6.82–7.93	5.11 ± 0.45	0.996	3
	Eugenol	0.48	0.34–0.67	2.73	2.46–3.08	3.61 ± 0.92	0.997	3
	Ethanolic extract	3.98		–	–	4.13 ± 0.85	0.988	3
	Amitraz	1.92	1.08–2.10	4.06	3.96–4.15	4.19 ± 0.79	0.980	3

[a] Lethal concentrations (LC_{50} and LC_{90}) values in mg/mL. LCL = lower confidence limit. UCL = upper confidence limit.

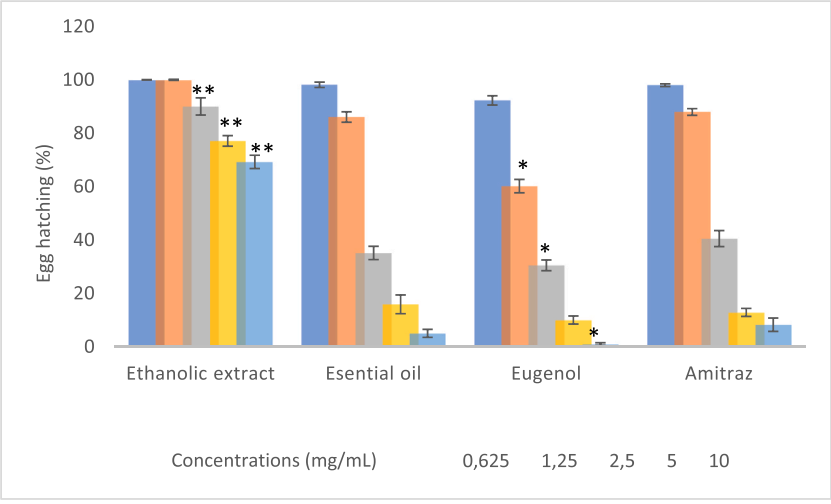

Fig. 3. Impact of the *Syzygium aromaticum* essential oil, eugenol and ethanolic extract on *Hyalomma scupense* egg hatching; amitraz was used as the positive control. *$P < 0.05$; **$P < 0.001$ compared to amitraz (ANOVA, Tukey's HSD test).

3.5. Acetylcholinesterase activity

AChE inhibitory activities of clove ethanolic extract, essential oil, eugenol and galantamine are shown in Fig. 6. AChE inhibition study showed that eugenol exhibited inhibition of the enzyme (96.36%) better than extract (18.04%) and essential oil (91.79%).

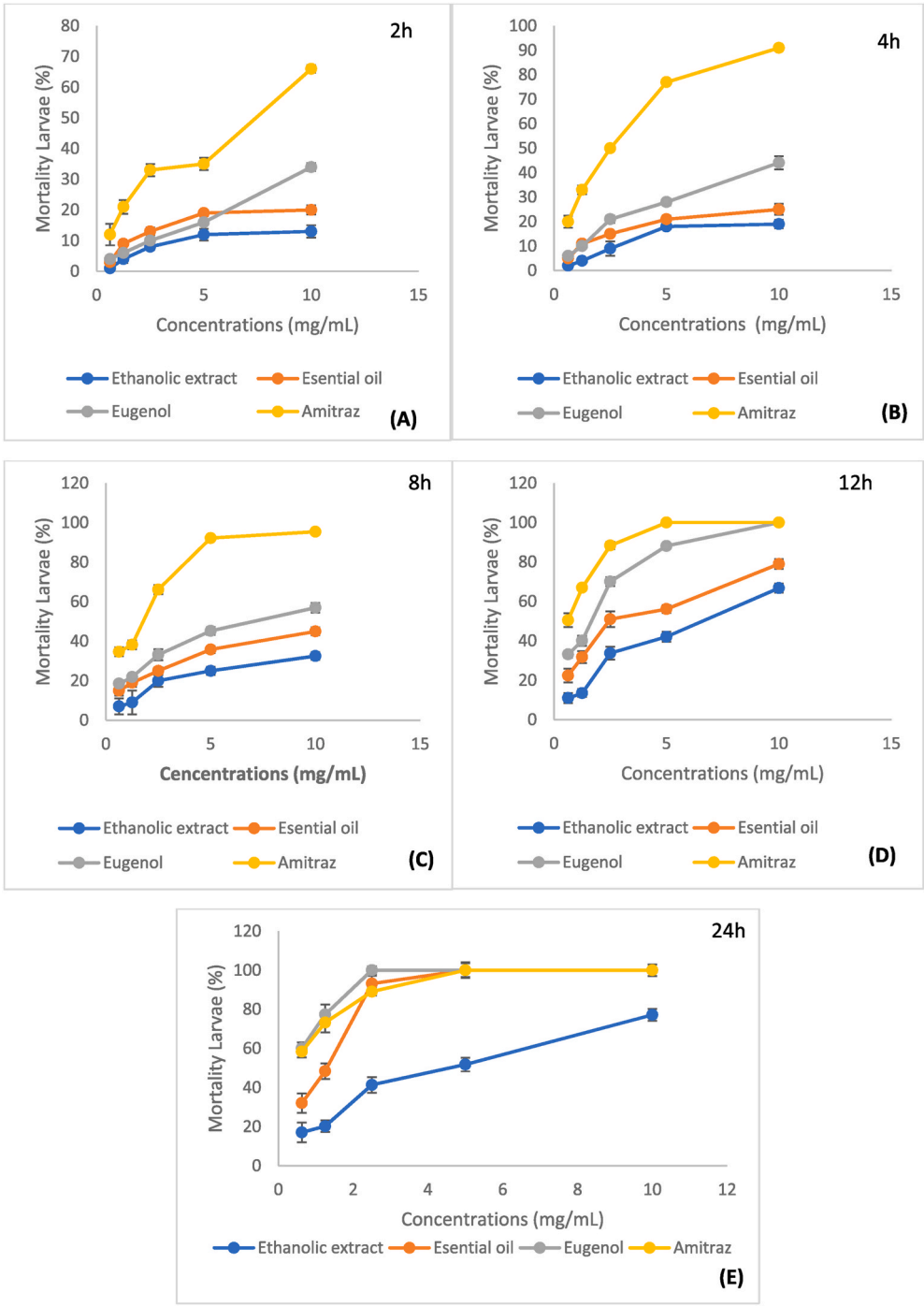

Fig. 4. The *in vitro* effects of essential oil, eugenol and ethanolic extract from *Syzygium aromaticum* against *Hyalomma scupense* larvae by assessment of mortality rate after different exposure times: 2h **(A)**, 4h **(B)**, 8h **(C)**, 12h **(D)** and 24h **(E)**. Data are stated as mean ± SD (n = 3). Amitraz was used as a positive control.

Likewise, as noticed from their IC_{50} values, eugenol, was found the most active AChE inhibitor (IC_{50} = 0.178 mg/mL) (Table 6).

4. Discussion

The extensive use of chemical acaricides has resulted in the development of tick resistance, in addition to producing residues in meat and milk and having negative effects on non-target species [3]. These problems underscore the critical necessity for the creation

Table 5
Lethal concentrations of *Syzygium aromaticum* essential oil, eugenol and ethanolic extract against larvae *Hyalomma scupense*.

Tests	Tested compounds	LC_{50}^{a}	LCL-UCL	LC_{90}^{a}	LCL-UCL	Slope±SE	R^2	df
larvicidal	Essential oil	1.67	1.84–2.58	2.36	2.28–2.47	2.84 ± 0.15	0.981	2
	Eugenol	0.51	0.44–0.81	2.11	2.09–2.24	1.77 ± 0.22	0.998	2
	Ethanolic extract	3.89	2.94–4.22	–	–	2.16 ± 0.77	0.993	2
	Amitraz	0.60	0.54–0.68	2.72	2.67–2.82	1.09 ± 0.66	0.997	2

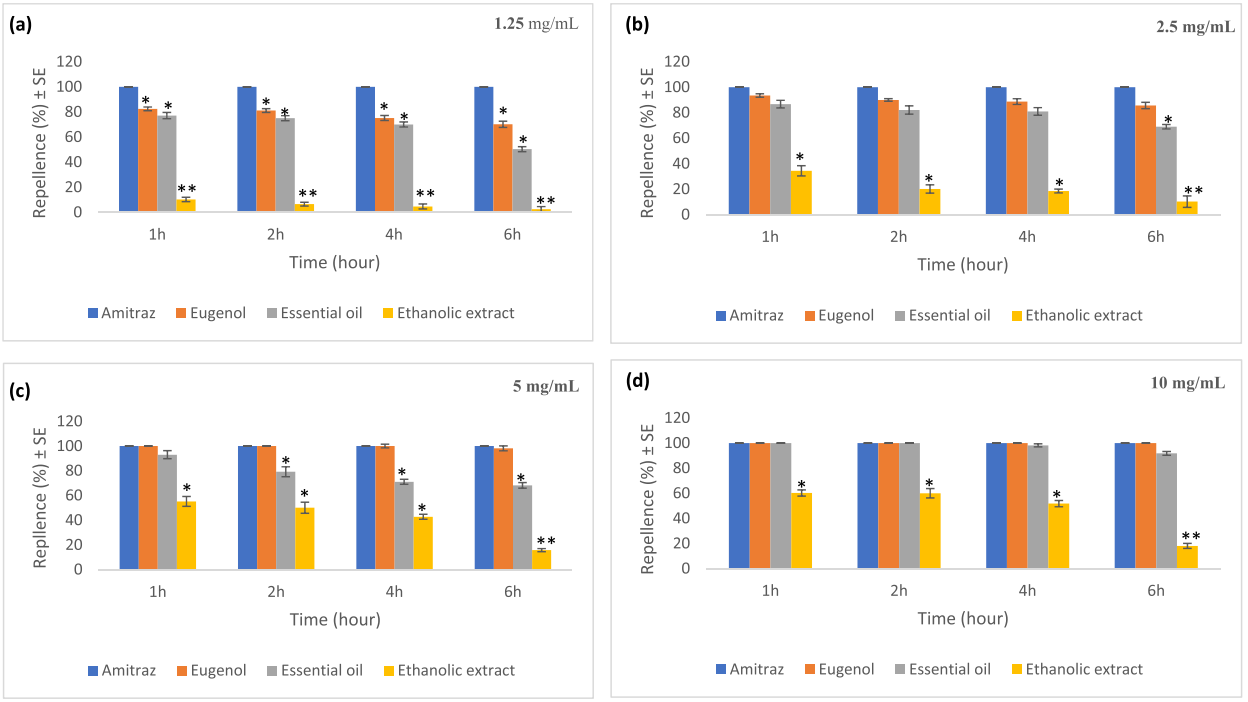

Fig. 5. Repellency percent of *Hyalomma scupense* adults exposed to essential oil, eugenol and ethanolic extract from *Syzygium aromaticum* in different concentrations (1.25 **(a)**; 2.5 **(b)**; 5 **(c)** and 10 mg/mL **(d)**) after various exposure times in the filter paper test. *$P < 0.05$; **$P < 0.001$ compared to amitraz (ANOVA, Tukey's HSD test).

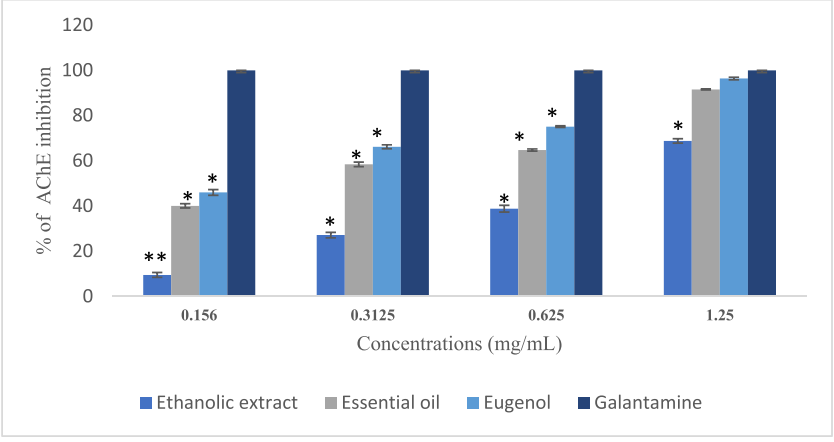

Fig. 6. Dose-dependent inhibitory activity of essential oil, ethanolic extract, and eugenol from *Syzygium aromaticum* against *Hyalomma scupense* acetylcholinesterase. Data are given as mean ± SD (n = 3). Galantamine was used as the positive control. *$P < 0.05$; **$P < 0.001$ compared to galantamine (ANOVA, Tukey's HSD test).

Table 6
IC_{50} values of ethanolic extract, essential oil and eugenol from *Syzygium aromaticum* against *Hyalomma scupense* acetylcholinesterase.

Sample	IC_{50} (mg/mL)	Slope	95% CI	χ^2
Ethanolic extract	1.20	1.18 ± 1.33	0.247–0.346	3.27
Essential oil	0.191	1.22 ± 0.21	0.179–0.238	4.18
Eugenol	0.178	0.67 ± 8.47	0.161–0.1	1.04
Galantamine	0.097	0.54 ± 0.69	0.078–0.120	

Data are given as mean ± SD (n = 3).
CI: Confidence interval.

of innovative and environmentally acaricides. Many investigations on the anti-tick properties of plants have been conducted for many years [22]. In this experiment, we aimed to determine the effectiveness of ethanolic extract, essential oil, and eugenol from *S. aromaticus* on *H. scupense*, a known vector of tropical bovine theileriosis, in terms of its adulticidal, ovicidal, larvicidal, and repellent activities.

Secondary compounds of clove were identified both quantitatively and qualitatively. The GC/MS revealed that eugenol was the main component in clove oil with other phytochemicals only being found in trace amounts or at very low concentrations. Compared with earlier reports on Indian clove which include 70% eugenol [23]. While, as reported by Ref. [24], Monado clove oil contained eugenol 55.60%. Different clove samples, varied distillation techniques, and different sample preparation procedures all contribute to varying percentage compositions [24]. Also, it has been claimed that the results of separating the components of clove bud oil can be impacted by the type of operating column utilized in the GC-MS study [25]. In addition, in the current study, total polyphenols, flavonoids and condensed tannins amounts of *S. aromaticum* extract were comparable to earlier research using samples of Egyptian clove [26].

Herein, adulticidal propriety of clove essential oil and its major compound, eugenol reached to 93.76% and 100%, respectively at 10 mg/mL concentration and their LC_{50} was 0.48 and 1.17, respectively on adult ticks. In considering the action of clove essential oil on the adults of *R. microplus* [27], observed a 100% mortality of engorged females *R. microplus* treated with the highest concentration, 10 mg/mL, which confirms the results obtained in the present study. Moreover, *S. aromaticum* oil showed significant toxicity against the poultry red mite *D. gallinae* with LC_{50} value of 8.9 μg/cm^3 [28]. In another study, the effect of clove, basil oil, and peppermint oils on the mortality of immature stages of two-spotted spider mites, *Tetranychus urticae* Koch, was tested. Clove oil was significantly the most effective achieved mortality percentages of 74.80% and 80.20% after 24 h at concentrations of 150 and 200 μL/L. Clove oil is also reported to be an excellent larvicide, adulticide, repellent, and ovicide against two mosquito vectors (*Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus*) [29]. The mechanism of *S. aromaticum* essential oil involves lowering the acetylcholine-esterase "AchE" and increasing alkaline-phosphatase "ALP", hence killing the pests [30].

In the present study, eugenol exhibited ovicidal effect and inhibited egg hatching at concentration of 10 mg/mL. However, in contrast with our findings [12], found that eugenol at concentrations 50 mg/mL led to high inhibition of egg hatching of *R. microplus*.

In considering the action of eugenol and clove essential oil on the larvae of *H. scupense*, a mortality rates of 100 and 93.17%, respectively in larvae, at 2.5 mg/mL, after 24 h of exposure. While ethanolic extract showed moderate repellent activity that lasted for a shorter period of time. Our findings are consistent with [12] who observed that they caused 97.9 and 100%, *R. microplus* larval mortality at concentration 2.5 mg/mL for eugenol and clove essential oil respectively. According to research by Ref. [31] clove oil and eugenol needed to be used at higher concentrations (40 mg/mL) in order to cause *Rhipicephalus sanguineus* larvae mortality (95%). It has been reported that difference in mortality rates may be caused by a variety of factors including tick species, study method, exposure time and environmental circumstances [32].

On the other hand, current results revealed that, *S. aromaticum* oil and its major compound showed potential for use against *H. scupense* cattle ticks as a repellent agent, where they exhibited 71.09 and 100% repellency by 5 mg/mL concentration at 4 h, respectively. Present data are consistent with earlier investigations concerning the repellent activities of essential oils where indicated that eugenol, is better toxicant and repellent to the insects than monoterpenes [33–36]. It has been established that the presence of monoterpenoids, sesquiterpenes, and alcohols contribute to the repellent effects.

Eugenol demonstrated highest AChE inhibitory among the extract and oil. Our research shows that the main mechanism by which eugenol acts on *H. scupense* by the potent activity revealed is acetylcholinesterase inhibition. It has been claimed that, essential oil components like eugenol were found to perform docking with acetylcholinesterase protein model and this may be the probable target site of this terpene compound [37]. Furthermore, according to Ref. [38] AChE inhibition is mainly responsible for the harmful processes of essential oils. It has been reported that eugenol is the main component of clove essential oil that is effective against mites [39]. This phenylpropanoid has demonstrated potent acaricidal effects on mites, where the double bond's position in the molecule's side chain plays a pivotal role in the bioactivity [40,41]. Eugenol is also effective for treating bee products against varroosis [42]. Regarding the mode of action, it has been assumed that its functional group may interfere with the mitochondrial respiration of the target mite [43,44].

5. Conclusion

This study showed promising acaricidal and repellent effects of *S. aromaticum on H. scupense*. Although *S. aromaticum* essential oil and its major compound displayed its acaricidal and repellent activity through inhibiting AChE, additional investigations are required

to confirm the accurate mechanisms and the suitability of *S. aromaticum* in practical application.

Author contribution statement

Dhouha Alimi: Performed the experiments; Wrote the paper.
Azhar Hajri: Analyzed and interpreted the data.
Selim Jallouli: Contributed reagents, materials, analysis tools or data.
Hichem Sebai: Conceived and designed the experiments.

Data availability statement

Data included in article/supp. material/referenced in article.

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No additional information is available for this paper.

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.

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