Punjab University Journal of Zoology

38(2): 211-219 (2023)
https://dx.doi.org/10.17582/journal.pujz/2023.38.2.211.219

Research Article

In vitro Acaricidal and Repellent Effects of *Amomum subulatum* Essential Oil Against *Hyalomma* Ticks

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Article History
Received: October 21, 2023
Revised: November 05, 2023
Accepted: November 24, 2023
Published: December, 09, 2023

Authors' Contributions
AMAK analysed the data and wrote the manuscript. RZA supervised the research, revised and edited the manuscript and aquired funds. ZDS and MSM gave suggestions about writing, reviewing and editing the manuscript.

Keywords
Hyalomma, Ticks, Mortality, Repellency, Essential oils, *Amomum subulatum*

Abstract | The experiments were conducted to assess the acaricidal and repellent activities of *Amomum subulatum* (*A. subulatum*) (Black cardamom) essential oil against *Hyalomma* ticks in bovines. Gas chromatography-flame ionization detection (GC-FID) was performed to identify the chemical components of *A. subulatum* essential oil. The acaricidal and repellent activities of the *A. subulatum* essential oil against *Hyalomma* spp. were observed via adult immersion test (AIT), the larval immersion test (LIT), egg hatchability test (EHT), and the tick repellency assay. GC-FID provided that the main compounds of *A. subulatum* essential oil were monoterpenoids (limonene and α-terpinene, and α-terpineol) (33.8%) while other chemical compounds were α-terpinolene (9.5%), sabinene (9.3%), 1- Carveol (8.9%), α-phellandrene (7.8%), linalool (4.7%), myrtenol (4.7%), nerolidol (4.6%), β-pinene (4.6%), terphenyl acetate (3.6%), 1, 8-cineole (2.7%), and traces (1.7%). Five different concentrations of *A. subulatum* essential oil (0.31, 0.62, 1.25, 2.50, and 5% v/v) along with positive (0.1% Cypermethrin) and negative control (absolute alcohol) were prepared to check the acaricidal and repellent effects. *A. subulatum* essential oil significantly ($p<0.05$) enhanced the rates of mortality in a dose-dependent manner of adult and larvae of *Hyalomma* spp. Egg number, egg mass, and larval hatching were also reduced significantly ($p<0.05$) in a dose-dependent manner. As a result, reproductive index (RI), reproductive efficiency index (REI), and nutrient index (NI) were also decreased significantly ($p<0.05$) in a dose-dependent manner. Similarly, *A. subulatum* essential oil showed dose-dependent response against repellency of *Hyalomma* spp. The LC_{50} and LC_{90}, RC_{50} and RC_{90} values for *A. subulatum* essential oil were also calculated. Excellent larvicidal, adulticidal, and repellent outcomes of *A. subulatum* essential oil against *Hyalomma* ticks were achieved.

Novelty Statement | This is the first time used *Amomum subulatum* essential oil against Hyalomma ticks and result indicates that *A. subulatum* essential oil is a potential approach for either eliminating or suppressing Hyalomma ticks' infestation.

To cite this article: Khan, A.M.A., Abbas, R.Z., Sindhu, Z.D. and Mahmood, M.S., 2023. *In vitro* acaricidal and repellent effects of *Amomum subulatum* essential oil against *Hyalomma* ticks. *Punjab Univ. J. Zool.*, **38**(2): 211-219. https://dx.doi.org/10.17582/journal.pujz/2023.38.2.211.219

Introduction

Ticks are the main reservoirs of tick-borne pathogens of medical and veterinary concern. Tick- and tick-borne diseases prevail all over the world, typically in tropical and sub-tropical areas and affect almost 30% of the total cattle population (Basit *et al*., 2022). Among devastating parasitic infections of livestock, tick- and tick-borne diseases are ranked after mosquitoes and are considered as the most typical arthropod-borne diseases of livestock, humans, and companion animals (Abdelbaset *et al*., 2022). These ticks and tick-borne infections have doubled in the

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last two decades (Sanchez-Vicente *et al.*, 2019). Ticks cause direct and indirect losses to the livestock e.g., they induce fever, anemia, irritation leads to chronic stress, immunodepression, hide damage, poor feeding leading to lethargic condition, weight loss, decreased milk production, and in females increased calving interval (Kasaija *et al.*, 2021). Multiple species of ticks infect humans and animals, but the *Hyalomma* spp. are among the most common ticks infesting and serving as a vector for zoonotic agents (Rjeibi *et al.*, 2022). *Hyalomma* spp. are among the most considered species of arachnids to be controlled by the farmers and researchers (Valcárcel *et al.*, 2023).

Various acaricides like synthetic pyrethroids organophosphates, carbamates, and organochlorines are being used to control these ticks but their frequent use is resulting in the development of resistant tick species (Selles *et al.*, 2021). Acaricidal resistance has threatened the welfare of livestock farmers globally because it led to decreased productivity and the emergence of infections (Ahmed *et al.*, 2022; Betelhem *et al.*, 2022; Saeed and Alkheraije, 2023; Sikander *et al.*, 2023). Vaccines for the control of ticks have also been developed and being used commercially, but their efficacy is questionable, moreover they have limited value for the control of ticks (Bonnet *et al.*, 2022). The vaccines in the future, if work successfully, cannot replace the therapeutic control measures. In this scenario, the need for alternatives is at prime for the control of ticks (Semenza *et al.*, 2022).

Multiple alternative strategies are being suggested by the scientists, including metallic nanoparticles, entomophagous fungi, botanical products etc. (Raheel *et al.*, 2021; Srisanyong *et al.*, 2021; Hussain *et al.*, 2022). Botanicals have always been a priority for the researchers to use as control of parasitic diseases (Abbas *et al.*, 2020; Mubashir *et al.*, 2022; Tanda *et al.*, 2022). Multiple forms of botanicals i.e., whole plants, plant parts, extracts and essential oils etc. are in consideration (Sobhy *et al.*, 2021; Bangulzai *et al.*, 2022). The essential oils have the most promising importance among the botanicals because of the active compounds present in them (Radwan *et al.*, 2022; Saeed and Alsayeqh, 2023). The essential oils of various plants have been proven to contain multiple medicinal properties (Ali *et al.*, 2022; Bangulzai *et al.*, 2022; Salman and Imran, 2022). Multiple essential oils have been tested for their acaricidal activities (Shezryna *et al.*, 2020; Chaimanee *et al.*, 2021; El-Sayed *et al.*, 2022).

Amomum subulatum is commonly known as the Indian black cardamom or large cardamom (Saeed *et al.*, 2023). It is commonly found in the sub-continent region, and it is famous for its medicinal and culinary usage (Joshi and Piya, 2019). Essential oil of *A. subulatum* is a dark liquid having a pungent smell (Kumar *et al.*, 2022). *A. subulatum* has been found to be antiparasitic and acaricidal in multiple experiments (Al-Hoshani *et al.*, 2023a). This experiment was designed to evaluate the acaricidal activities of essential oil of *A. subulatum* against *Hyalomma* spp. because of its medicinal properties of *A. subulatum.* Researchers also checked its effects against arthropods but not particularly against *Hyalomma* ticks.

Materials and Methods

Collection of ticks and their identification

Hyalomma anatolicum (H. anatolicum), Hyalomma dromedarii (H. dromedarii), and *Hyalomma marginatum* (*H. marginatum*) were collected carefully from various parts of Faisalabad by dragging technique, as described by Falco and Fish (1992). The ticks were kept in perforated plastic bottles with labels to allow for air and moisture exchange. The ticks were then brought to the Chemotherapy Laboratory, Department of Parasitology, University of Agriculture, Faisalabad, Pakistan. An identification guidebook was used for the identification of ticks based on morphological traits (Estrada-Peña *et al.*, 2017).

Chemical composition of essential oils by gas chromatography-fluorescent

Ionization detection (GC-FID)

GC-FID technique was performed for the evaluation of the composition of *A. subulatum*. This technique was performed at Central Hi-tech Laboratory, University of Agriculture Faisalabad, Pakistan, using a GC-17 SHIMADZU® spectrophotometer. The columns were adjusted at DB WEX 30M 0.25, and nitrogen was used as a mobile phase at the flow rate of 20mL/min. Adjustment of temperature was done at 90°C for 120 sec, then at 180°C for the same time and then at 240°C for 180 sec. The observations were recorded and maintained accordingly. The compositions verified the essential oil's purity, and the active compounds present in them (Zhao *et al.*, 2021).

Experimental design

In vitro acaricidal activity of *A. subulatum* essential oils was performed by preparing different dilutions. For this purpose, 0.31, 0.62, 1.25, 2.50, and 5% dilutions of *A. subulatum* essential oil were prepared with absolute alcohol (volume/volume; v/v), for adult immersion, larval immersion, egg hatch and tick repellency assays. For the adult immersion, larval immersion and egg hatch tests, absolute alcohol was maintained as negative control and 0.1% cypermethrin (Alphakill® by Agrichem Pharmauticlas, China) was used as a positive control. While for the acaricidal assay, N, N-diethyl-meta-toluamide (Pakistan Chemicals®; DEET) was used as positive control for the repellent assay and absolute alcohol remained as negative control. All the groups in all the test were replicated thrice for each test.

Adult immersion test

The Drummond *et al.* (1973) methodology was adhered to for adult mortality. Ten female ticks were initially weighed and immersed in various A concentration in accordance with this methodology five min with essential oil of subulatum. Ticks were dipped, then put in petri dishes with moist filter sheets and in a biological oxygen demand incubator set to maintain a temperature of 27°C and a humidity of 90% for a full day. Subsequently, the adult mortality was determined by counting both dead and live female ticks. After receiving dilutions, live female ticks were placed in perforated Eppendorf tubes and incubated for 20 days at 90% humidity and 27°C. Tick females deposited their eggs in Eppendorf tubes. To calculate reproductive parameters, egg mass and the residual weight of each individual female tick were recorded. For every concentration, the experiment was run three times, with a positive and a negative control. In this test, the following parameters were noted:

Adult mortality

Adult mortality was performed according to Drummond *et al.* (1973) guideline sand calculated according to the following formula:

$$\text{Mortality (\%)} = \frac{\text{Number of dead ticks}}{\text{Total number of ticks}} \times 100$$

Reproductive Index (RI (%))

RI (%) was calculated Toro-Ortiz *et al.* (1997) using the following formula:

$$\text{RI (\%)} = \frac{\text{Weight of eggs laid (mg)}}{\text{Weight of adult females before treatment (mg)}} \times 100$$

$$\text{REI (\%)} = \frac{\text{egg mass weight} \times \text{\% egg hatching}}{\text{engorged female tick weight}} \times 20000$$

Nutrient index (NI%)

NI% was calculated using the following formula:

$$\text{NI (\%)} = \frac{\text{mass egg weight (mg)}}{\text{initial female tick weight(mg)} - \text{residual weight of female tick}} \times 10$$

Inhibition of oviposition (IO%)

IO% was calculated by using the following formula:

$$\text{IO (\%)} = \frac{\text{IE control} - \text{IE treated}}{\text{IE control}} \times 100$$

$$\text{PE} = \frac{\text{RE control} - \text{RE treatment}}{\text{RE control}} \times 100$$

Where IO and PE represented the inhibition of oviposition and product effectiveness, respectively.

Larval immersion test

The larval immersion test was used to calculate the effectiveness of *A. subulatum* essential oil on the larvae of *Hyalomma* spp. modified by Rosado-Aguilar *et al.* (2010). Tick larvae (7–14 days old) were utilized in this investigation. All the treatments and control groups were added into micro-centrifuge tube and 100 larvae were kept in each tube. After that, each tube was closed tightly and shaked vigorously for 5 sec and then gently for 5 min. Tubes were then opened and all larvae were shifted over filter paper to dry. These filter papers were then placed in an incubator for 24 h at 27°C and 90% relative humidity. Data were collected by counting dead and alive larvae with the help of a stereomicroscope (Rosado-Aguilar *et al.*, 2010).

$$\text{Larval mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae}} \times 100$$

Egg hatchability test

In glass tubes, 300 *Hyalomma* eggs were submerged for 5 min in 5mL of *A. subulatum* essential oil. After decanting the solutions, the tubes were sealed with cotton plugs and incubated for roughly 14 days at 27 to 28°C and a relative humidity of 70 to 80%, until the eggs began to hatch. Ethanol was utilized as negative control and cypermethrin was used as positive control. There were three replications of each treatment. Larval hatchability and inhibition of larval hatchability percentages were recorded (Kaaya *et al.*, 1996).

$$\text{Hatchability (\%)} = \frac{\text{Number of eggs hatched}}{\text{total number of eggs}} \times 100$$

$$\text{Inhibition of Hatchability (\%)} = \frac{\text{Hc} - \text{Ht}}{\text{Hc}} \times 100$$

Where Hc and Ht represented the hatchability in control and treated groups, respectively.

Tick repellency assay

The vertical migratory kind of behavior of adult ticks, which was created and redesigned by Tabari *et al.* (2020), was utilized to evaluate repellant activity. For this purpose, ten ticks were observed to check the repellency behavior of *Hyalomma* ticks against *A. subulatum* essential oil with absolute alcohol as negative control and DEET as a positive control. The whole experiment was conducted at 27°C and 90% relative humidity. After one hour, the number of ticks above and below the filter paper strips were counted for 15 min and the percentage repellency was calculated.

$$\text{Repellency (\%)} = \frac{100 - \text{(mean number of ticks not repelled)}}{\text{mean number of ticks not repelled by control}} \times 100$$

Statistical analysis

Statistical analysis was done through one-way analysis of variance (ANOVA) and Tukey's test by Minitab software while Probit Analysis by IBM SPSS software by keeping 95% confidence level and considering the results to be non-significant when P>0.05.

Results and Discussion

Chemical composition of A. subulatum

In the essential oil of *A. subulatum*, 13 chemical compounds were found among which the Limonene was present in greater concentration. The compounds are represented in Table 1; the peeks in the GC-FID assay are given in Figure 1.

Table 1: Phytochemical composition of *A. subulatum* essential oil through gas chromatography-flame ionization detection.

Sr. No	Time of retention	Name of the component	Concentration (%)
1	2.317	Limonene	11.9
2	7.783	α-Terpinene	11.1
3	19.367	α- Terpnineol	10.8
4	5.150	α-Terpinolene	9.5
5	13.050	Sabinene	9.3
6	16.567	1- Carveol	8.9
7	22.567	α-Phellandrene	7.8
8	37.300	Linalool	4.7
9	40.550	Myrtenol	4.7
10	25.700	Nerolidol	4.6
11	32.967	β-Pinene	4.6
12	28.750	Terphenyl acetate	3.6
13	31.133	1, 8-cineole	2.7
14	1.433	Traces/noise	1.7

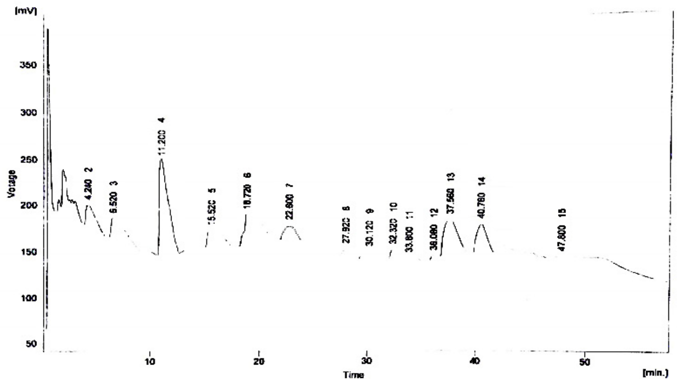

Figure 1: Gas chromatography-flame ionization detection resultant chromatogram showing peaks against the time.

Adult tick mortality

An adult immersion test was used to assess *A. subulatum's* acaricidal activity against adult Hyalomma ticks at five different concentrations (0.31, 0.62, 1.25, 2.50, and 5%). The outcome showed that 5% of *A. subulatum* exhibited a significant ($p<0.05$) acaricidal effects against adult ticks as compared to negative control. Table 2 shows the lethal concentrations, or LC_{50} and LC_{90}, that have also been found to be 2.80 and 21.11%, respectively. Probit analysis with a regression equation revealed a positive correlation between the concentration of *A. subulatum* essential oil and adult mortality. This showed that the concentration of essential oils increased together with an increase in adult mortality (Figure 2).

Table 2: Effect of various concentrations of *A. subulatum* essential oil against adults and larval stages.

Treatments	Adult tick mortality	Larval tick mortality
C1	6.66 ± 5.77^{e}	12.33 ± 3.05^{f}
C2	10 ± 0^{de}	21.33 ± 2.08^{f}
C3	23.33 ± 5.77^{cd}	31.33 ± 2.51^{d}
C4	36.66 ± 5.77^{cd}	45.33 ± 3.05^{c}
C5	53.33 ± 5.77^{b}	68.33 ± 4.04^{b}
CP	86.66 ± 5.77^{a}	90 ± 4.58^{a}
CN	6.66 ± 5.77^{e}	3.66 ± 0.57^{g}

C1, *A. subulatum* oil 0.31%; C2, *A. subulatum* oil 0.625%; C3, *A. subulatum* oil 1.5%; C4, *A. subulatum* oil 2.5%; C5, *A. subulatum* oil 5%; CN, Control Negative; CP, Control Positive. Mean±SD along with the same superscripts have a non-significant difference ($p>0.05$) from each other.

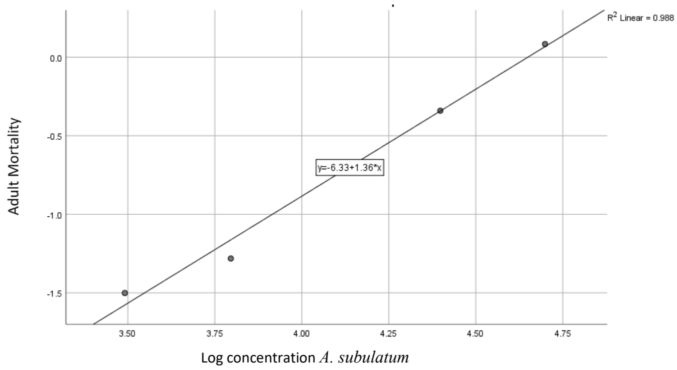

Figure 2: Dose-response model of *A. subulatum* essential oil against adults of *Hyalomma* spp.

Larval tick mortality

The larvicidal effect of *A. subulatum* essential oil at five different concentrations (0.31, 0.62, 1.25, 2.50, and 5%) was evaluated against the larvae of *Hyalomma* ticks by using a larval immersion test. Result revealed that 5% concentration of *A. subulatum* showed a significant ($p<0.05$) larvicidal effect against larvae of *Hyalomma* ticks. LC_{50} and LC_{90} values were also calculated which were 2.08 and 19.75%, respectively (Table 2). *A. subulatum* essential oil concentration and larval mortality were found to be positively correlated by probit analysis using a regression equation. This indicated that as essential oil concentration increased, adult mortality also did so, as illustrated in Figure 3.

Effect of various concentrations of A. subulatum essential oil on reproductive parameters and larval hatchability

There is evidence showing that the essential oil extracted from *A. subulatum* can decrease the *Hyalomma* tick's reproductive capabilities. At 5% concentration, *A. subulatum* essential oil reduced *Hyalomma* tick oviposition by 64.88%. Additionally, it induced larval hatching at 38% and inhibited larval hatching at 62%. As the concentration of *A. subulatum* essential oil increased, the effect on oviposition reduction increased, but the egg hatching rate had been dropped from 80 to 38% when $p< 0.05$. Data revealed that as the concentration of the essential oil grew, the rate of larval hatching dropped, which affected the reproductive index and, thus, the rate of reproduction, as illustrated in Table 3.

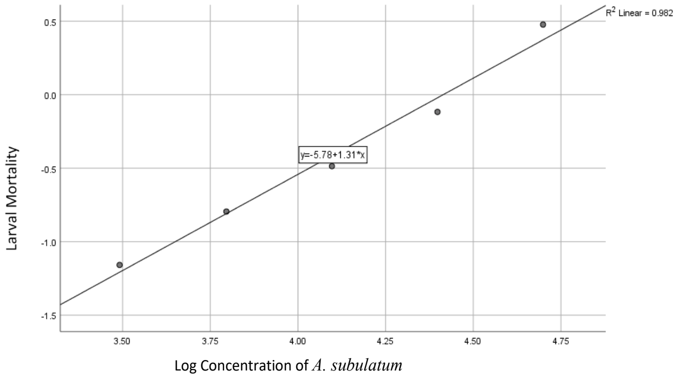

Figure 3: Dose-response model of *A. subulatum* essential oil against larvae of *Hyalomma* spp.

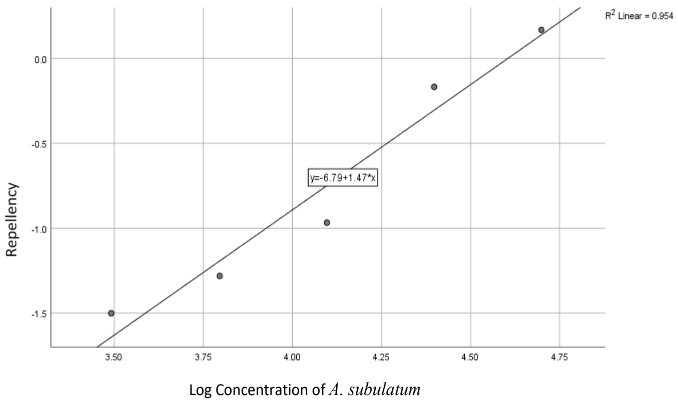

Figure 4: Dose-response model of *A. subulatum* essential oil against adults of *Hyalomma* spp.

Percentage repellency of Hyalomma ticks

As illustrated in Figure 4, all concentrations of *A. subulatum* essential oil showed significant ($p < 0.05$) results against repellency of *Hyalomma* ticks except 0.31% concentration. The highest activity was observed at 5% concentrations of *A. subulatum* essential oil. RC_{50} and RC_{90} were also calculated by using probit analysis with regression equation which showed 2.68 and 19.49% values.

Product effectiveness

The efficacy of a test substance against ticks was measured through the product effectiveness parameter. Different concentrations of *A. subulatum* essential oil were used and proved to have different results in relation to effectiveness against *Hyalomma* species. The 5% concentration showed the best results against *Hyalomma* ticks, which had non-significant ($p > 0.05$) results from the positive control (0.1% cypermethrin) as shown in Figure 5.

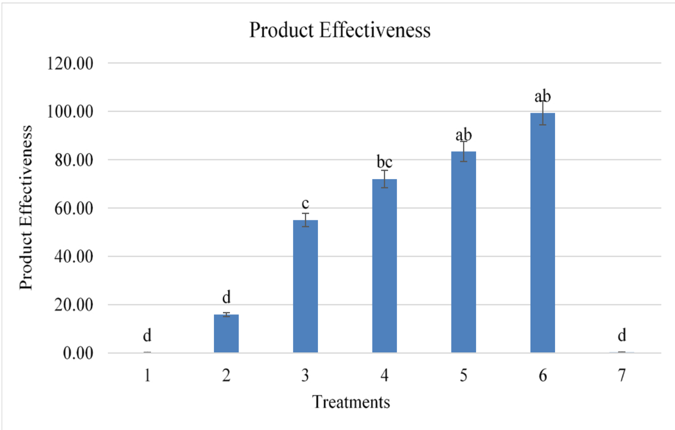

Figure 5: Product effectiveness of *A. subulatum* essential oil against *Hyalomma* spp. 1: *A. subulatum* oil 0.31%; 2: *A. subulatum* oil 0.62%; 3: *A. subulatum* oil 1.25%; 4: *A. subulatum* 2.50%; 5: *A. subulatum* oil 5%; 6: positive control; 7: negative control; Bars with the same superscript symbols differ non-significantly from each other (P>0.05).

Infestation of arthropods, especially arachnids, causes huge economic loss globally (Jabeen *et al*., 2022; Naseer *et al*., 2022; Mehnaz *et al*., 2023). The tick infestations have been controlled by synthetic acaricides over the years, but ticks have developed resistance, which led to the discovery of alternative methods of tick control, especially

Table 3: Effect of various concentrations of *A. subulatum* essential oil on reproductive parameters and larval hatchability.

Treatments	IO (%)	EH/LH (%)	ILH (%)	RI (%)	REI (%)	NI (%)
C1	0.49±8.29[d]	80.33±2.4[a]	19.66±2.4[d]	51.06±4.25[a]	81.9±4.48[a]	72.85±0.4[a]
C2	12.05±10.6[d]	76.55±1.0[a]	23.44±1.01[d]	45.13±5.44[a]	69.06± 7.96[a]	69.77±1.9[a]
C3	38.1±7.66[c]	57.55±9.82[b]	42.44±9.82[c]	31.76±3.93[b]	36.95±10.49[b]	61.47±2.6[b]
C4	47.21±7.4[bc]	42.11±5.09[c]	57.88±5.09[b]	27.09±3.8[bc]	23.01±5.87[bc]	57.86±2.9[b]
C5	64.88±2.57[b]	38±1.52[c]	62±1.52[b]	18.02±1.3[c]	13.67±0.44[cd]	47.23±2[c]
CP	95.41±1.7[a]	11.33±2.51[d]	88.66±2.5[a]	2.35±0.87[d]	0.55±0.28[d]	8.82±2.9[d]
CN	0±0.94[d]	80±1.52[a]	20±1.52[d]	51.31±0.4[a]	82.09±1.09[a]	73.02±0[a]

IO, Inhibition of oviposition; EH/LH, Egg hatchability/larval hatchability; ILH, Inhibition of larval hatchability; RI, Reproductive index; REI, Reproductive efficiency index; NI, Nutrient index; C1, *A. subulatum* oil 0.31%; C2, *A. subulatum* oil 0.625%; C3, *A. subulatum* oil 1.5%; C4, *A. subulatum* oil 2.5%; C5, *A. subulatum* oil 5%; CN, control negative; CP: control positive. Mean±SD along with the same superscripts have a non-significant difference (p>0.05) from each other.

botanical agents, including essential oil (Ibrahium *et al.*, 2022; Akhtar *et al.*, 2023). These essential oils are a volatile mixture of organic compounds that have a mechanism of action and target the ticks in a variety of ways (Faraone *et al.*, 2020; Catani *et al.*, 2022; Özüiçli *et al.*, 2023). *A. subulatum* essential oil was extracted by hydro-distillation technique and its composition was determined by GC-FID. The major components found in *A. subulatum* were limonene and α-terpinene. These compositions are like previous studies (Thinh *et al.*, 2021; Bhutia *et al.*, 2022) but variation in the quantity of different components is because of the various factors such as genotype, geographic conditions, harvest time, drying techniques, extraction techniques, and storage conditions (Li *et al.*, 2021; da Silva *et al.*, 2022; Jimayu, 2022). These factors have an impact on the content and composition of the essential oils (Abou Chehade *et al.*, 2022). The other GC-FID detected components along with their quantity in percentage and retention time are listed in Table 1. In the present study, different dilutions of *A. subulatum* essential oils were prepared in absolute alcohol. The response to adult and larval *Hyalomma* ticks was dose-dependent (Figure 2 and 3). In the mortality time graph, a significant (P<0.05) difference was observed between higher concentrations (2.5 and 5%) of *A. subulatum* and the negative control (absolute alcohol) and concluded that higher doses caused greater mortality of adult ticks and larvae. Alruhaili *et al.* (2023) reported that chemical constituents obtained from *A. subulatum* were very effective against insects, particularly *Tribolium castaneum*. In a similar study, *Syzygium aromaticum* essential oil had shown 100% tick mortality when it was used in 100mg/mL dose (Ferreira *et al.*, 2018). On the other hand, a dose-dependent response was also observed in terms of egg production, egg hatchability, inhibition of larval hatchability, reproductive index, reproductive efficiency, and nutrient index. Nutrient index value was also decreased when the concentration of *A. subulatum* increased. The acaricidal activity of tested essential oil may be because of the main component's limonene and α-terpinene which cause the inhibition of acetylcholinesterase enzyme activity in ticks (da Silva Lunguinho *et al.*, 2021) while other compounds such as α- phellandrene, linalool, β-pinene, and 1, 8-cineole have minor inhibitory activity, but they have synergistic action (Wojtunik-Kulesza *et al.*, 2019). The reduction in the egg numbers was because of the female mortality in the first few days after *A. subulatum* oil treatment. The reduction in oviposition led to a drastic reduction in reproductive index and reproductive efficiency of the *Hyalomma* ticks when concentrations of essential oil are increased. Essential oils induce cuticular waxes to break and plug the ticks' respiratory spiracles, which causes water stress and asphyxia (Al-Hoshani *et al.*, 2023b). Additionally, these oils enter the cuticle, diffuse into the haemolymph, and are then transported to internal organs including the ovaries and salivary glands, impairing the digestion and reproductive systems (Jesser *et al.*, 2017).

Like acaricidal activity, a dose-dependent response of *A. subulatum* against the repellency of *Hyalomma* ticks was observed for 5 different concentrations. 5% concentration of *A. subulatum* produced effective response against *Hyalomma* ticks and showed significant (p<0.05) difference from the DEET treatment as shown in Figure 4. RC_{50} and RC_{90} were also calculated by using probit analysis with regression equation which showed 2.68 and 19.49% values. The repellency of *A. subulatum* is due to the active component limonene and other active components present in essential oil. Terpinene and limonene in the essential oils have proven repellent efficacy against Ixodes ticks (da Silva Lunguinho *et al.*, 2021; Oladipupo, 2022) and these were major components of essential oil of *A. subulatum* in this study (Table 1). These volatile substances create vapor barriers, driving arthropods away from the essential oil and giving them repelling properties (Salman *et al.*, 2020).

Conclusions and Recommendations

The result of this study indicates that *A. subulatum* essential oil is a possible approach option for either eliminating or suppressing *Hyalomma* tick infestation. Further investigation is required to confirm these findings before recommending for commercial application. Moreover, methods to improve the longevity of essential oils as well as processes for improving oil yield following extraction needs to be improved.

Consent for publication

All authors are fine with the current version of the manuscript and give their consent for publication.

Ethical approval

Not applicable.

Conflict of interest

The authors have declared no conflict of interest.

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