



## ACARICIDAL EFFICACY OF NANO-ENCAPASULATED MENTHA PIPERITA ESSENTIAL OIL AGAINST RHIPICEPHALUS MICROPLUS: ECO-FRIENDLY ALTERNATIVE FOR TICK CONTROL IN LIVESTOCK

Shabab Ahmad<sup>1</sup>, Muhammad Oneeb<sup>2\*</sup>, Muhammad Lateef<sup>3</sup>, Muhammad Ijaz<sup>4</sup>, Muhammad Irfan Siddique<sup>5</sup>, Sajida Nawaz<sup>6</sup>

<sup>1,2\*,3</sup>Department of Parasitology, Faculty of Veterinary Sciences, University of Veterinary Sciences, Lahore, Pakistan

<sup>4</sup>Department of Veterinary Medicine, Faculty of Veterinary Sciences, University of Veterinary Sciences, Lahore, Pakistan

<sup>5</sup>Institute of Pharmaceutical Sciences, Faculty of Biosciences, University of Veterinary Sciences, Lahore, Pakistan

<sup>6</sup>Department of Physics, University of Engineering and Technology, Lahore, Pakistan/ Centre for Nanofibers and Nanotechnology, National University of Singapore

\*Corresponding author: Muhammad Oneeb (MO),

Department of Parasitology, Faculty of Veterinary Sciences, University of Veterinary Sciences, Lahore, Pakistan, Email: muhammad.oneeb@uvas.edu.pk (MO)

### Abstract

Ticks and tick-borne diseases (TTBDs) are among the global health challenges. The irrational use of synthetic chemicals poses serious threats in terms of toxicity, environmental hazards and resistance. The use of essential oils as an augmentative approach can be highly important for combatting this issue. However, their volatile nature, low stability and direct exposure to extreme environmental conditions compromise their efficacy. The use of suitable polymers to encapsulate essential oils is one way forward. *Mentha (M.) piperita* essential oil (EO) is a possible alternative for the control of TTBDs. The aim of this study was to evaluate the *in vitro* acaricidal efficacy of chitosan (CS)-encapsulated *M. piperita* EO against *R. microplus*. The *M. piperita* EO was encapsulated in Preparation of CS nanoparticles (NPs) following emulsification/ionic gelation. The adult immersion bioassay was employed to analyse the acaricidal activity of encapsulated *M. piperita* EO (8 mg/ml to 0.5 mg/ml). Furthermore, the toxicity of *M. piperita* EO NPs against non-target species was evaluated by three tests including: Acute dermal irritation test, acute dermal toxicity study and skin sensitization test. It exhibited excellent acaricidal activity against *R. microplus*, the efficacy of encapsulated *M. piperita* EO was the highest, increasing up to 100% (RC<sub>50</sub>=0.877, RC<sub>90</sub>=5.231) at the highest concentration. Similarly, the inhibition of oviposition was maximum at highest concentration. While, hatching rate of laid eggs increased with the decrease of concentration. Moreover, the *M. piperita* EO NPs were found nontoxic for non-target species. The present study provides the first description of the acaricidal activity of nanoencapsulated *M. piperita* EO against adult *R. microplus*, which is an eco-friendly alternate control strategy for ticks.

**Keywords:** *Rhipicephalus microplus*. *Mentha piperita*. Nanoencapsulation. acaricidal activity. Toxicity evaluation

## Introduction

The widespread distribution of parasitic diseases is a global issue that is thought to be an important challenge to the overall health and productivity of animals (1). Livestock production, with around 1.49 billion cattle globally, is the most significant and highly profitable economic industry (2). *Rhipicephalus* (*Boophilus*) *microplus*, bovine tick, is the well-known ectoparasite with the highest global economic significance for livestock (3). The feeding behavior of the tick, including blood loss, and stress irritability leads to decreased production of milk and meat, weakened immune system, and damage to skin and leather. Moreover, they can spread a variety of diseases, theileriosis, babesiosis, and anaplasmosis, in the animals leading to morbidity and mortality (4-7).

The use of synthetic chemical acaricides has long been regarded the primary approach of tick management(8). Most commercial ectoparasiticides, including pyrethroids, organochlorines, and isoxazolines are used in tick control approaches (9, 10). However, its negligent application leads to adverse impacts on hosts and high expenses for farmers (11, 12). Usually, application does not adhere to basic safety guidelines and dosages are more than prescribed, which results in a decrease in effectiveness (13). Furthermore, drug residues harmful to human health may be found in meat and milk (14).

Alternative control strategies, especially plant-derived products are significant that are less toxic to hosts, more accessible, and less expensive because they typically possess acaricidal properties (15). A variety of essential oils (EOs) are effective acaricidal options because of their direct actions, breakdown, and low degree of toxicity to mammals (16). EOs are composed of terpenes, terpenoids, and phenylpropanoids which are volatile chemicals with a variety of therapeutic uses. EOs and their constituents may be extracted from plants and their applications in veterinary (17). Therefore they can be used as an alternative of the synthetic products that already exist in the market. Meanwhile, bio-insecticides especially, essential oils which are comprised of hundreds of compounds should be used in a sensible way because they may cause harm to non-target species when used topically (18-20). Hence, it is necessary to evaluate the toxicity of bio-insecticides against non-target species.

A common taxonomic group in the Mediterranean flora is the genus *Mentha* (21). Usually, the aerial portions of the flowering plant undergo steam distillation to extract the essential oil of *M. piperita* (22). Menthol, menthon, carvon and limonene are the major constituents of *M. piperita* EO (23, 24). It has been one of the most widely utilized aromatic plant for medicinal uses (25, 26). Moreover, it has been observed to have cytotoxic, insecticidal, nematicidal, antifungal, and antibacterial properties (27-30). Furthermore, several studies have confirmed the acaricidal effectiveness of its EO against ticks (31, 32). But its effectiveness is compromised due to the volatile nature of essential oils and harsh environmental conditions (33).

To combat with the concern, nanobiotechnology enhance can improve the medicinal efficacy of traditional bio-pesticides by enhancing their bioavailability in various ways against the targeted species. As a result, a variety of novel nano-products, such as nanoparticles, nanofibres, nanoemulsions, and nanocapsules have been developed using nanotechnology in many disciplines (34-36). Nano encapsulation increase the duration of its availability and improve efficacy. The oil's volatility is considerably reduced by encasing it in nanoparticles, ensuring a more lasting effect (37). This improvement in delivery methods not only makes *M. piperita* more effective, but it also makes it safer and more suitable for use in a range of situations. Therefore, *M.piperita* EOs nanoencapsulation offers a viable approach to the ongoing fight against illnesses spread by ticks. Its effectiveness and longer lifespan indicate a significant breakthrough in integrated pest management techniques.

However, its acaricidal activity against the cattle tick has not yet been demonstrated to date. Therefore, the present work is aimed to check the acaricidal efficacy of *M.piperita* EO nanoparticles and their toxicity evaluation against non-target species for topical application to provide an effective, greener and safer acaricidal product against *R.microplus* ticks.

## 2. Materials and Methods

### 2.1. Extraction of Essential oil

The procured *M. piperita* herbs were sent for taxonomic identification to Government College University's Botany Department, Lahore, Pakistan. Herbs were cleaned and allowed to air dry followed by steam-distillation to obtain the *M. piperita* EO (38).

### 2.2. Nanoparticle synthesis

*M. piperita* EO NPs were synthesized using the technique adopted by (40) with minor modifications. Briefly, chitosan (CS) solution (1% w/v) was prepared in glacial acetic acid, followed by addition of Tween 80 and *M. piperita* EO in dichloromethane. Emulsification was done using a rotor-stator homogenizer (D-500, DLAB, US), followed by ionic gelation with TPP solution. The resulting emulsion was centrifuged, washed, and pH-adjusted before freeze-drying. Lastly, the resulting solution was then freeze-dried for 72 hours at  $-65^{\circ}\text{C}$  using a Freeze Dryer (Labconco Corporation, USA). The NPs were subsequently preserved in the refrigerator for further bioassay.

### 2.3. Collection and Identification of Ticks

Engorged female *Rhipicephalus microplus* ticks from infested animals were collected and transported to the Entomology laboratory, Department of Parasitology, University of Veterinary and Animal Sciences, Lahore, Pakistan. Only ticks with an undamaged body parts and the ability to move were picked for the experiment. The ticks were thoroughly washed with tap water and dried on filter paper towel. Taxonomic keys of Aydin (1994, 2000) were used to identify *R. microplus* ticks. They were incubated at 27 to 28  $^{\circ}\text{C}$  and 80–85% relative humidity for further bioassay (41–43).

### 2.4. Adult immersion test (AIT)

The procedure was performed as described by (44–46) with slight modifications. The acaricidal efficacy of five concentrations of *M. piperita* EO NPs and pure (8 mg/ml, 4 mg/ml, 2 mg/ml, 1 mg/ml, and 0.5 mg/ml) was assessed by immersing adult ticks in the mixtures. Trichlorophon and distilled water (DW) were served as control positive and negative respectively. In total twelve (12) groups were formed for the experiment. All concentrations were administered to the relative groups. Ten engorged ticks were chosen for each group and there were three replicates of each treatment. Treated ticks were placed in an incubator under controlled environmental conditions and mortality was observed after 14 days of treatment. Reproductive index inhibition of oviposition were calculated through following formula and the hatching rate was visually observed after 30 days of treatment (44, 47).

$$\text{Reproductive Index(RI)} = \frac{\text{Average weight of eggs laid (mg)}}{\text{average weight of live ticks (mg)}}$$

$$\text{Inhibition of oviposition (IO) (\%)} = \frac{\text{RI of control ticks} - \text{RI of treated ticks}}{\text{RI of control ticks}} \times 100$$

### 2.5. Toxicity evaluation against non-targeted species

The toxic effects of *M. piperita* EO NPs against non-target species were determined through skin sensitization, acute dermal toxicity, and acute dermal irritation assays. The tests were carried out in conjunction with Tara Laboratories (ISO 17025-2017) Lahore, Pakistan (OECD, 2015, 2017, 2021).

#### 2.5.1. Acute Dermal Irritation Test

Three healthy albino rabbits, of 2.5 kg each, were used in the investigation. The animals were weighed, given an 8-day acclimatization period, retained in pre-labelled cage. The animals were given the test formulation (*M. piperita* EO NPs) at a dosage of 0.5gm, and they were subjected to a

12-hour cycle of light and dark. Every rabbit's skin area was measured twice a day, and the test formulation was assessed at prearranged intervals (Figure 1). Table 1 provides reference criteria for evaluation. After the patch was removed, the data were examined 24, 48, and 72 hours (48).



**Figure 1.** Acute dermal irritation test procedure

**Table 1:** Erythema and oedema formation score card

Erythema/Eschar Formation		Oedema Formation	
No Erythema	0	No Oedema	0
Very slight Erythema (barely perceptible)	1	Very slight Oedema	1
Well defined Erythema	2	Slight Oedema	2
Moderate to severe Erythema	3	Moderate Oedema (approximately 1 mm)	3
Severe Erythema	4	Severe Oedema (more than 1mm )	4

### 2.5.2. Acute Dermal Toxicity Study

The healthy adult Wistar albino rats, acclimatized to the study conditions for a week. The animals were divided into three groups: group I (200 mg/kg), group II (1000 mg/kg), and group III (2000 mg/kg). The *M. piperita* EO nanoformulation was applied to the shaved area in the groups, and the animals were observed for 24 hrs. Changes in skin, behavioral patterns, eyes, salivation, diarrhea, and mucous membranes were observed. Mortality was observed during the study duration (Figure 2). The surviving animals were weighed, sacrificed, and subjected to necropsy. Standard table provides the GHS criteria used to evaluate the toxicity (49).



**Figure 2.** Acute dermal toxicity study procedure



### 2.5.3. Skin Sensitization Test

The skin sensitization test was conducted on 20 Guinea pigs, following OECD (406) guidelines. The animals were housed at  $20 \pm 3$  °C and 30-70% humidity with an artificial light interval of 12 hours light and 12 hours dark. The concentration of the test chemical *M. piperita* EO NPs used for each induction exposure was kept the highest (17%) to cause mild irritation. The test was performed on the same test surface on days 6-8 and 13-15. On days 27-29, an occlusive patch containing 6% of the test nanoformulation was applied to the posterior untreated flank of both treated and control group animals. The patches were dressed for 6 hrs. The skin reactions were observed and recorded approximately 30 hrs after the application of the challenge patch. About 24 hours after the 30hr observation period, skin reactions were once more noted and examined according to the grades (Figure 3) Changes in weight at the end of the experiment were also recorded.



**Figure 3.** Acute dermal irritation test procedure

### 2.6. Statistical Analysis

Three replicate experiments were performed, and the data are presented as the means  $\pm$  SEs. Lethal concentrations ( $LC_{50}$  and  $LC_{90}$ ), along with their confidence limits for pure *M. piperita* EO and *M. piperita* EO NPs, were determined via probit analysis using SPSS (20.0).

## 3. RESULTS:

### 3.1. Adult Immersion Test

The criteria for evaluating the anti-tick activity of the *M. piperita* NPs in the current study was its effect on the mortality percentage of adult ticks, egg laying, egg hatching, reproductive index (RI), and hatching percentage of eggs. The percentage inhibition of oviposition (IO) % and reproductive index (RI) were computed for various doses and treatments. The concentrations comprised Trichlorfon and distilled water (DW) controls in addition to varying quantities of *M. piperita* EO NPs ranging from 8 mg/ml to 0.5 mg/ml. Assessments of RI and IO% provide clarification on how the interventions affect reproduction. Increased IO% and lower RI were often correlated with higher concentrations, indicating possible impairment of reproductive processes. Differential efficacy was shown by the variation in mortality % among concentrations and treatments (Table 2). The Probit analysis was performed to determine the  $LC_{50}$  and  $LC_{90}$  values. The  $LC_{50}$  was 0.877, and the  $LC_{90}$  was 5.231 (Table 3).

**Table 2.** Mean values ( $\pm$  standard error) of live tick weight, egg weight, mortality percentage, RI, IO%, and hatching rate across different concentrations.

Concentration	Live tick wt.mg	Mortality (%)	Egg wt (mg)	RI	IO(%)	Hatching rate (%)
	(Mean $\pm$ SE)					
8 mg/ml <i>M. piperita</i> EO NPs	42.06 $\pm$ 3.104	100	0	0	0	0
4 mg/ml <i>M. piperita</i> EO NPs	37.19 $\pm$ 1.428	80	10.06 $\pm$ 3.405	0.27	61.64	0

2 mg/ml <i>M. piperita</i> EO NPs	41.86± 4.62	70	12.05±3.725	0.316	53.31	0
1 mg/ml <i>M. piperita</i> EO NPs	37.43±1.984	50	18.03±4.40	0.372	47.15	5
0.5/mg <i>M. piperita</i> EO NPs	34.57±3.524	40	19.38±4,149	0.442	37.21	45
Trichlorfon	32.03±1.914	100	0	0	0	0
DW	27.15±1.66	10	19.14±2.483	0.704	0	100

**Table 3.** LC value of *M. Piperita* EO NPs with 95 % confidence interval against *R. microplus*

Compound	LC <sub>50</sub> value with 95% confidence interval			LC <sub>90</sub> CI value with 95% confidence interval		
	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
<i>M. piperita</i> EO NPs	0.877	0.247	1.525	5.231	2.693	52.869

### 3.2. Toxicity Analysis

#### 3.2.1. Acute Dermal Irritation Test

No redness (erythema) or edematous lesions were observed at any interval in the three rabbits compared with those in the control group (Table 4).

**Table 4:** Erythema and oedema formation at different time intervals in experimental rabbits

Animal ID	Grading and time interval		
	24 hrs	48 hrs	72 hrs
1	0	0	0
2	0	0	0
3	0	0	0

#### 3.2.2. Acute Dermal Toxicity Test

All three groups treated with *M. piperita* EO NPs at all concentrations showed no mortality. Furthermore, no clinical findings were observed in any of the treated groups of animals. No necropsy findings were observed. Throughout this period, body weight changes (10±5 g) were observed in the experimental animals. Thus, the total DT score of the test formulation was 0, and our formulation was deemed nontoxic for dermal use (Table 5).

**Table 5:** Results of acute dermal toxicity of *M. piperita* EO NPs in Wister rats

Sr. no	No of animals exposed	Dose level mg/kg	Mortality	Clinical findings	Necropsy findings	Body weight changes (g)
1	2	200	0	No	Nil	10±5
2	2	1000	0	No	Nil	10±5
3	2	2000	0	No	Nil	10±5

#### 3.2.3. Skin Sensitization Test

No clinical findings were observed in the treated area, and 10±5 g changes in the test animals were noted. The results mentioned below depict the use of the *M piperita* EO nanoformulation as a nonsensitizer.

**Table 6:** Mean erythema score on different days

Patch No.	Mean Erythema Score	
	Incidences of positive effect/animal	
	Control	Experimental group
1(Day1)	0	1
2(Day6)	0	1
3(Day 3-15)	0	1
4(Day27)	0	0

### Discussion:

The uncontrolled application of chemical control agents to eradicate ticks, particularly *R. microplus*, has resulted in the emergence of resistant strains. For this reason, it's essential to search for alternatives that keep ticks under control. Among various control measure, plant extracts are a possible source of novel acaricides because of their excellent efficacy and safety.

Numerous studies have been conducted on essential oils as substitutes for control vectors. The objective of this study was to determine the efficacy of active component for the development of a novel acaricidal product for the control of *R. microplus* by evaluating the acaricidal activity of *M. piperita* EO NPs against engorged female ticks.

*M. piperita* is a medicinal plant that has been examined in extensive detail across the world (50). It is rich in a variety of physiologically active chemicals and has been tested for insecticidal and acaricidal properties (51, 52). In a previous research, experimentally infected cattle were treated with 2% peppermint oil (EO), 10% rosemary oil (EO), and 5% geraniol (EO) ,21 days later, a response rate of 70% was observed against engorged female *R. microplus* (53).

Similarly, excellent acaricidal activity against *R. microplus* was demonstrated by the essential oils of *Backhousia citriodora*, *Callistemon viminalis*, and *Cinnamodendron dinisii*. Of these, the EO of *B. citriodora* had the highest acaricidal activity, followed by that of *C. dinisii* and *C. viminalis* (54). However, there are several studies in the literature on the Lamiaceae family. The acaricidal activity of essential oils from *Thymbra sintenisii* Bornm. & Azn. subsp. isaurica P.H. Davis (TSI) and *Thymbra sintenisii* Bornm. & Aznav. sub sp. sintenisii Bornm. & Aznav (TSS) species have shown effectiveness against *Hyalomma marginatum* ticks.

These oils may be helpful in controlling ticks also have ability to create nanoparticles when combined with AgNO<sub>3</sub>, which was also assessed and compared (55). In this study, the *M. piperita* EO NPs showed varying mortality, from 8 mg/ml (100%), 4 mg/ml (80%), 2 mg/ml (70%), 1 mg/ml (50%) and 0.5 (40%) which shows potential acaricidal activity. Results reveal higher mortality of ticks in high doses.

As well as, our studies show remarkable effects on hatching rate (%), RI and IO (%).In the present study, 8, 4 and 2 mg/ml concentrations of *M. piperita* EO NPs show 0% hatching, which represents excellent results, as the concentration decreases and hatching increases gradually.

The acaricidal activity of *Hesperozygis myrtoides* oil was evaluated by different concentrations (mg/ml) of *H. myrtoides* oil used in AIT on *R. microplus* which results in the concentration decreases in the percentage of egg hatching (% hatching), reproductive efficiency index (REI) and egg weight increase gradually (56).

In this research, *M. piperita* EO Nanoparticle formulations show brilliant results in the inhibition of oviposition, the concentrations have a direct relationship with inhibition; the highest concentration of 4mg shows 61.64 % inhibition; as the concentration decreases, the IO percentage decreases.

A study examines the effectiveness of hydroethanolic and hexanic extracts from *Randia aculeata*, *Moringa oleifera*, and *Carica papaya* against *R. microplus* ticks. *R. aculeata* seed extracts caused the most mortality (55-85.5%) and prevented egg hatching, but *M. oleifera* and *C. papaya* had lesser effectiveness. These extracts significantly reduced tick mortality, egg laying, and overall reproductive efficiency (57).

Our results showed that the topical application of this nanoformulation in experimental animals was not toxic. Additionally, we did not observe any dermal reactions after the nanoformulations were applied to the skin of the experimental animals. Researchers have shown that nanoformulations containing natural products have no dermal toxicity (58). Similar results were shown by (59), in which researchers found the peppermint nanoemulsion nontoxic.

Similarly, the acute dermal irritation and acute dermal toxicity test results also revealed that the drug was not sensitive. This difference might be due to the encapsulation of *M. piperita* EO NPs, which results in the slow and controlled release of entrapped material (60).

For a toxicity analysis, the toxicity study of *M. piperita* EO NPs did not show any signs of irritation in acute skin irritation tests performed on rabbits over a 24- to 72-hour period. Moreover, acute cutaneous toxicity studies at several doses (200 mg/kg, 1000 mg/kg, and 2000 mg/kg) on rats revealed no mortality or adverse effects, indicating the safety of *M. piperita* EO NPs for topical administration. Experiments on skin sensitization verified that *M. piperita* EO NPs are safe to use without causing significant skin irritation or sensitization, and they further supported this by demonstrating little erythema. Additionally, to ensure the safety of *M. piperita* EO NPs in livestock, field experiments are required for the study's toxicity evaluations that concentrated on non-target species.

### Conclusion

In conclusion, EO nanoformulations are gaining much attention as alternate control strategy for insects. In this study, *M. piperita* EO nanoformulation was prepared using two-step ionic gelation technique. Additionally, *M. piperita* EO NPs have been identified to possess a stronger acaricidal activity against *R. microplus* ticks. Furthermore, they were found non-toxic for non-target species. This attempt enhances our knowledge on development of an innovative design and safer *M. piperita* EO based nano-acaricidal for *R. microplus* ticks control.

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### Author Contributions

Shabab Ahmad conducted the experiments and wrote the initial draft of this manuscript. Muhammad Oneeb designed the research, supervised the study and edited the manuscript draft. Muhammad Lateef assisted in performing the repellent bioassay, while Muhammad Ijaz helped in tick collection and rearing. Muhammad Irfan Siddique and Sajida Nawaz assisted in nanoformulation.

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### Declarations

**Competing Interests** The authors declare that they have no conflict of interest of any sort. **Ethics approval** The study was approved by the Research Ethics Committee of University of Veterinary and Animal Sciences, Lahore, Pakistan (DR. number 400, September 30, 2021). All applicable International, National, and/or Institutional guidelines for the care and use of animals have been followed.

### 4. References

1. Nepveu-Traversy M-E, Fausther-Bovendo H, Babuadze GJV. Human Tick-Borne Diseases and Advances in Anti-Tick Vaccine Approaches: A Comprehensive Review. 2024;12(2):141.



2. Reis AC, Konig IFM, Mendonça GHA, Lunguinho AdS, Cardoso MdG, Remedio RNJE, et al. Toxic effect of *Callistemon viminalis* essential oil on the ovary of engorged *Rhipicephalus microplus* female ticks (Acari: Ixodidae). 2023;90(1):99-117.
3. Cruz RR, García DID, Silva SL, Domínguez FR. Integrated management of the cattle tick *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) and the Acaricide resistance mitigation. *Insecticides-Impact and Benefits of Its Use for Humanity*: IntechOpen; 2021.
4. Jain P, Satapathy T, Pandey RKJB, biotechnology a. *Rhipicephalus microplus*: A parasite threatening cattle health and consequences of herbal acaricides for upliftment of livelihood of cattle rearing communities in Chhattisgarh. 2020;26:101611.
5. Jamil A, Yu Z, Wang Y, Xin Q, Gao S, Abdul Wahab M, et al. Tick-borne Rickettsia, Anaplasma, Theileria, and enzootic nasal tumor virus in ruminant, PET, and poultry animals in Pakistan. 2024;15:1359492.
6. Kebede A, Mitiku A, Tilahun A, Oli Y, Gutata MJW. Prevalence of Ixodidae Ticks and its Associated Risk Factors in Local Breed (Zebu) Cattle at Mao Komo Special District Benishangul Gumuz Regional States, Western Ethiopia. 2024;12:1-7.
7. Tagel W. Prevalence and Associated Risk Factors of Ixodid Ticks Infestation And Tick Borne Haemoparasites of Small Ruminants In Enarje Enawuga District, North Western Ethiopia 2022.
8. Reshma KR, Prakasan KJE, Letters AS. Synthetic Acaricides As A Promising Tool in Tick Control Program-The Present Scenario. 2020;7(2-2020):58-69.
9. Stafford KA, Coles GCCJADRMoDR, Volume 1. Drug resistance in ectoparasites of medical and veterinary importance. 2017:735-44.
10. Gonçalves IL, das Neves GM, Kagami LP, Eifler-Lima VL, Merlo AAJB, Chemistry M. Discovery, development, chemical diversity and design of isoxazoline-based insecticides. 2021;30:115934.
11. Rodriguez-Vivas RI, Jonsson NN, Bhushan CJPr. Strategies for the control of *Rhipicephalus microplus* ticks in a world of conventional acaricide and macrocyclic lactone resistance. 2018;117:3-29.
12. Pelletier J, Rocheleau J-P, Aenishaenslin C, Dimitri Masson G, Lindsay LR, Ogden NH, et al. Fluralaner baits reduce the infestation of *Peromyscus* spp. mice (Rodentia: Cricetidae) by *Ixodes scapularis* (Acari: Ixodidae) larvae and nymphs in a natural environment. 2022;59(6):2080-9.
13. Ren Y, Dou W, Wang JJ, Yuan GJPMS. Lethal and sublethal effects of fluralaner on the citrus red mite, *Panonychus citri* (McGregor). 2024.
14. van Asselt E, Pikkemaat M, Jansen L, Hoek-van den Hil E. Prioritisation of chemical substances for national monitoring: Applied to antibiotics, antiparasitics, carbamates and NSAIDs in bovine, porcine and poultry products. RIKILT Wageningen University & Research; 2019.
15. Rodrigues L, Giglioti R, Morita Katiki L, Franceschini Sarria AL, José Veríssimo CJIJoA. Screening of volatile compounds of essential oils with acaricidal activity in *Rhipicephalus microplus* larvae using a new in vitro evaluation method. 2024:1-9.
16. Valcárcel F, Olmeda AS, González MG, Andrés MF, Navarro-Rocha J, González-Coloma AJFiA. Acaricidal and insect antifeedant effects of essential oils from selected aromatic plants and their main components. 2021;3:662802.
17. Štrbac F, Krnjajić S, Stojanović D, Ratajac R, Simin N, Orčić D, et al. In vitro and in vivo anthelmintic efficacy of peppermint (*Mentha x piperita* L.) essential oil against gastrointestinal nematodes of sheep. 2023;10.
18. Isman MBJPr. Commercial development of plant essential oils and their constituents as active ingredients in bioinsecticides. 2020;19:235-41.
19. Assadpour E, Can Karaça A, Fasamanesh M, Mahdavi SA, Shariat-Alavi M, Feng J, et al. Application of essential oils as natural biopesticides; recent advances. 2023:1-21.

20. Shan MI, Ali RMM, Zeshan A, Bilal S, Shakoor K, Riaz S, et al. ESSENTIAL OILS AS BIOPESTICIDES IN PEST MANAGEMENT: SAFER ALTERNATIVES TO SYNTHETIC PESTICIDES.
21. Wu Z, Tan B, Liu Y, Dunn J, Martorell Guerola P, Tortajada M, et al. Chemical composition and antioxidant properties of essential oils from peppermint, native spearmint and scotch spearmint. 2019;24(15):2825.
22. Katekar VP, Rao AB, Sardeshpande VRJSC, Pharmacy. A hydrodistillation-based essential oils extraction: A quest for the most effective and cleaner technology. 2023;36:101270.
23. Karpiński TMJB. Essential oils of Lamiaceae family plants as antifungals. 2020;10(1):103.
24. Hudz N, Kobylinska L, Pokajewicz K, Horčinová Sedláčková V, Fedin R, Voloshyn M, et al. *Mentha piperita*: essential oil and extracts, their biological activities, and perspectives on the development of new medicinal and cosmetic products. 2023;28(21):7444.
25. Mahendran G, Rahman LUJPR. Ethnomedicinal, phytochemical and pharmacological updates on Peppermint (*Mentha*× *piperita* L.)—A review. 2020;34(9):2088-139.
26. Sartoratto A, Machado ALM, Delarmelina C, Figueira GM, Duarte MCT, Rehder VLGJBoM. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. 2004;35:275-80.
27. Al-Marby A. Nematicidal and antimicrobial evaluation of extracts, nanosized materials and fractions of selected plants and the identification of the bioactive phytochemicals. 2017.
28. Rodrigues MMG. Control of the Root-Knot Nematode, *Meloidogyne Incognita*, Using Plant Bioproducts: Universidade do Minho (Portugal); 2022.
29. Pereira BDF. Activity of essential oils and hydroalcoholic extracts from 12 plants against food spoilage yeasts: evaluation of their potential as new biopesticides: Universidade do Minho (Portugal); 2021.
30. Raveau R, Fontaine J, Lounès-Hadj Sahraoui AJF. Essential oils as potential alternative biocontrol products against plant pathogens and weeds: A review. 2020;9(3):365.
31. Noaman V, Bahreininejad BJJoa-PE. Evaluation of the acaricide activity of extracts from eight species of medicinal plants on larvae of *Hyalomma anatolicum* (Acari: Ixodidae) ticks. 2024:102231.
32. Salman M, Abbas RZ, Israr M, Abbas A, Mehmood K, Khan MK, et al. Repellent and acaricidal activity of essential oils and their components against *Rhipicephalus* ticks in cattle. 2020;283:109178.
33. Aljaafari MN, Yap PSX, Seboussi R, Lai KS, Lim SHE. The potential use of essential oils as natural biocides against plant pathogens. *Pesticides in the Natural Environment*: Elsevier; 2022. p. 317-28.
34. Shah MA, Wani SH, Khan AAJJoFB, Nanoprocessing. Nanotechnology and insecticidal formulations. 2016;1(3):285-310.
35. Soudy BA, Allam SF, Hassan M, Khalil N, Youssef DAJEJoC. A new philosophy in fabrication pesticides (Abamectin) and Essential oils (Orange oil) into nano-form. 2021;64(7):3951-9.
36. Radwan IT, Khater HF, Mohammed SH, Khalil A, Farghali MA, Mahmoud MG, et al. Synthesis of eco-friendly layered double hydroxide and nanoemulsion for jasmine and peppermint oils and their larvicidal activities against *Culex pipiens* Linnaeus. 2024;14(1):6884.
37. Machado S, Pereira R, Sousa RMOJSoTTE. Nanobiopesticides: Are they the future of phytosanitary treatments in modern agriculture? 2023:166401.
38. Muntean D, Licker M, Alexa E, Popescu I, Jianu C, Buda V, et al. Evaluation of essential oil obtained from *Mentha*× *piperita* L. against multidrug-resistant strains. 2019:2905-14.
39. Harke M, Somkuwar A, Dubey S, Limsay R, Umam S, Sawarkar A, et al. Qualitative and quantitative phytochemical evaluation of ethanolic extract of *Mentha piperita* (Linn.). 2021;10(5):996-1000.

40. Shetta A, Ali IH, Sharaf NS, Mamdouh WJJoBM. Review of strategic methods for encapsulating essential oils into chitosan nanosystems and their applications. 2024;129212.
41. Bandaranayaka K, Dissanayaka U, Rajakaruna RJCJoS. Experimental aspects of life cycles of two hard tick species, *Rhipicephalus (Boophilus) microplus* and *Hyalomma isaaci* (Acari: Ixodidae), on New Zealand white rabbits. 2021;50(1):47-56.
42. Udobi MI. Time Since Blood-feeding (hunger) in *Ixodes Ricinus* Ticks: Measurement and Consequences: University of Bristol; 2023.
43. Njoroge PW. Effect of East Coast Fever Marikebuni Vaccine on Feeding and Reproductive Success of *Rhipicephalus Appendiculatus*: University of Nairobi; 2023.
44. Shakya M, Singh M, Gautam K, Jamra S, Agrawal V, Jayraw A, et al. Assessment of Antitick and Repellent Activity of *Azadirachta indica* Oil against Adults and Larvae Stages of *Rhipicephalus microplus*. 2024;2024.
45. Youssef AA, Abdelgawad AA, Abdel-Ghany HS, Abdelshafy SJEJoVS. Chemical Composition of the Essential Oil from *Ruta chalepensis* L. Growing Wild and its Acaricidal Activity Against the Cattle Tick *Rhipicephalus annulatus* (Acari: Ixodidae). 2024;55(4):955-64.
46. Jain P, Satapathy T, Pandey RKJEP. *Rhipicephalus microplus* (acari: Ixodidae): Clinical safety and potential control by topical application of cottonseed oil (*Gossypium* sp.) on cattle. 2020;219:108017.
47. Dzemo WD, Vudriko P, Ramatla T, Thekiso OJP. Acaricide Resistance Development in *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) Populations against Amitraz and Deltamethrin on Communal Farms of the King Sabata Dalindyebo Municipality, South Africa. 2023;12(7):875.
48. OECD. Test No. 404: Acute Dermal Irritation/Corrosion2015.
49. OECD. Test No. 402: Acute Dermal Toxicity2017.
50. Rajput S, Sharma P, Malviya RJRAiFN, Agriculture. Utilization of Herbal Components as Insecticidal and Repellent Effects. 2023;14(3):144-54.
51. Tourabi M, Nouioura G, Touijer H, Baghouz A, El Ghouzi A, Chebaibi M, et al. Antioxidant, Antimicrobial, and Insecticidal Properties of Chemically Characterized Essential Oils Extracted from *Mentha longifolia*: In Vitro and In Silico Analysis. 2023;12(21):3783.
52. Kalembe D, Synowiec AJM. Agrobiological interactions of essential oils of two menthol mints: *Mentha piperita* and *Mentha arvensis*. 2019;25(1):59.
53. Klafke GM, Thomas DB, Miller RJ, de León AAPJT, Diseases T-b. Efficacy of a water-based botanical acaricide formulation applied in portable spray box against the southern cattle tick, *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae), infesting cattle. 2021;12(4):101721.
54. da Silva Lunguinho A, das Graças Cardoso M, Ferreira VRF, König IFM, Gonçalves RRP, Brandão RM, et al. Acaricidal and repellent activity of the essential oils of *Backhousia citriodora*, *Callistemon viminalis* and *Cinnamodendron dinisii* against *Rhipicephalus* spp. 2021;300:109594.
55. Kaya EC, Akdeniz M, Ugurlu P, Yener I, Firat M, Ertas AJJoEObP. Content analysis, nanoparticle forming potential and acaricidal effects on Tick (*Hyalomma marginatum*) of essential oils from two common *Thymbra* species. 2024:1-22.
56. Castilho CV, Fantatto RR, Gaínza YA, Bizzo HR, Barbi NS, Leitão SG, et al. In vitro activity of the essential oil from *Hesperozygis myrtoides* on *Rhipicephalus (Boophilus) microplus* and *Haemonchus contortus*. 2017;27:70-6.
57. Bravo-Ramos J, Flores-Primo A, Paniagua-Vega D, Sánchez-Otero M, Cruz-Romero A, Romero-Salas DJE, et al. Acaricidal activity of the hexanic and hydroethanolic extracts of three medicinal plants against southern cattle tick *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae). 2021;85(1):113-29.
58. Rai VK, Mishra N, Yadav KS, Yadav NP. Nanoemulsion as pharmaceutical carrier for dermal and transdermal drug delivery: Formulation development, stability issues, basic considerations and applications. *Journal of controlled release*. 2018;270:203-25.

59. Mohammadifar M, Aarabi MH, Aghighi F, Kazemi M, Vakili Z, Memarzadeh MR, et al. Anti-osteoarthritis potential of peppermint and rosemary essential oils in a nanoemulsion form: behavioral, biochemical, and histopathological evidence. *BMC Complementary Medicine and Therapies*. 2021;21(1):1-12.
60. Teshome K, Gebre-Mariam T, Asres K, Perry F, Engidawork E. Toxicity studies on dermal application of plant extract of *Plumbago zeylanica* used in Ethiopian traditional medicine. *Journal of ethnopharmacology*. 2008;117(2):236-48.