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Comparative Study of Two Monoterpenes Effect on *Rhipicephalus microplus* Tick

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Abstract

The cattle tick Rhipicephalus microplus is one of the most important ectoparasites for livestock in tropical and subtropical areas worldwide. This tick hurts the economy of the milk and meat production chain. In addition, it constitutes a vector for the transmission of Anaplasmosis and Babesiosis pathogens. The control of R. microplus populations is mainly based on the use of synthetic acaricides. However, using this control method presents a danger to humans and the environment and leads to the emergence of resistant tick populations. In this situation, searching for ecological and effective control

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alternatives is essential. Thus, plant extracts constitute a promising solution, particularly essential oils and their active compounds. Thus, the present study aims to assess the acaricidal activity of two monoterpenes (Thymol and 1.8 cineole) abundantly found in essential oils to find an alternative to synthetic acaricides. The acaricidal activity was determined according to the method of larval immersion test (LIT). Eight concentrations were tested and R software version 4.0.3 was used for data analysis. Results showed 100 % larval mortality rates for the two monoterpenes with LC50 and LC90 values of (0.28 and 0.64) and (0.64 and 2.66) respectively, for thymol and 1.8 cineole for the immersion time of 5 min. For 10 min of immersion, all LC values decreased for the two monoterpenes. These findings highlight the potential of the thymol and 1.8 cineole as an alternative for managing R. microplus tick.

Keywords : Acaricidal activity, Thymol, 1.8 cineole, Ticks, Rhipicephalus microplus

1. Introduction

Rhipicephalus microplus is an endemic important tick specie causing significant loss and damage to livestock production in tropical and subtropical regions of the world (Rodriguez-Vivas et al., 2018; Diaz et al., 2019). This tick affects animal welfare, resulting in stress, anemia due to blood spoliation, reduction of productive and reproductive performance, and transmits hemiparasites causing bovine babesiosis and anaplasmosis (Chagas, 2012). The main method of controlling ticks is the use of synthetic acaricides (Quadros et al., 2020 ; Obaid et al., 2022). However, this control method has led to the emergence of resistant tick populations (Gupta et al., 2021 ; Dzemo et al., 2022). In searching for alternatives to synthetic acaricides, plant extracts in particular essential oils (EOs) appear promising (Rodriguez-Vivas et al., 2018 ; Selles et al., 2021). Indeed, EOs are particularly advantageous because of their low toxicity, low persistence, and their complex chemistry that hinders the development of resistance in parasites (Mello-Peixoto et al., 2013). Furthermore, previous work has shown that the biological activity of EOs is closely related to their chemical composition (Adenubi et al., 2016 ; Djebir et al., 2019). In addition, other studies have shown that this activity is mainly due to monoterpene compounds in essential oils (Zielińska-Błajet et al., 2020 ; Liu et al., 2022). Thymol and 1,8-cineole are monoterpenes in several EOs. Thymol (2-isopropyl-5-methylphenol) is mainly present in EOs of plants of the *Lamiaceae*, *Verbenaceae*, *Scrophulariaceae*, *Ranuncolaceae* and *Apiaceae* families (Marchese et al., 2016). Many biological properties have been reported on this compound, including antioxidant, anti-inflammatory, antibacterial and antifungal properties (Marchese et al., 2016 ; Kowalczyk et al., 2020).

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1.8 cineole (1,8-Epoxy-*p*-menthane) or eucalyptol is mainly derived from the EOs of plants in the *Lamiaceae*, *Myrtaceae* and *Zingiberaceae* families (Cai et al., 2021). Many pharmacological properties have been reported on thymol, including anti-inflammatory, antioxidant, antimicrobial, mucolytic, broncholytic, anticancer, etc. (Cai et al., 2021).

The present study aims to evaluate the acaricidal activity of thymol and 1.8 cineole on *R. microplus* larvae to find a natural and effective bio-acaricide.

2. Materials and methods

2.1 *Origin of ticks and chemical material*

The Adult females of *R. microplus* ticks were collected from Kimini (10.100000, -4.783330), a locality located in the Southwest region of Burkina Faso. The strain has been identified in the laboratory of CIRDES (Walker et al., 2003). These were immediately oviposited under optimal temperature (28 ± 1 °C) and relative humidity (85 ± 5%) to produce eggs and then larvae after approximately 14 and 22 days of incubation, respectively. Fourteen days after the first observation of larvae, bioassays were performed. Thymol and 1.8 cineole standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2 *Acaricidal activity*

The LIT test was used to evaluate the acaricidal activity of thymol and 1.8 cineole against *R. microplus* larvae with minor modifications according to the protocol described by Klafke et al. (2012). Eight concentrations (0.25, 0.5, 1, 1.75, 2.5, 3.75, 4.25 and 5 mg/mL) were used and the experiment was carried out in three replicates for each treatment. A solution (positive control) containing 1.0% of ethanol and the tween 20 at 3 % was used to dilute the standards. Thus, larvae (approximately 300 to 500) aged 14 days were previously placed with a brush between two pieces of Whatman paper No.1. 3 ml of the control solution was first applied to these larvae, which remained immersed for 5 minutes. They were then transferred to filter-paper packages (Whatman No.1) and sealed with “Bulldog” clips. This process was done in triplicate for all the treated larvae and controls. The test is repeated with a larval immersion time of 10 minutes. Both packages containing control and treated groups with thymol and 1.8 cineole were placed in the incubator under 27-28°C and 80-95% relative humidity for 24 h. After this period, dead and live larvae were counted to calculate mortality rates according to the following formula :

$$\textbf{Mortality (\%)} = \frac{\textbf{dead larvae}}{\textbf{total of larvae}} \textbf{ x 100}$$

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The Abbott formula has corrected the mortality rate of control groups when this rate exceeds 5 % (Abbot, 1925). Larvae without movement were diagnosed dead, and only larvae that could move around were recorded as alive (Perez-Cogollo et al., 2010).

2.3 *Statistical analysis*

Statistical analysis was performed using R software version 4.0.3. The mean values for each treatment were subjected to an analysis of variance (one-way ANOVA) followed by Tukey's test at the 5% significance level. The lethal LC_{50} and LC_{90} concentrations were determined with their respective 95% confidence intervals by probit analysis with GraphPad prism 7.0.

3. Results

Results of larval mortality rates of thymol and 1.8 cineole at different concentrations during 5 and 10 minutes were recorded in Table 1.

Table 1. Larval mortality rates of thymol and 1.8 cineole

Concentrations (mg/mL)	5 min		10 min	
	Mortality rate (% ± SD)		Mortality rate (% ± SD)	
	Thymol	1.8 cineole	Thymol	1.8 cineole
0.25	42.3 ± 0.11^{d}	18.6 ± 0.07^{d}	51.3 ± 0.11^{c}	27.6 ± 0.01^{d}
0.50	60.4 ± 0.70^{c}	44.3 ± 0.21^{c}	68.4 ± 0.70^{b}	48.3 ± 0.52^{c}
1.00	88.7 ± 0.56^{b}	65.2 ± 0.53^{b}	93.7 ± 0.56^{a}	71.2 ± 0.26^{b}
1.75	96.7 ± 0.56^{a}	73.6 ± 0.61^{b}	98.3 ± 0.56^{a}	82.6 ± 0.22^{a}
2.50	100^{a}	81.3 ± 0.46^{b}	100^{a}	88.3 ± 0.83^{a}
3.75	100^{a}	94.1 ± 0.73^{a}	100^{a}	97.6 ± 0.83^{a}
4.25	100^{a}	100^{a}	100^{a}	100^{a}
5.00	100^{a}	100^{a}	100^{a}	100^{a}

a, b, c, d: Letters indicating different statistical groups. Identical letters in the same column indicate homogeneous statistical groups. Different letters indicate different statistical groups; **SD**: Standard deviation.

The larval mortality rates of thymol and 1.8 cineole for 5 and 10 minutes of immersion were represented as a function of concentration (Figure 1).

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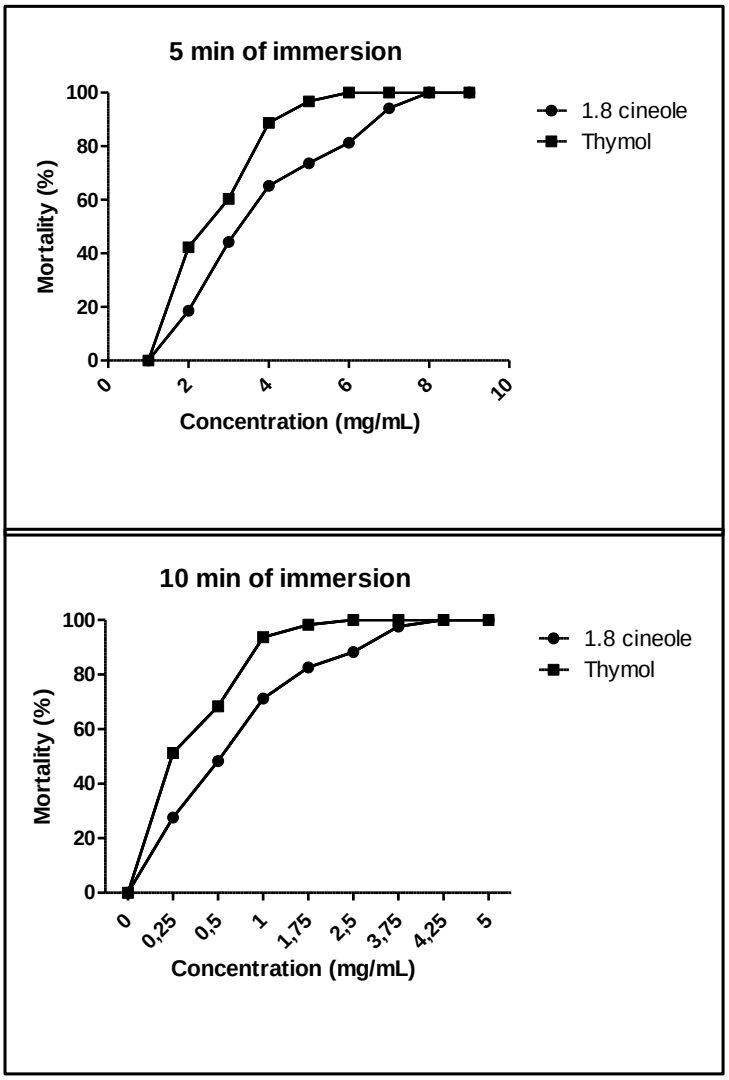

Figure 1. Mortality rate of thymol and 1.8 cineole for 5 and 10 min of immersion

Both monoterpenes caused 100% mortality rates on the larvae of the *R. microplus* tick. Thymol caused more larval mortality compared to 1.8 cineole. The lethal concentrations LC_{50} and LC_{90} were determined and results have been reported in Table 2.

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Table 2. LC_{50} et LC_{90} of thymol and 1.8 cineole

Monoterpenes		5 min		10 min	
		LC (mg/mL ± SD)	CI	LC (mg/mL± SD)	CI
Thymol	LC_{50}	0.28 ± 0.03	[0.11- 0.50]	-	-
	LC_{90}	1.21 ± 0.01	[1.01- 3.02]	0.95 ± 0.07	[1.01- 2.07]
1.8 cineole	LC_{50}	0.64 ± 0.05	[0.50 -0.83]	0.52 ± 0.01	[0.36 -0.73]
	LC_{90}	2.66 ± 0.02	[2.50 - 3.81]	2.53 ± 0.03	[1.40 - 2.91]

LC : Lethal concentrations **; CI :** Confidence intervals**; LC_{50}**: dose causing the mortality of 50% of larvae; **LC_{90}**: dose causing the mortality of 90% of larvae, **SD**: Standard deviation.

4. Discussion

In the current study, the LIT test was carried out twice. The larvae were immersed for 5 min the first time, and the second time, they were immersed for 10 min. For the 5 min immersion time, the effect of 1.8 cineole caused larval mortality rates ranging from 18.6 at the concentration of 0.25 mg/mL to 100% at 4.25 mg/mL. Thymol's lowest concentration caused 42.3% mortality; at 2.5% concentration, all larvae were eliminated. For the 10 min immersion time, 27.6 % mortality was recorded by 1.8 cineole at the lowest concentration, and at 4.25 mg/mL, this compound caused 100% larval mortality. As for thymol, larval mortality rates ranged from over 50% at the lowest concentration (0.25 mg/mL) to 100% mortality from 2.5 mg/mL.

Only a few studies have been realized on the acaricidal activity of 1.8-cineole. However, its acaricidal activity against *Sarcoptes scabiei* has been reported (Hu et al., 2015). In addition, It has been demonstrated that 1.8 cineole was active on the reproductive parameters of the tick *R. microplus* (Queiroz et al., 2020). Indeed, these authors showed that this compound caused an inhibition of the oviposition and hatching rates. Also, other authors have reported the acaricidal activity of an essential oil of *Eucalyptus globulus* with 1.8 cineole as the most abundant compound against the tick *Rhipicephalus (Boophilus) annulatus* (Adenubi et al., 2021).

For thymol, several previous studies have reported its acaricidal effects on the tick *R. microplus*. Indeed, it has been shown that the thymol was effective on the tick *R. microplus* with an LC_{50} value of 1.81 mg/mL (Lima et al., 2017). Similarly, a study showed that this compound was active on *R. microplus* tick with an LC_{50} of 4.46 mg/mL (Cruz et al., 2013). In addition, another study found over 99% mortality of *R. microplus* larvae due to thymol (Scoralik et al., 2012). Furthermore, acaricide and ovicide activities of thymol on engorged females and eggs of *R. microplus* have been proven (De Oliveira Monteiro et al., 2010).

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In the current study, the value of the lethal concentration LC_{50} was 0.28 mg/mL for thymol. This value was lower than those obtained in the previously mentioned studies performed on *R. microplus* larvae (De Oliveira Monteiro et al., 2010 ; Scoralik et al., 2012 ; Cruz et al., 2013 ; Lima et al., 2017). This shows that the thymol used in the present study was more active. The sensitivity of the larvae to the product can explain this. Indeed, some larvae can be more sensitive than others when faced with a given product.

The lethal concentrations (LC_{50} and LC_{90}) of 1.8 cineole were higher than that of thymol, indicating that thymol was more active on *R. microplus* larvae than 1.8 cineole. Indeed, numerous studies have been reported on the activity of thymol as an insecticide, acaricide, and animal repellent (Escobar et al., 2020). The acaricidal efficacy of thymol compared to 1.8 cineole could also be linked to the fact that thymol is a phenolic compound. A hydroxyl group on its aromatic ring confers a high reactivity to this compound compared to 1.8 cineole which is chemically more stable from a structural point of view (Platzer et al.,2022).

Furthermore, the results showed that the larval mortality rates of thymol and 1.8-cineole differed when the larvae were immersed for 5 or 10 min. Indeed, for 1.8 cineole, the LC_{50} concentration increased from 0.64 to 0.52 mg/mL, and the LC_{90} concentration increased from 2.66 to 2.53 mg/mL. For thymol, the LC_{50} concentration could not be determined because mortality rates at the lowest concentration exceeded 50% mortality. As for the LC_{90} concentration, its value decreased from 1.21 to 0.95 mg/mL. These results indicate that the mortality rates increased with increasing immersion time of the larvae under both compounds.

Previous studies have revealed the molecular targets of 1.8-cineole and thymol on the tick *R. microplus*. Indeed, it has been shown that these two compounds act on *R. microplus* larvae as acetylcholinesterase inhibitors (Cardoso et al., 2020). For thymol, another study showed that this compound induces the increased activity of antioxidant and detoxifying enzymes in *R. microplus* larvae (Tavares et al., 2022). The acaricidal effect observed by thymol and 1.8 cineole in our study may be related to these mechanisms of action. However, further investigations are needed.

Conclusion

The present study has permitted us to compare the acaricidal activity of 1.8 cineole and thymol on the larvae of the tick *R. microplus*. Thymol was more active than 1.8 cineole but both compounds showed interesting mortality rates. The efficacy of thymol could probably be explained by its chemical structure making it more active than 1.8 cineole. The results of this study could lead to the development of new effective acaricide formulations based on these two monoterpenes for the control of *R. microplus* tick. However, questions

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relating to the mechanisms of action of these products and their toxicity need to be addressed.

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Data Availability: The data used to support the findings of this study are included in this article. Any required further information can be provided by the corresponding author upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

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