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Acaricidal Activity of *Illicium verum, Laurus nobilis* and *Origanum vulgare* Essential Oils Against *Rhipicephalus microplus* (Acari: Ixodidae)

Atividade Acaricida dos Óleos Essenciais de *Illicium verum, Laurus nobilis* e *Origanum vulgare* Contra *Rhipicephalus microplus* (Acari:Ixodidae)

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Abstract
Due to the increase of *Rhipicephalus microplus* populations resistant to synthetic acaricides, as well as the need for safer products to be used in cattle breeding, it becomes necessary to search for new products, such as those to be obtained from plants. Thus, this study aimed to determine the chemical composition and evaluate the acaricidal activity of *Illicium verum*, *Laurus nobilis* and *Origanum vulgare* essential oils against *R. microplus in vitro*. The essential oils were obtained by hydrodistillation, and chemical characterization was performed by gas chromatography coupled with mass spectrometry (GC-MS). The acaricidal activity of each essential oil was first evaluated on larvae at concentrations 2.5, 5.0, 10.0, 15.0 and 20.0 mg/mL. The results of the larval treatment indicated that only the essential oil of *I. verum* caused mortality. Thus, immersion tests of this oil were conducted on engorged females of *R. microplus* at concentrations of 10.0, 20.0, 40.0, and 60.0 mg/mL. GC-MS analysis of *I. verum, L. nobilis* and *O. vulgare* essential oils showed E-anethole (88.32%), 4-terpineol (24.92%) and 1.8-cineol (74.23%) as the major components, respectively. Regarding the acaricidal activity both in larvae and *R. microplus* engorged females, only *I. verum* essential oil showed effective, causing 99.8% of larvae mortality at concentration 15 mg/mL and achieved 84.9% efficacy on engorged females at concentration 60mg/mL. This study provides support for further investigation of *I. verum* essential oil components as novel natural products to *R. microplus* control.

Keywords: Bay Laurel. Cattle Tick. E-Anethole. Oregano. Star Anise.

Resumo
Devido ao aumento de populações de *Rhipicephalus microplus* resistentes aos acaricidas sintéticos, bem como à necessidade de produtos mais seguros para serem utilizados na pecuária, torna-se necessária a busca por novos produtos, como aqueles a serem obtidos a partir de plantas. Assim, este estudo teve como objetivo determinar a composição química e avaliar a atividade acaricida dos óleos essenciais de *Illicium verum, Laurus nobilis* e *Origanum vulgare* contra *R. microplus* in vitro. Os óleos essenciais foram obtidos por hidrodestilação e a caracterização química foi realizada por cromatografia gasosa acoplada à espectrometria de massas (GC-MS). A atividade acaricida de cada óleo essencial foi avaliada primeiramente em larvas nas concentrações 2,5, 5,0, 10,0, 15,0 e 20,0 mg/mL. Os resultados do tratamento das larvas indicaram que apenas o óleo essencial de *I. verum* causou mortalidade. Assim, foram realizados testes de imersão desse óleo em fêmeas ingurgitadas de *R. microplus* nas concentrações de 10,0, 20,0, 40,0 e 60,0 mg/mL. A análise por GC-MS dos óleos essenciais de *I. verum, L. nobilis* e *O. vulgare* mostrou E-anetol (88,32%), 4-terpineol (24,92%) e 1,8-cineol (74,23%) como componentes majoritários, respectivamente. Quanto à atividade acaricida tanto em larvas quanto em fêmeas ingurgitadas de *R. microplus*, apenas o óleo essencial de *I. verum* se mostrou eficaz, causando 99,8% de mortalidade larval na concentração 15 mg/mL e alcançou 84,9% de eficácia em fêmeas ingurgitadas na concentração 60mg/mL. Este estudo

fornece suporte para investigações adicionais dos componentes do óleo essencial de *I. verum* como novos produtos naturais para o controle de *R. microplus*.

Palavras-chave: Louro. Carrapato Bovino. E-Anetol. Orégano. Anis Estrelado.

1 Introduction

Bovine ixodidiosis caused by *Rhipicephalus microplus* (Acari: Ixodidae) is an important parasitic infestation in Brazilian herds, it can develop with anemia, concomitant diseases caused by the pathogenic agents as *Anaplasma marginale, Babesia bovis* and *B. bigemina* transmitted during the blood meal of the ticks in animals, reduce the quality of animal leather, in addition to compromising productivity (Reck *et al*., 2014). The economic losses caused to Brazilian livestock by *R. microplus* were estimated at 3.24 billion/year (Grisi *et al*., 2014). The control of this ectoparasite in Brazil is realized mainly through the use of synthetic acaricide, often empirically and erroneously, without veterinary guidance, which has accelerated the development of resistant tick populations (Klafke *et al*., 2017).

Although chemicals are important to cattle tick control, they are considered expensive and can be detrimental to the environment, animal and dangerous for the consumers if the recommended residual periods for food of animal origins are not followed : in this perspective, the use of acaricides should be minimized and integrated with alternative tick control approaches (Walker, 2014).

Some new approaches of tick control have been studied, such as biological control (Perinotto *et al*., 2017). Selection of cattle tick resistant (Biegelmeyer *et al*., 2012), homeopathy (Aurnheimer *et al*., 2011), association between entomopathogenic fungi and bacteria to plants secondary metabolites (Nardoni *et al*., 2018) and phytotherapy using essential oils (EOs) (Chagas *et al*., 2016). Among the alternative measures mentioned above, the EOs have been widely studied due to be considered eco-friendly, has high biodegradability, easy obtaining and environmental safety, in comparison to synthetic agents (Singh; Pandey, 2018). Natural products have been the origin of many important molecules in drug discoveries. Different culinary herbs have been screened for their biological activities. The active ingredients from plants are known to possess insecticidal, growth inhibiting, antimoulting, and repellent activities (Ghosh; Azhahianambi; Yadav, 2007).

Previous studies have been demonstrated that *Illicium verum* L. (star anise) (Austrobaileyales: Illiciaceae)*, Origanum vulgare* L. (oregano) (Lamiales: Lamiaceae) and *Laurus nobilis* L. (bay laurel) (Laurales: Lauraceae) OEs have high concentration of E-anethole (Tuan and Ilangantileke, 1997), Carvacrol (Nostro *et al*., 2007) e 1.8-Cineol (Fernandez *et al*., 2018), respectively, components responsible by biological activities such as antioxidant, antifungal and antibacterial. The *I. verum* EO

has been demonstrated activity against *Ixodes ricinus* unfed nymphs (Elmhalli *et al*., 2018) and a relevant acaricidal activity of *Dermacentor. nitens* engorged females (Dos Santos Lima Junior *et al*, 2020). *O. vulgare* has been reported to repel the tick *Amblyomma americanum* (Meng *et al*., 2016) and another species of oregano, *Origanum onites* L. has been found to be toxic to the tick *Rhipicephalus turanicus* (Coskun *et al*., 2008). However, there is little information about the acaricidal activity of these OEs on tick control, mainly for the cattle tick; there are available few studies with *L. nobilis* in current literature (Fernandez *et al*., 2018; Vinturelle *et al.*, 2021). Therefore, the objective of this work was to determine the chemical composition and evaluate the acaricidal activity of *I. verum*, *L. nobilis* and *O. vulgare* essential oils against *R. microplus in vitro*.

2 Material and Methods

2.1 Plant material

The plant material of *L. nobilis* L. (bay laurel leaves), *O. vulgare* L. (oregano leaves), and the star *I. verum* L. (star anise fruit) were acquired drily at a fair located in the city of Cuiabá, Mato Grosso, Brazil. The plants were chosen through previous research in the literature, and which have not yet been tested against ticks, but which have already shown activity or repellency effects on bacteria, fungi, helminths, mites or insects

2.2 Essential oils extraction

Leaves and fruits were air-dried, ground, and subjected to hydrodistillation using a Clevenger-type apparatus (100 g, 3h). The obtained oils were dried over hydrous sodium sulfate for 24h, filtered, and then stored at 4 °C in brown sealed glass vials until tested.

2.3 Chemical composition of essential oil

The chemical composition of the essential oils was annalyzed using a gas chromatograph coupled to a mass spectrometer (GC/MS – Shimadzu QP-2010 Plus) equipped with a Factor Four/ VF – 5ms fused-silica capillary columm (30 m x 0.25 mm x 0.25 μm film thickness), using hellium as carrier gas at 1mL/min. The inicial oven temperature was 60 ºC, which after being held constant for 2 min was increased at a rate of 3 ºC min^{-1} to 260 ºC, followed by 10ºC min^{-1} to 290 ºC, with a final isotherm (290 ºC) for 10 min. The sample injection was 1μ (1:50 split mode). The injector and detector temperatures were of 220 ºC and 250 ºC, respectively. The mas spectra were obtained in a range of m/z 10-300, by the electron impact technique at 70 eV.

The quantitative analysis of the oils chemical composition was carried out in a gas chromatograph coupled to an HP5890 Series II flame ionization detector (FID), using the same operational conditions and the same type of columm as in the GC/MS analysis.

The percentage of each constituent was calculated by the integral area under the respective peaks in relation to the total area of all the sample constituents. The various chemical constituents of the essential oil were identified by visual comparison of their mass spectra with those in the literature (Adams, 2001) and spectra supplied by the equipment database (NIST 08), as well as bay comparison of the retention indices with those in the literature (Adams, 2001). A standard solution of n-alkanes (C8-C20) was injected under the same chromatographic conditions as the sample and used to obtain the retention indices as described by Van den Dool and Kratz (1963).

2.4 Larval packet test (LPT)

For the test, engorged females of R. microplus were collected through natural infestation in dairy cattle raised on a private property in the city of Juiz de Fora (MG), without prior contact with chemical products. These females were then collected and taken to the Parasitology laboratory at the Federal University of Juiz de Fora (MG).

The methodology adapted by Monteiro *et al.* (2012), where approximately 100 larvae were placed between 6x6 cm filter papers, closed with binder clips, and impregnated with 180 µL (90 µL in each side) of essential oils at concentrations of 2.5; 5; 10; 15 and 20 mg/mL, the emulsification of this oil is achieved with Tween 80 at a concentration of 30 mg/mL (3%), using distilled water as the vehicle, in addition a negative control group was established with distilled water and Tween 80 at 30 mg/mL. Ten packets were used for each treatment determined.

The experimental groups were placed in a climate-controlled incubator at 27±1 °C and relative humidity (RH) of 80±10%. To prevent any possible cross-interference, the control group was kept in a different chamber from the treatment groups under the same conditions. After 24h, the packets were opened, and numbers of living and dead larvae were counted with a vacuum pump connected to a pipette tip attached to the end of a rubber hose. Average mortality in each packet was expressed as the percentage and calculated as: mortality (%) = (number of dead larvae/total number of larvae) ×100.

2.5 Adult immersion test (AIT)

The acaricidal activity on engorged female was tested only with EO that had activity on larvae. Thus, adult immersion tests with *I. verum* EO at concentrations of 10.0, 20.0, 40.0, and 60.0 mg/mL were performed, as proposed by Drummond *et al*. (1973). Females were divided into groups

composed of 10 ticks of homogeneous weight. Each group was immersed for 5 min in EO or control group (distilled water and Tween 80 at 3%). After immersion, the females were removed and placed on sheets of paper towel to remove excess liquid. Subsequently, the females were individually placed in Petri dishes and kept in a climate-controlled incubator at 27±1 ∘C and RH of 80±10%.

After the incubation period (15 days), the egg masses laid by each female were weighed. The eggs were transferred to test-tube, sealed with absorbent cotton, and kept under the same conditions as the females for 20 days to allow the observation of larvae hatching. The values of the oviposited mass were used to calculate the egg production index (EPI), obtained by the formula proposed by Bennett (1971), in which EPI=egg mass/initial female weight before oviposition×100. Subsequently, the index of estimated reproduction (ER) was calculated as: (egg mass weight/female weight before oviposition) × hatching percentage × 20000. The efficacy of treatment as a percentage referring to offspring inhibition was obtained according to Drummond *et al*. (1973), where efficacy (%) = (ER of the control group − ER of the treated group)/ER of the control group×100.

2.6 Statistical analysis

The data were analyzed using the *Instat* 3.0 software. The treatment means were compared by analysis of variance (ANOVA), followed by the Tukey test, except in cases of nonparametric data, which were analyzed by the Kruskal-Walli's test followed by Student-Newman-Keuls ($p<0.05$).

3 Results and Discussion

3.1 Chemical composition of essential oils

A total of 18 compounds were identified from *I. verum* essential oil, composed to monoterpenes hydrocarbons (4.85%) and oxygenated monoterpenes (95.14%), the E-anethole was identified as the major compound, representing 88.32% of this oil; the sesquiterpenes were not identified. In the EO of *O. vulgare*, 28 chemical components were identified, composed of monoterpenes hydrocarbons (33.82%), oxygenated monoterpenes (63.62%) and sesquiterpenes (2.34%), the major compounds were Terpinen-4-ol (24.92%), carvacrol (19.67%), γ-Terpinene (11.82%), Z-4-Thujanol (8.31%) and γ-Terpinene (7.32%). In the EO of *L. nobilis*, 23 chemical components were identified, all monoterpenes, being 10.82% of monoterpene hydrocarbons and 84.38% of oxygenated monoterpenes, the major component was 1.8-cineole (74.23%). The sesquiterpenes and benzene derivatives were not identified (Table1).

Table 1 - Chemical composition, calculated Kovats index (KI_C), Kovats index obtained from of literature ($KI_{Lit.}$), percentages of identifies components and classes of the same (%) in the essentials oils from fruits of *Illicium verum* and leaves of *Origanum vulgare* and *Laurus nobilis*

Compounds	**KI_C**	**$KI_{Lit.}$**	**%**		
			Iv	**Ov**	**Ln**
Monoterpene hydrocarbons			4.85	33.82	10.82
α-Thujene	931	930	-	1.30	0.34
α-*Pinene*	940	939	0.38	0.75	2.64
Sabinene	979	975	-	2.53	2.64
α-Pinene	984	979	-	1.04	2.51
Myrcene	992	990	0.19	1.17	-
α- Phellandrene	1011	1002	0.15	0.43	-
δ-3-Carene	1013	1011	0.35	-	-
α-Terpinene	1023	1017	0.10	7.32	0.32
p-Cymene	1036	1024	0.33	3.64	1.67
Limonene	1035	1029	3.05	-	-
Sylvestrene	1038	1030	-	1.49	-
ɣ-*Terpinene*	1065	1059	0.15	11.82	0.58
Terpinolene	1088	1090	0.15	2.33	0.12
Oxygenated monoterpenes			95.14	63.62	84.38
Dehydro-1,8-cineole	995	991	-	-	0.36
1.8-*Cineole*	1047	1031	0.83	-	74.23
E-4-*Thujanol*	1077	1070	-	1.64	0.24
Linalool oxide	1075	1072	0.07	-	-
Linalool	1107	1096	1.45	-	0.21
Z-4-*Thujanol*	1103	1098	-	8.31	0.31
Z-p-*Menth-2-en-1-ol*	1132	1121	-	1.46	0.42
E-pinocarveol	1150	1139	-	-	0.86
E-p-*Menth-2-en-1-ol*	1151	1140	-	1.00	-
Sabina ketone	1157	1159	-	-	0.26
Pinocarvone	1171	1164	-	-	0.53
ɣ-*Terpineol*	1178	1166	-	-	0.59
Terpinen-4-ol	1189	1177	0.55	24.92	3.75
ɣ-*Terpineol*	1194	1188	0.29	3.88	0.30
Myrtenol	1202	1195	-	-	1.65
Estragole	1203	1196	2.52	-	-
E-*Piperitol*	1216	1208	-	0.46	-
Anisole	1245	1235	-	1.70	-
p-Anisaldehyde	1268	1250	0.87	-	-
Linalyl acetate	1254	1257	-	0.58	-
E-*Anethole*	1304	1284	88.32	-	-
Carvavrol	1311	1299	-	19.67	-
δ- *Terpinyl acetate*	1318	1317	-	-	0.49
α- Terpinyl acetate	1344	1349	-	-	0.18
Eugenol	1359	1359	0.24	-	-
Sesquiterpene hydrocarbons			0.00	1.91	0.00
E-*Caryophyllene*	1426	1419	-	1.23	-
Germacrene D	1487	1485	-	0.13	-
Byciclogermacrene	1501	1500	-	0.33	-
B-Bisabolene	1512	1505	-	0.22	-

Oxygenated sesquiterpenes			0.00	0.43	0.00
Spathulenol	1585	1578	-	0.22	-
Caryophyllene oxide	1591	1583	-	0.21	-
Non isoprenoid components			0.00	0.21	0.00
3-Octanol	999	991	-	0.21	-
Total			99.99	99.99	95.20

Source: research data.

3.2 Larval packet test

In the LPT, only the EO of *I. verum* demonstrated activity, resulting in a mortality rate of 72.2, 99.8 and 94.8% at concentrations 10.0, 15.0 and 20.0 mg/mL, respectively, with differences in relation to the control group (p <0,05). Statistically, there was not a difference between the concentrations 15.0 and 20.0 mg/mL. In the treatment with EOs of *O. vulgare* and *L. nobilis* in all concentrations and the control group was not observed larval mortality (Table 2).

Table 2 - Mean ± standard deviation of mortality percentage in larvae of *Rhipicephalus microplus* treated with different concentrations of essential oil of *Illicium verum*, *Origanum vulgare e Laurus nobilis*, under laboratory conditions (27±1 °C and relative humidity of 80±10%)

Concentration	Illicium verum	Origanum vulgare	Laurus nobilis
Control*	0 ± 0a	0 ± 0	0 ± 0
2.5 mg/mL	0 ± 0a	0 ± 0	0 ± 0
5.0 mg/mL	0 ± 0a	0 ± 0	0 ± 0
10.0 mg/mL	72.2± 10.0b	0 ± 0	0 ± 0
15.0 mg/mL	99.8± 0.003c	0 ± 0	0 ± 0
20.0 mg/mL	94.80 ± 0.07c	0 ± 0	0 ± 0

Mean followed by a different letter in the same column presents a significant difference ($p < 0.05$). (*)Tween 80 (3%).
Source: research data

3.3 Adult immersion test

As previously explained, only the EO of *I. verum* was tested against *R. microplus* engorged females at concentrations of 10, 20, 40, and 60 mg/mL. In the statistical analysis, difference was not observed between all groups on the weight of females before oviposition, showing that the ticks were homogeneously distributed to form the groups. Numerically, the treatment of females with the EO of *I. verum* reduced egg production and egg production index in all concentrations when compared with the control group but was not observed statistical difference among the treatments. Larval hatching was reduced at all concentrations tested, except at concentration of 10.0 mg/mL. Treatment achieved efficacy (Percent control) of 56.9, 77.2, 78.5 and 84.9% at concentrations of 10, 20, 40, and 60 mg/mL, respectively (Table 3).

Table 3 - Mean ± standard deviation of weight of females before oviposition (WFBO), egg mass weight (EMW), egg production index (EPI), larval hatching (LH) and percent control (PC) of *Rhipicephalus microplus* treated with different concentrations of *Illicium verum*, under laboratory conditions (27±1 °C and relative humidity of 80±10%)

Treatments	WFBO (mg)	EMW (mg)	EPI (%)	LH (%)
Control*	232.9 ± 37.1a	108.0 ± 47.5a	44.0 ± 15.0a	93.0 ± 8.0a
10.0 mg/mL	233.7 ± 34.6a	92.9 ± 32.7a	40.0 ± 12.0a	46.8 ± 37.7ab
20.0 mg/mL	233.8 ± 40.2a	82.5 ± 34.8a	31.0 ± 17.0a	27.9 ± 32.8b
40.0 mg/mL	233.3 ± 27.1a	85.6 ± 30.2a	37.0 ± 12.0a	25.3 ± 33.1b
60.0 mg/mL	233.7 ± 50.3a	79.1 ± 45.1a	32.0 ± 15.0a	19.2 ± 24.6b

Mean followed by a different letter in the same column presents a significant difference ($p < 0.05$).(*) Tween 80 (3%).
Source: research data.

The analysis of chemical composition of *I. verum, O. vulgare* and *L. nobilis* essential oils by GC/MS showed as major compounds E-anethole (88.32%); Terpinen-4-ol (24.92%), carvacrol (19.67%); 1.8-cineole (74.23%), respectively. Similar results showing E-anethole as a major component in EO of *I. verum* are present in the literature, such as Matos *et al*. (2020), who observed E-anethole in the content of 88.85%, presented a potential fumigant effect on *C. maculatus* with relatively low lethal concentration (LC_{50}) (22.36 µL/L of air), is an alternative to synthetic chemical insecticides for the control of stored grain pests. Scopel *et al*. (2016), obtained approximately 80% of E-anethole in EO of *I. verum*, extracted by supercritical fluid, varying the concentration of CO_2 and the temperature. However, Pandiyan, Mathew and Munusamy (2019) found a different content of E-anethole (53.05%), in addition to another constituent, p-anisaldehyde (12.47%), in the essential oil of *I. verum*.

Similar results showing carvacrol as the major component in *O. vulgare* are present in the literature, such as Souza, Stamford and Lima (2006), who observed carvacrol in a concentration of 68.06% in their studies followed by p-cymene (15.91%), α-pinene (2.56%) and myrcene (1.87%). Other studies as Govindarajan *et al*. (2016) had a content of carvacrol (38.30%) higher than that of terpinene-4-ol (28.70%). Morshedloo *et al*. (2018) demonstrated that there are seven populations of Iranian oregano, originating from different geographical and bioclimatic zones, with great variability in the chemical profile of essential oil, such as Carvacrol (0.3-46.8%), linalyl acetate (0.2-44.3%), (Z)-α-Bisabolene (0.0-40.3%), (E)-β-Caryophyllene (0.0-24.0%) and Caryophyllene oxide (0.1-21.3%).

In a previous phytochemical study the presence has been revealed of terpenoids, anthocyanins and glycosides in essential oil of *L. nobilis* (Patrakar; Mansuriya; Patil, 2012). According to Maatallah

et al. (2016), the water deficit stress effects increased the 1.8-cineole content in two laurel ecotypes and decreased the other chemical constituents in essential oils. In southern Brazil, in the state of Paraná, a species of laurel collected showed a chemical profile of its essential oil as the main constituent of isoeugenol (53.5%), followed by myrcene (16.6%) and chavicol (10.2%) (Peixoto *et al*., 2017). In another study, bay leaves collected in the countries of Tunisia, Algeria and Morocco, despite having variability in the content of the chemical constituents of essential oils, maintained the majority of 1,8-cineole, linalool and isovaleraldehyde (Jemâa *et al*., 2012).

The variations found at the chemical composition of essential oils between the studies may be because the quantities of secondary compounds can be affected by climate and growing conditions, the origin of the plant, development stage, interaction with insects and predators, cultivation techniques and genetic factors (Özcan; Erkmen, 2001).

The acaricidal activity assessed in the current study resulted in a positive effect only with the EO of *I. verum* both in larvae and engorged females of *R. microplus*. The EOs of *O. vulgare* and *L. nobilis*, were not able to cause mortality even in larvae. In ticks, the cuticle is more fragile in stage larval than an adult. This occurs probably by hormonal stimuli in the engorged females the increase of the thickness of the cuticle, to prevent the overflow of the proteins acquired during the blood repast, which makes it difficult to penetrate, differently from larvae (Furlong; Martins; Prata, 2007). Thus, the larval packet test may be used as screening to select the best essential oils to be tested on engorged females.

Information on the acaricidal activity of EO from *Origanum* species is very limited, en study the OE *O.vulgare* diluted in DMSO had a high mortality rate greater than 90 % in engorged females of *R. microplus* (Duque *et al*., 2021). There are few studies with EO of other species, as *O. onites* L., but on other species ticks (Coskun *et al*., 2008; Carroll *et al*., 2017). Coskun *et al*. (2008) tested the effect of EO of *O. onites* against *Rhipicephalus turanicus* engorged females, the authors showed that the EO was effective in killing the ticks at concentrations of 25.0, 50.0, and 100% (v/v). These results were attributed to the high carcravol concentration (64.3%) in the oil tested. In the present study, the carcravol concentration was lower (19.67%), besides this, mixed with other 27 chemical components.

Fernandez *et al*. (2018) evaluated the acaricidal effect of the EO obtained from *L. nobilis* leaves on larvae and engorged females of *R. microplus.* The authors achieved LC_{99} at a concentration of 5.94 µL/mL on larvae by packet test and effectiveness of 57.9% on engorged females by adult immersion test at a concentration of 200 µL/mL. In the chemical composition of this study cited above, the major class of EO was oxygenated monoterpenes (78.15%), with 1.8-cineole (40.14%) and linalool (15.69%) as major constituents. Although the major component found in the present study was also 1.8-cineole, here it was in greater concentration (74.23%) and did not have the linalool.

These factors could explain the difference between our results on acaricidal activity. Moreover, it is important to stand out that the variations in acaricidal efficacy may occur according to the origin of the colony of tick and environmental conditions (Raynal *et al*., 2013).

There are few studies about the relationship of synergism and antagonism between the compounds and the expressed effect on biological activity (Pavela, 2014). However, the potentiating and inhibitory effect among the chemical constituents of EO *L. nobis* was demonstrated by comparing the larvicidal potential between the different fractions in the study realized for Fernandez *et al*. (2018), where the authors observed that 1,8-cineole, linalool and terpinen-4-ol present potentiating effect, whereas 1,8-cineole and methyl eugenol promoted lower larvicidal effect.

The present study reports the effectiveness of *I. verum* essential oil against larvae and engorged females of cattle tick, *R. microplus*. In the literature, the work of Dos Santos Lima Junior *et al*. (2020) presented a relevant acaricidal activity of *I. verum* verified in engorged females of *D. nitens*, reaching a control percentage of 89.6% and decreasing the percentage of hatching larvae significantly, in another study of *I. verum* against unfed nymphs of *Ixodes ricinus* (Elmhalli *et al*., 2018), the authors reached 99% of mortality at the biggest concentration tested (0.4 μL/cm^2). Despite, the test has been realized with different tick species, both works demonstrate that the E-anethole was the major component, at concentration around the 85–90%. From a chemical point of view, the acaricidal activity of *I. verum* EO is suggested to be attributable to the presence of E-anethole, because this component has presented biological activities on different organisms such as fungi (Huang *et al*., 2010), aphid (Lima, 2014) and bacteria (Souza *et al*., 2016).

Through the results reached by EO of *I. verum* in both stages of *R. microplus* larvae and engorged females, demonstrate the capacity to be used to control the ticks' biological cycle. In the current study, the EO caused 99.8% rate mortality of larvae being thus considered as strategic targets in control systems, controlling the non-parasitic stage in pastures. Concerning the adult tick, the EO of *I. verum* achieved 84.9% of efficacy, this value can be considered promising, especially in times when the majority of tick populations are resistant to chemical acaricides. Besides that, this efficacy may be increased in future studies through formulations of this essential oil.

4 Conclusion

In conclusion, it was evidenced that the EOs of *L. nobilis* and *O. vulgare* did not have an acaricidal effect on *R. microplus*. While the EO of *I. verum* was considered quite promising to be used to control this tick. However, further research should be conducted testing this oil in formulations to improve their activity.

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