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Acaricide and ovicide activities of thymol on engorged females and eggs of *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae)

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Abstract The present work had the objective of evaluating the influence of different concentrations of thymol on the biological parameters of engorged females of *Rhipicephalus* (*Boophilus*) *microplus* and also its ovicide activity on eggs of this tick. In order to carry out the work, four groups were formed, each containing 20 engorged females, which were immersed for 5 min in different concentrations of thymol (1.0%, 1.5%, and 2.0%) and a control group (water + dimethylsulfoxide). The following biological parameters were observed: *initial weight* (mg); *egg mass weight* (mg); *pre-oviposition*, *oviposition*, and *survival period* (days); *hatching percentage* (%HP); *egg production* (%EPI) and *nutritiona*l (%NI) indexes; and the percentage of control (%C). In order to perform the second stage, thymol solutions were sprayed on the egg masses (50 mg). The parameters' initial weight and pre-oviposition did not present significant differences ($p>0.05$) among the groups. Thymol caused alterations in the parameters egg mass weight, oviposition, survival period, hatching percentage, EPI, and NI, presenting highly significant differences between the treatments and the control group ($p<0.01$). The control efficacy was higher than 95% in all the treatments, reaching 99% in the concentration of 2.0%. In the ovicide test, the hatching percentage was not affected in any of the treatments ($p>0.05$). In the second experiment, when eggs were treated directly, the thymol did not affect significantly this parameter. These results demonstrate that thymol showed deleterious action on most of the analyzed parameters; thus, it is possible to conclude that, in laboratorial conditions, this monoterpene was efficient in the control of engorged females of *R.* (*B.*) *microplus*.

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Introduction

From economical point of view, *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini 1887) (Acari, Ixodidae) is the most important tick of the Neotropical region and one of the most important of the world (Walker et al. 2003; Martins et al. 2006; Jonsson and Piper 2007). In Brazil, based on updated estimates, the economic losses caused by this ixodide reach two billion dollars per year (Grisi et al. 2002).

The main way of controlling this parasite involves the use of acaricides; however, most of the producers do not know about important aspects on the biology of this tick, its ecological interactions (with the host and environment), and about the safe and proper methods for managing the equipments used in the acaricidal bath (Furlong et al. 2004; Labruna 2008). In this way, the expected results in the combat against this arthropod are not obtained, and the producer, not satisfied, starts performing more frequent treatments. As a result, these actions make it easier the selection of populations of resistant ticks (Martins et al. 2006; Furlong et al. 2007).

The use of substances extracted from plants has arisen great interest among the scientific community due to the innumerous advantages, when compared to the use of synthetic chemicals: Natural insecticides are obtained from renewable resources and are rapidly degradable; the development of pest resistance to these substances—

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Table 1 Initial weight, egg mass weight, egg production (%EPI), and nutritional (%NI) indexes of engorged females of *Rhipicephalus* (*Boophilus*) *microplus* treated with different concentrations of thymol under laboratory conditions (27±1°C and UR > 80 ± 10%)

Treatments	Initial weight (mg)	Egg mass weight (mg)	Egg production index (%)	Nutritional index (%)
Water + DMSO (*n*)	204.05 a±35.43 (18)	119.82 a±19.62 (18)	59.16 a±7.51 (18)	74.16 a±10.88 (18)
Thymol 1.0% (*n*)	202.56 a±23.84 (20)	16.62 b±28.36 (20)	17.32 b±16.13 (9)	24.72 b±20.81 (9)
Thymol 1.5% (*n*)	204.20 a±26.64 (20)	14.67 b±24.11 (20)	16.53 b±13.51 (9)	26.52 b±18.42 (9)
Thymol 2.0% (*n*)	201.09 a±33.79 (20)	12.72 b±29.33 (20)	18.42 b±17.24 (7)	25.15 b±23.06 (6)
Statistical test	ANOVA	ANOVA/Tukey	Kruskal–Wallis/Student–Newman–Keuls	ANOVA/Tukey

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n sample size

composed of an association of various active principles—is a slow process; these pesticides may be easily accessed and obtained by agriculturists, and they do not leave residues in food, or damage the ecosystem, besides the fact that they have low production costs (Baladrin et al. 1985; Chagas 2004).

Having in mind the harmful effects of bovine tick parasitism and the serious scene at present concerning the resistance to chemical acaricides, and also the pressures at the market for residue-free products, a line of research has been developed at the Federal University of Juiz de Fora (UFJF), with the aim of evaluating the efficacy of thymol, a monoterpenoid found in plants of the Lamiacea family, on the control of this and other species of ixodides of economic importance. This monoterpene has already had its insecticide, bactericide, fungicide, and molluscicide potential evidenced by different authors (Imdorf et al. 1995; Mansour et al. 2000; Ferreira et al. 2009), though its acaricide effect was only demonstrated recently for immature of *R.* (*B*).*microplus* (Novelino et al. 2007a, b), *Rhipicephalus sanguineus* (Latrelle 1906) (Acari: Ixodidae) (Daemon et al. 2009; Monteiro et al. 2009). Its repellent activity on tick larvae of the bovine was also demonstrated by Novelino et al. (2007b). Thus, the objective of the present work was to evaluate the influence of the different concentrations of thymol on the biological parameters of engorged females of *R.* (*B.*) *microplus* and also its ovicide activity on eggs of this tick.

Materials and methods

The study was developed at the Laboratory of Parasitic Arthropods, located at the Advanced Laboratory of Zoology of the Department of Zoology of the Federal University of Juiz de Fora, Minas Gerais, Brazil. It was used a strain of *R.* (*B.*) *microplus* originating from the municipality of Campina Verde, Minas Gerais, Brazil. Due to their low water solubility, thymol solutions were emulsified under heating (60°C) in aqueous dimethylsulfoxide (DMSO 1%). Choice of concentrations was based on the results accomplished by Novelino et al. (2007a), where 1% of thymol was able to kill 100% of larvae of this ixodide. As engorged females are less susceptible than larvae, 1% was the lowest concentration, being also tested concentrations of 1.5% and 2.0%.

Experiment 1

To perform the test, 78 females were used, which were weighed and separated in four groups of (each female one repetition), and then submitted to immersion for 5 min in

Table 2 Pre-oviposition, oviposition, survival period, and hatching percentage of engorged females of *Rhipicephalus* (*Boophilus*) *microplus* treated with different concentrations of thymol under laboratory conditions (27±1°C and UR > 80 ± 10%)

Treatments	Pre-oviposition	Oviposition (days)	Survival period (days)	Hatching percentage -%EC
Water + DMSO (*n*)	2.00 a±0.00 (18)	13.89 a±1.41 (18)	17.44 a±4.23 (18)	91.64 a±9.08 (18)
Thymol 1.0% (*n*)	2.55 a±1.33 (9)	4.50 b±2.50 (8)	13.10 b±5.43 (20)	12.44 b±26.11 (9)
Thymol 1.5% (*n*)	2.30 a±0.67 (9)	4.75 b±3.01 (8)	11.55 b±3.74 (20)	11.60 b±25.74 (9)
Thymol 2.0% (*n*)	2.33 a±0.81 (6)	5.50 b±3.51 (6)	11.50 b±4.99 (20)	11.33 b±26.31 (6)
Statistical test	Kruskal–Wallis	ANOVA/Tukey	ANOVA/Tukey	Kruskal–Wallis/Student–Newman–Keuls

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n sample size

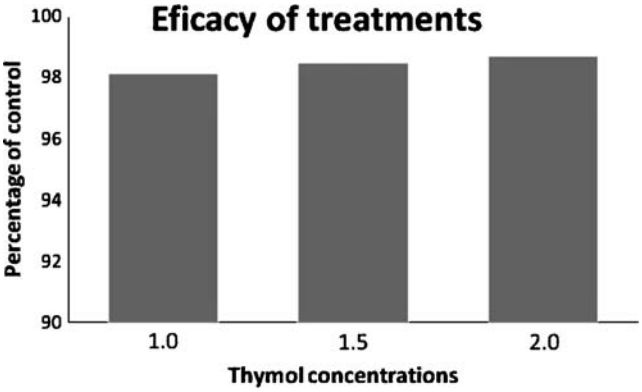

Fig. 1 Percentage of control of engorged females of *R.* (*B.*) *microplus* treated with different concentrations of thymol under laboratory conditions (27±1°C and UR > 80 ± 10%). Advanced Laboratory of Zoology of the Federal University of Juiz de Fora, Minas Gerais, Brazil

thymol solution in concentrations of 1.0%, 1.5%, and 2.0%, according to Drummond et al. (1973). A control group containing 18 ticks was also established (water + DMSO). After immersion, the engorged females were fixed, with the aid of double-sided adhesive tape, in dorsal decubitus position in Petri dishes properly identified and conditioned in a climatized room at 27±1°C and UR > 80 ± 10% for monitoring of the following parameters: *initial weight* (mg); *egg mass weight* (mg); *pre-oviposition*, *oviposition*, and *survival periods* (days); *hatching percentage* (%HP); *egg production index* (%EPI); *nutritional index* (%NI) (Bennett 1974); and the *percentage of control* (%C) obtained by the formula proposed by Drummond et al. (1973).

Experiment 2

In order to perform this second stage, engorged females were cleaned with a soft paintbrush, conditioned in Petri dishes in groups of five, and kept in a climatized room at 27±1°C and UR > 80 ± 10%. The oviposition process was checked daily to verify the initial date, and after the fourth oviposition day, the egg masses were gathered, weighed, divided in groups of 50 mg each, and interleaved on filter paper (4×4cm), constituting a total of 40 groups.

Thymol solutions in concentrations of 1.0%, 1.5%, and 2.0%, and the control (water + DMSO) were sprayed on the egg masses with 1.5 ml of the tested solution and ten repetitions each solution. After 10 min, the egg masses were gathered and kept in test tubes, being isolated with hydrophilic cotton and conditioned in a climatized room BOD under the conditions mentioned previously to verify occurrence of hatching.

Statistical analysis

The statistical analysis was performed using the software Biostat version 5.0. The percentage values were transformed into √arcsen *x*. The median values of each treatment were analyzed by ANOVA and Tukey test ($p<0.05$). In the case of nonparametric distribution, the values were compared through nonparametric tests of Kruskal–Wallis and Student–Newman–Keuls ($p<0.05$).

Results

The results of the first experiment are presented in Tables 1 and 2 and Fig. 1. There were no significant differences among the initial weight of the different groups ($p>0.05$) (Table 1). With reference to the parameters egg mass weight, EPI, and NI, the values obtained for the control group were of 119.82 mg, 59.16%, and 74.16%, respectively, which are higher numbers than those found for treated groups, resulting in highly significant differences between the treatments and the witness group ($p<0.01$) (Table 1).

Pre-oviposition period of treated groups did not present significant differences in relation to the control ($p>0.05$) (Table 2). Oviposition and survival periods of treated groups varied from 4.50 to 5.50 days and from 11.10 to 13.50 days, respectively, being statistically different from the values obtained for the control group (13.89 to 17.44 days) (Table 2). Hatching percentage of treated groups varied between 12.44% and 11.33%, differing statistically ($p<0.01$) from the control (91.64%) (Table 2).

The best control efficacy was of 99%, obtained in thymol concentration of 2.0%, and in the other concentrations (1.0% and 1.5%) efficacy was of 98% (Fig. 1). It is worth to

Table 3 Hatching percentage of larvae of *R.* (*B.*) *microplus* originating from eggs treated with different concentrations of thymol under laboratory conditions (27±1°C and UR > 80 ± 10%)

Treatments	Water + DMSO	Thymol 1%	Thymol 1.5%	Thymol 2%
% of hatching	96.3 a±2.4 (10)	94.0 a±5.9 (10)	95.3 a±6.0 (10)	93.8 a±4.2 (10)

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n sample size

emphasize that all treatments reached a percentage of control higher than 95%.

The results obtained in the second experiment are presented in Table 3. Hatching percentage of treated groups varied from 93.8% to 95.3%, being statistically similar ($p>0.05$) to the value obtained for the control group (96.3%) (Table 3).

Discussion

There is a correlation between the initial weight of engorged females of bovine ticks and the parameters referring to the oviposition process (Borges et al. 2001; Santos and Furlong 2002). The inexistence of statistical differences among the initial weight of engorged females in different groups makes it evident that the other alterations in the analyzed parameters are probably correlated with the thymol effect (Table 1).

Thymol, in every concentration used, interfered significantly on most of the analyzed parameters. Values referring to egg mass weight, EPI, and NI in the treated groups were lower than those obtained for the control group, proving that thymol had deleterious action in the oviposition process, interfering on the conversion of ingested blood into eggs (Table 1). Monteiro et al. (2009), evaluating the efficacy of different concentrations of thymol (0.25%, 0.5%, 1.0%, 1.5%, and 2.0%) on females of *R. sanguineus*, noticed that the egg mass weight, EPI, and NI were not affected in any of the treatments.

Thus, as observed by Monteiro et al. (2009), the use of different concentrations of thymol did not cause any alterations during pre-oviposition period (Table 2), demonstrating that this monoterpene was not able to reduce nor to accelerate the beginning of the oviposition process. Oviposition and survival periods were affected in all treatments. However, reduction was more accentuated during oviposition period (Table 2), when it was noticed that, for many times, females which were still alive were not able to produce eggs. These parameters were not analyzed for *R. sanguineus* in the study conducted by Monteiro et al. (2009); thus, this comparison is not capable of being accomplished.

Hatching percentage was affected in the first experiment, and it was lower than the control values (Table 2), a fact that was not observed in the study conducted by Monteiro et al. (2009). However, in the second experiment, when eggs were treated directly, thymol did not affect significantly this parameter, wherein similar values for hatching percentage in treatments and the control were being noticed (Table 3). This way, it can be inferred that thymol did not have a direct deleterious action in egg incubation, but it did in the ovoposition and/or embryonic processes. Interferences on these processes may lead to the production of inviable eggs (Pereira and Labruna 2008). For better understanding the real effect of thymol on egg viability of *R.* (*B.*) *microplus*, it is necessary to perform studies containing histological analyses of the structures involved in this process.

The percentage of control obtained in the concentration of 1.0% was of 98% (Table 1), higher than that demonstrated by Monteiro et al. (2009), where the best control efficacy for *R. sanguineus* was of 41%, obtained with the concentration of thymol at 2.0%. Previous studies had already demonstrated that non-engorged larvae of bovine ticks are more susceptible to the effect of thymol than larvae of *R. sanguineus* (Novelino et al. 2007a, b; Daemon et al. 2009), and this also is evidenced regarding engorged females of these ixodides, since the lowest concentration of thymol used in the present work resulted in a control efficiency higher than that obtained with the use of the highest concentration (2.0%) for *R. sanguineus*. The fact that it is a tick that originated from xeric regions may give *R. sanguineus* higher resistance, allowing better impermeability of the cuticle (Daemon et al. 2009).

These results added to the previous works developed by this group of research (Novelino et al. 2007a, b) and may conclude that thymol has acaricide activity on different stages of *R.* (*B.*) *microplus* (non-engorged larvae and engorged females) and repellent activity on non-engorged larvae. Since most of the acaricides commercially available show efficacies lower than 70% (Furlong et al. 2007), thymol may be indicated as a promising alternative to be used in the control of bovine ticks. In this context, new research strategies will be focused on the formulation of stable and less volatile dilutions in environmental conditions and field tests with stabulated animals with the aim of developing new technologies able to be used in the strategic control of this ixodide, taking into account the efficacy of the product, the reduction on production costs, and less environmental pollution.

References

Baladrin NF, Klocke JA, Wurtle ES, Bollinger WH (1985) Natural 259 plant chemicals: sources of industrial and medical materials. Science 228:1154–1660

Bennett GF (1974) Oviposition of *Boophilus microplus* (Canestrini, 1887) (Acarina: Ixodidae): influence of tick size on egg production. Acarol 16:52–61

Borges LMF, Carneiro JR, Gomes AG, Moreira PC (2001) Influência do peso inicial e da estação do ano na conversão em ovos de fêmeas de *Boophilus microplus* (Acari: Ixodidae). Cienc Animal Bras 2:127–131

Chagas ACS (2004) Controle de parasitas utilizando extratos vegetais. Rev Bras Parasitol Vet 13:156–160

Daemon E, Monteiro CMO, Rosa LS, Clemente MA, Arcoverde A (2009) Evaluation of the acaricide activity of thymol on engorged and unengorged larvae of Rhipicephalus sanguineus (Latreille, 1808) (Acari: Ixodidae). Parasitol Res 105:495–497

Drummond RO, Ernest SE, Trevino JL, Gradney WJ, Graham OH (1973) *Boophilus anulatus* and *Boophilus microplus*: laboratory tests of insecticides. J Econ Entomol 66:30–133

Ferreira P, Soares GLG, D'avila S, ECA BESSA (2009) The influence of caffeine and thymol on the survival, growth and reproduction of *Subulina octona* (BRUGÜIÈRE, 1789) (MOLLUSCA, SUBULINIDAE). Braz Arch Biol Technol 52(4):945–952

Furlong J, Martins JRS, Prata MCA (2004) Controle estratégico do carrapato dos bovinos. A Hora Vet 23:53–56

Furlong J, Martins JRS, Prata MCA (2007) O carrapato dos bovinos e a resistência: temos o que comemorar? Controle estratégico do carrapato dos bovinos. A Hora Vet 27:53–56

Grisi L, Massard CL, Moya-Borja GE, Pereira JB (2002) Impacto econômico das principais ectoparasitoses em bovinos no Brasil. A Hora Vet 21:8–10

Imdorf A, Kilchenman V, Bogdanov S (1995) Toxiziät von thymol; campher, menthol and eucaliptol auf Varroa jacobsoni oud ind Apis mellifera L. in labortest. Apidol 26:27–31

Jonsson NN, Piper EK (2007) Integrated control programs for ticks on catle, The University of Queensland, Queensland

Labruna MB (2008) Combate contra *Rhipicephalus* (*Boophilus*) *microplus*. In: Pereira MC, Labruna MB, Szabo MPJ, Klafke GM (eds) *Rhipicephalus* (*Boophilus*) *microplus:* Biologia, Controle e Resistência. MEDVET, São Paulo, pp 57–64

Mansour SA, Messehea SS, El-Gengaihi SE (2000) Botanical biocides. 4. Mosquitocidal activity of certain *Thymus capitatus* constituents. J Nat Toxins 9:49–62

Martins JRS, Furlong J, Leite RC (2006) Controle de carrapatos. In: Barros-Battesti DMB, Arzua M, Bechara GH (eds) Carrapatos de importância médico-veterinária da Região Neotropical. Um guia ilustrado para a identificação de espécies. Instituto Butantan, São Paulo, pp 145–153

Monteiro CMO, Daemon E, Clemente MA, Rosa LS, Maturano R (2009) Acaricidal efficacy of thymol on engorged nymphs and females of *Rhipicephalus sanguineus* (LATREILLE, 1808) (ACARI: IXODIDAE). Parasitol Res 105:1093–1097

Novelino AMS, Daemon E, Soares GLG (2007a) Evaluation of acaricide effect of thymol, menthol, salycilic acid and methyl salycilate on Boophilus microplus (Canestrini, 1887) (Acari: Ixodidae) larvae. Parasitol Res 101:809–811

Novelino AM S, Daemon E, Soares GLG (2007b) Avaliação da atividade repelente do timol, mentol, salicilato de metila e ácido salicílico sobre larvas de *Boophilus microplus* (CANESTRINI 1887) (ACARI: IXODIDAE). Arq Bras Med Vet Zootec 59:700–704

Pereira MC, Labruna MB (2008) *Rhipicephalus* (*Boophilus*) *microplus*. In: Pereira MC, Labruna MB, Szabo MPJ, Klafke GM (eds) *Rhipicephalus* (*Boophilus*) *microplus:* Biologia, Controle e Resistência. MEDVET, São Paulo, pp 15–56

Santos AP, Furlong J (2002) Competição intraespecífica em *Boophilus microplus*. Cienc Rural 32:1033–1038

Walker AR, Bouarttour A, Camicas JL, Estrada-Peña A, Horak I, Latif A, Pegram R, Preston P (2003) Ticks of Domestic Animals in Africa. A Guide to identification of Species. University of Edinburgh, Edinburgh