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EFEITOS DE CARVACROL E TIMOL, LIVRES OU EM COMBINAÇÃO COM CIPERMETRINA, SOBRE O CARRAPATO *Rhipicephalus microplus*

CAIO PAVÃO TAVARES

São Luís
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Orientador (a): Prof. Dr. Livio Martins Costa Junior

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Dedico à minha mãe, família e amigos.

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RESUMO

O carrapato bovino, *Rhipicephalus microplus*, é um ectoparasito de grande importância econômica na pecuária bovina. A ocorrência e disseminação deste carrapato são controladas principalmente com carrapaticidas sintéticos, no entanto, este método de controle está associado a vários problemas, incluindo a seleção de populações multirresistentes. Novos compostos carrapaticidas, incluindo produtos de origem natural, devem ser caracterizados. Os monoterpenos, carvacrol e timol, se apresentam como potencial alternativa carrapaticida, tanto isolados quanto combinados com outras moléculas; entretanto, tanto o mecanismo de ação desses monoterpenos quanto os mecanismos envolvidos no efeito sinérgico desses compostos, precisam ser melhor elucidados em carrapatos. O presente estudo tem como objetivo avaliar a eficácia e o potencial modo de ação da combinação de cipermetrina e timol em carrapatos de duas populações com diferentes níveis de suscetibilidade à cipermetrina (baixa e alta suscetibilidade), assim como avaliar os efeitos de carvacrol e timol sobre as enzimas antioxidantes e detoxificantes das larvas dessas mesmas populações. A determinação da atividade carrapaticida de cipermetrina e timol em larvas foi realizada em diferentes concentrações. As fêmeas ingurgitadas foram divididas em cinco grupos experimentais (n = 20): 1) Grupo controle não tratado; 2) Grupo controle: 2,0% (v/v) DMSO; 3) Grupo timol: 1300 µg/mL de timol; 4) Grupo Cipermetrina: 3700 µg/mL de cipermetrina; 5) Associação de cipermetrina (3700 µg/mL) + timol (1300 µg/mL). Um subgrupo foi utilizado para determinar o percentual de controle e outro subgrupo, com dez adultos de cada tratamento, foi utilizado para quantificar timol e cipermetrina por análise cromatográfica de HPLC. Carvacrol e timol foram testados em concentrações variando de 0,14 a 5,0 mg·mL-1 em ambas as populações para determinar as CL_1, CL_{25}, CL_{50} e CL_{75}. Larvas de ambas as populações de *R. microplus* foram tratadas com as quatro concentrações de cada monoterpeno, para a determinação da atividade das enzimas glutationa-S-transferase (GST), catalase (CAT), superóxido dismutase (SOD) e glutationa peroxidase (GPX). Todos os compostos testados foram eficazes em larvas de ambas as populações, e a combinação com timol diminuiu a CL_{50} da cipermetrina (232,4 a 52,7 µg/mL) na população de baixa suscetibilidade. A combinação de timol e cipermetrina também foi efetiva em teleóginas, com percentuais de controle superior ao grupo de teleóginas tratadas apenas com cipermetrina. A partir das análises de HPLC, uma concentração mais alta de cipermetrina (pop. 1: 30,3 ± 6,9 e pop. 2: 45,4 ± 17,7 ng/mg) foi detectada nos tecidos de fêmeas ingurgitadas tratadas com a combinação em comparação com as concentrações do analito nos grupos tratados com apenas cipermetrina (pop. 1: 12,4 ± 4,4 pop. 2: 25,5 ± 9,4 ng/mg). Larvas de *R. microplus* da população Jaguar tratadas com diferentes concentrações letais de carvacrol e timol apresentaram um aumento dose-dependente de CAT, GPX, SOD e GST após o tratamento com CL_{25}. Além disso, as larvas tratadas com CL_{75} apresentaram os maiores níveis de atividade enzimática para carvacrol (1,76 $mg \cdot mL^{-1}$) e timol (1,32 $mg \cdot mL^{-1}$). A atividade de CAT, GPX, SOD e GST em larvas da população Porto Alegre tratadas com carvacrol e timol também aumentou significativamente até a CL_{50} de cada

monoterpeno. No entanto, na CL_{75} de carvacrol e timol, observou-se diminuição da atividade de todas as enzimas. De acordo com os resultados obtidos nesta tese, demonstramos pela primeira vez a eficácia carrapaticida da combinação de timol + cipermetrina em *R. microplus*, e que a presença de timol aumenta a concentração de cipermetrina nos tecidos internos de fêmeas ingurgitadas através de um possível mecanismo para aumentar a penetração de cipermetrina ao nível cuticular. Da mesma forma, demonstramos pela primeira vez o efeito do carvacrol e do timol em importantes enzimas antioxidantes e detoxificantes do carrapato *R. microplus*.

Palavras-chave: Monoterpeno; Piretroide; Penetração cuticular; Sistema antioxidante; Sistema detoxificante; Carrapato bovino.

ABSTRACT

The cattle tick, *Rhipicephalus microplus*, is an ectoparasite of great economic importance in livestock. The occurrence and spread of this tick are mainly controlled using synthetic acaricides. However, this control method is associated with several problems, including the selection of multiresistant populations. New acaricide compounds, including products of natural origin, must be characterized. The monoterpenes, carvacrol and thymol, present themselves as a potential alternative acaricide, both isolated and combined with other molecules; however, the mechanism of action of these monoterpenes, as well as the mechanisms involved in the synergistic effect of these compounds, need to be better elucidated in ticks. The present study aims to evaluate the efficacy and potential mode of action of the combination of cypermethrin and thymol in ticks from two populations with different levels of susceptibility to cypermethrin (low and high susceptibility), as well to evaluate the effects of carvacrol and thymol on the antioxidant and detoxifying enzymes of larvae from the same populations. The determination of the acaricide activity of cypermethrin and thymol in larvae was performed at different concentrations. The engorged females were divided into five experimental groups (n = 20): 1) Untreated control group; 2) Control group: 2.0% (v/v) DMSO; 3) Thymol group: 1300 µg/mL thymol; 4) Cypermethrin Group: 3700 µg/mL of cypermethrin; 5) Combination of cypermethrin (3700 µg/mL) + thymol (1300 µg/mL). A subgroup was used to determine the control percentage and another subgroup, with ten adults from each treatment, was used to quantify thymol and cypermethrin by HPLC chromatographic analysis. Carvacrol and thymol were tested at concentrations ranging from 0.14 to 5.0 $mg \cdot mL^{-1}$ in both populations to determine LC_1, LC_{25}, LC_{50}, and LC_{75}. Larvae of both populations of *R. microplus* were treated with four letal concentrations of each monoterpene to determine the activity of the enzymes, glutathione-S-transferase (GST), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX). All compounds tested were effective on larvae from both populations, and the combination with thymol decreased the LC50 of cypermethrin (232.4 to 52.7 µg/mL) on the low-susceptibility population. The combination of thymol and cypermethrin was also effective in engorged females, with a higher control percentage than the group of ticks treated with cypermethrin alone. From HPLC analyzes, a higher concentration of cypermethrin (pop. 1: 30.3 ± 6.9 and pop. 2: 45.4 ± 17.7 ng/mg) was detected in the tissues of engorged females treated with the combination compared to analyte concentrations in groups treated with cypermethrin only (pop. 1: 12.4 ± 4.4 pop. 2: 25.5 ± 9.4 ng/mg). *R. microplus* larvae from the Jaguar population treated with different lethal concentrations of carvacrol and thymol displayed a dose-dependent increase in CAT, GPX, SOD, and GST after treatment with LC_{25}. Further, larvae treated with the LC_{75} had the highest levels of enzyme activity for carvacrol (1.76 $mg \cdot mL^{-1}$) and thymol (1.32 $mg \cdot mL^{-1}$). CAT, GPX, SOD, and GST activity in Porto Alegre strain larvae treated with carvacrol and thymol also increased significantly up to the LC_{50} of each monoterpene. However, at the LC_{75} of carvacrol and thymol, a decrease in the activity of all enzymes was observed. According to the results obtained in this thesis, we demonstrated for the first time the

acaricide effect of the combination of thymol + cypermethrin in *R. microplus*, and that the presence of thymol increases the concentration of cypermethrin in the internal tissues of engorged females through a possible mechanism for increase the penetration of cypermethrin at the cuticular level. Likewise, we demonstrated for the first time the effect of carvacrol and thymol on important antioxidant and detoxifying enzymes in the tick *R. microplus*.

Keywords: Monoterpene; Pyrethroids; Cuticular penetration; Antioxidant system; Detoxifying system; Cattle tick.

SUMÁRIO

LISTA DE ABREVIATURAS E SIGLAS

CAT: Catalase
CTE: Cadeia transportadora de elétrons
EROS: Espécies reativas de oxigênio
GABA: Ácido gama-aminobutírico
GPX: Glutationa peroxidase
GST: Glutationa-S-transferase
H_2O_2: Peróxido de hidrogênio
HO: radical hidroxila
O_2^-: Ânion superóxido
O_2: Oxigênio molecular
OH^-: ânion hidroxila
SOD: Superóxido dismutase

CAPÍTULO I

%Rhatch: Percentage of hatching reduction
%Rovip: Percentage of reduction in oviposition
°C: Degrees Celsius
µg: microgram
µL: microliter
µm:micrometer
AChe: Enzyme acetylcholinesterase
AIT: Adult Immersion Test
ANOVA: analysis of variance
ATP: Adenosine Triphosphate
B.O.D: Biochemical oxygen demand
C%: Control percentage
CI: Confidence interval
cm: centimeter
DMSO: Dimethyl sulfoxide
EPI: Egg production index
EW: Egg mass Weight
FW: Females Weight
g: gram
h: hour
HPLC: High Performance Liquid Cromatography
HR: Egg hatchability rate
LC_{50}: 50% lethal concentration
mg: milligram
min: minutes
mL: milliliter
mm: millimeter
ng: nanogram

nm: nanometer
PBO: Piperonyl butoxide
R^2: Regression Correlation Coefficient
RH: Relative Humidity
SD: Standard Deviation
UV: Ultraviolet

CAPÍTULO II

°C: Degrees Celsius
µL: microliter
µM:microMolar
AIT: Adult Immersion Test
B.O.D: Biochemical oxygen demand
CAT: Catalase
CDNB: 1-chloro-2,4-dinitrobenzene
cm: centimeter
CO_2: Carbon Dioxide
E64: trans-Epoxysuccinyl-L-leucylamido(4-guanidino)butane
EC_{50}: Effective concentration
EDTA: Ethylenediamine tetracetic acid
ETC: Electron transport chain
GABA: Gamma aminobutyric acid
GPX: Glutathione peroxidase
GST: Glutathione-S-transferase
h: hour
H_2O_2: Hydrogen Peroxide
HO: Hydroxyl radical
INT: 2-(4-iodophenyl) 3-(4-nitrophenol) 5-phenyl- tetrazolium chloride
JAG: Jaguar population
LC_1: 1% lethal concentration
LC_{25}: 25% lethal concentration
LC_{50}: 50% lethal concentration
LC_{75}: 75% lethal concentration
LPO: Lipid peroxidation
M: Molar
mg: milligram
min: minutes
mL: milliliter
mM:milliMolar
MNP: Mitochondrial membrane potential
NaCl: Sodium Chloride
NaH_2PO_4: Monosodium phosphate
nm: nanometer
O_2^-: Superoxide anion

PBS: Phosphate Buffered Saline
pH: Hydrogen potential
POA: Porto alegre population
POX: Peroxidase
R^2: Regression Correlation Coefficient
RH: Relative Humidity
ROS: Reactive oxygen species
SE: Standard Error
SOD: Superoxide dismutase
TPKC: N-tosil-fenilalanina clorometil cetona
U/µg: Unit per microgram
U/g: Unit per gram
U/mg: Unit per milligram

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1. INTRODUÇÃO

O carrapato bovino, *Rhipicephalus microplus*, é o ectoparasito responsável pelas maiores perdas econômicas na pecuária bovina, atingindo a marca de US$3.24 bilhões por ano, apenas no Brasil. Por conta do seu hábito hematófago, *R. microplus* pode causar anemia, perda de peso e danos à pele do animal, assim como transmitir agentes patogênicos e conferir riscos de infecções secundárias. Estes prejuízos econômicos ainda se relacionam com os gastos para o controle do carrapato. Este controle é realizado principalmente através do uso de carrapaticidas sintéticos, no entanto, o uso constante e às vezes indiscriminado desses produtos tem causado forte pressão seletiva, acelerando o surgimento de populações resistentes à todas as classes químicas disponíveis no mercado.

Diante deste cenário, a busca por novas tecnologias de controle do carrapato, se faz extremamente necessária. Estratégias como o uso de novas moléculas, assim como a combinação de moléculas vêm sendo bastante exploradas na pesquisa básica afim de serem desenvolvidos novos produtos. Os monoterpenos carvacrol e timol, moléculas naturais encontradas nos óleos essenciais (OEs), se caracterizam como alternativas promissoras para o controle de *R. microplus*. OEs e seus constituintes podem atuar como sinergistas, potencializando o efeito de outras moléculas. O efeito sinérgico observado na combinação de monoterpenos e fenilpropanóides tem se caracterizado como uma importante estratégia para o desenvolvimento de produtos carrapaticidas

O mecanismo de ação dos monoterpenos, sobre carrapatos e insetos, envolve a inibição do receptor de tiramina, inibição da enzima acetilcolinesterase, e modulação dos receptores GABA. Alguns mecanismos envolvidos no efeito sinérgico de compostos naturais são: Efeitos em diferentes sítios alvo; Aumento de solubilidade e penetração; Inibição de enzimas detoxificantes; e neutralização de efeitos adversos.

No entanto, os mecanismos de ação e efeitos fisiológicos do carvacrol e do timol, assim como os mecanismos envolvidos nos efeitos sinérgicos dessas

moléculas, principalmente em carrapatos, foram muito pouco esclarecidos.

Neste sentido, acreditamos que devido ao efeito carrapaticida já relatado para os monoterpenos carvacrol e timol, podem ocorrer diversas alterações a nível bioquímico e fisiológico em *R. microplus*, o que pode elucidar possíveis mecanismos de ação dessas moléculas. Além disso, supomos que a combinação de um monoterpeno com piretróides sintéticos pode potencializar o efeito destes carrapaticidas por algum dos mecanismos descritos anteriormente.

O objetivo deste estudo foi avaliar os efeitos do carvacrol e do timol na atividade enzimática da superóxido dismutase (SOD), catalase (CAT), glutationa S-transferase (GST) e glutationa peroxidase (GPX) em *R. microplus*. Além de avaliar o efeito da combinação de cipermetrina e timol em carrapatos de duas populações com diferentes níveis de suscetibilidade à cipermetrina (baixa e alta suscetibilidade), determinando a eficácia e penetração cuticular dos compostos em fêmeas, como um potencial modo de ação dessa combinação de moléculas.

Este estudo gerou dos artigos, os quais foram foram publicados no periódico Ticks and Tick-borne Diseases (Qualis B1 em Medicina I; Fator de Impacto: 3.744 - Doi: https://doi:10.1016/j.ttbdis.2021.101874; Doi: https://doi.org/10.1016/j.ttbdis.2022.101929).

2. REFERENCIAL TEÓRICO

2.1. O carrapato *Rhipicephalus microplus*

Os carrapatos (classe Arachnida, ordem Ixodida) são artrópodes ectoparasitos, de nutrição hematófaga e com alto grau de especificidade, podendo, no entanto, apresentar hopedeiros alternativos como o ser humano, caracterizando estes parasitos como organismos de grande importância para a saúde pública e animal, tendo em vista que podem ser vetores de diversos agentes etiológicos causadores de doenças como babesiose, erliquiose, febre maculosa e doença de

Lyme (MASSARD; FONSECA, 2004; ARAÚJO et al., 2016).

Os carrapatos são divididos em três famílias distintas: Ixodidae, Argasidae e Nuttalliellidae, compreendendo cerca de 950 espécies descritas (DANTAS-TORRES et al. 2019; GARCIA et al., 2019). Especificamente, a família Ixodidae compreende os “carrapatos duros”, sendo composta por 14 gêneros, dos quais, 5 são encontrados na região neotropical, sendo eles: *Amblyomma, Dermacentor, Haemaphysalis, Ixodes e Rhipicephalus*. Esta família é a que compreende o maior número de espécies de carrapatos (NAVA et al., 2017).

R. microplus pertence ao gênero *Rhipicephalus*, o qual possui 82 espécies descritas (GUGLIELMONE et al., 2010). Esta espécie, foi classificada anteriormente como *Boophilus microplus*, sendo reclassificada mediante análises filogenéticas, passando a ser descrita como *R. (Boophilus) microplus*. O gênero *Boophilus* foi reclassificado como subgênero, que ainda possui outras espécies, como *R.* (Boophilus) *annulatus* e *R.* (Boophilus) *australis* (BURGER et al., 2014).

O carrapato *R. microplus*, popularmente conhecido como carrapato-do-boi, é de origem asiática e difundiu-se por vários países de clima tropical e subtropical, apresentando atualmente um ampla distribuição geográfica. Com relação à sua biologia, é um carrapato monoxeno, que parasita preferencialmente bovinos, mas podem parasitar outras espécies como equídeos, ovinos e cervídeos (ROCHA, 1984; GARCIA et al., 2019).

O carrapato-do-boi é o ectoparasito de maior importância econômica para a pecuária bovina nas regiões tropicais e subtropicais do mundo, por ocasionar perdas econômicas relacionadas à redução na produção de leite e no ganho de peso vivo, aumento nas taxas de mortalidade dos rebanhos, danos à pele dos animais, custo referentes ao seu controle e pelos efeitos da transmissão de hemoparasitas como *Babesia bigemina, Babesia bovis* e *Anaplasma marginale*, dos quais pode ser vetor (RODRIGUEZ-VIVAS et al., 2018).

R. microplus apresenta um ciclo de vida (Figura 1) dividido em duas etapas distintas: uma fase parasitária com período médio de 21 dias sobre um único hospedeiro e uma fase não parasitária que ocorre no solo com duração média de

dois a três meses, dependendo fundamentalmente das condições climáticas existentes. Em locais com estações do ano bem definidas, esta fase costuma ser menor no verão e na primavera (meses mais quentes) (GONZALES et al., 1974; PEREIRA; LABRUNA, 2008; GARCIA et al., 2019).

Durante a fase parasitária, o carrapato apresenta três estágios morfologicamente distintos: larva, ninfa e adulto. A larva apresenta três pares de patas, é bastante ativa, pois necessita encontrar o hospedeiro para nele se fixar, e sobrevive das reservas energéticas acumuladas na fase de ovo enquanto realiza a busca por um hospedeiro. Fixa-se no hospedeiro como o auxílio de estruturas, como as quelíceras, as quais seccionam a pele para a introdução do hipostômio, órgão responsável pela fixação da larva na pele do hospedeiro (GONZALES et al., 1974).

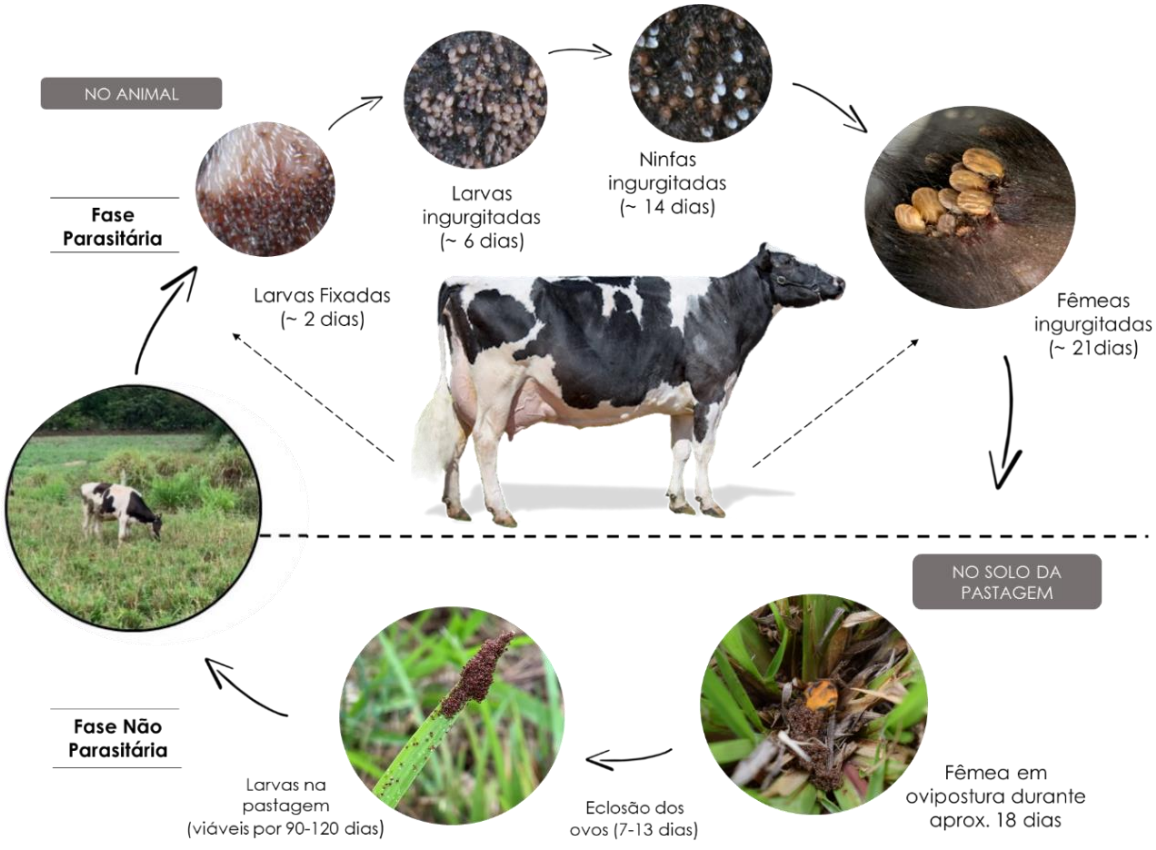

Figura 1: Ciclo de vida do carrapato bovino *Rhipicephalus microplus*. Ilustração: Isabella C. Sousa.

A larva inicia o processo de desenvolvimento e crescimento tegumentário com a alimentação, e entre o quarto e quinto dia (período de inércia), atinge a fase de metalarva. Por volta do sexto dia, já alimentada (ingurgitada) é formada uma nova estrutura, com um outro tegumento, mais um par de patas e uma fileira de dentição do hipostômio entre outras alterações: é a fase de ninfa. Esta fase dura em média dois a quatro dias e ao continuar seu desenvolvimento, uma nova alteração no exoesqueleto se processa, havendo um período igual de inatividade, denominado de metaninfa (GONZALES et al., 1974).

Em torno do décimo segundo dia surge o indivíduo adulto, sexualmente diferenciado, iniciando o processo de maturação dos machos e das fêmeas. Por volta do décimo sétimo dia os machos já estão aptos à cópula. As fêmeas, após serem fecundadas, passam de metaninfa para neógina num período médio de 17 dias. Em seguida, em um período de três dias, passam a partenógena (parcialmente ingurgitada) e em mais dois dias, a teleógina (ingurgitamento máximo) (GONZALES et al., 1974). Após a alimentação, as fêmeas chegam a apresentar um tamanho cerca de 10 vezes superior ao dos machos, se desprendem dos hospedeiros e caem no solo, concluindo um ciclo de aproximadamente 21 dias (fase parasitária).

Com a queda da fêmea ingurgitada se inicia a fase não parasitária do ciclo de *R. microplus*, fase extremamente dependente de fatores ambientais (temperatura e umidade). Em condições climáticas adequadas, logo após a queda, ocorre o período de pré-postura (com duração de 3 a 5 dias), tempo necessário para que ocorra maturação dos ovários, produção e maturação dos ovos. Em seguida, a ovipostura se inicia, com duração média de 18 dias. E após esse período, a fêmea morre, finalizando assim seu ciclo de vida. Os ovos passam por um processo de incubação até a eclosão e surgimento das larvas, que após alguns dias, tornam-se viáveis e sobem para a ponta da pastagem à espera de um hospedeiro para que possam se fixar. Cada teleógina pode converter até 50% de seu peso corporal em massa de ovos, e realizar oviposturas de aproximadamente 3.000 ovos (GARCIA et al., 2019).

2.2. Controle de *R. microplus* e o problema da resistência

O uso de carrapaticidas sintéticos das classes dos organofosforados, carbamatos, formamidinas, piretróides, lactonas macrocíclicas, fenilpirazóis e benzoilfeniluréias, constitui a principal forma de controle do carrapato bovino (PETER et al., 2005, BAFFI et al., 2008; RODRÍGUEZ-VIVAS et al., 2010, PRULLAGE et al., 2011; RODRÍGUEZ-VIVAS et al., 2018). Estes compostos agem de forma sistêmica, após absorção pelos tecidos do hospedeiro, ou por contato direto com os parasitos alvo após aplicação externa (RODRÍGUEZ-VIVAS et al., 2014). No entanto, o uso constante, às vezes indiscriminado e inadequado desses carrapaticidas tem causado forte pressão seletiva sobre populações de *R.* microplus, acelerando o surgimento de populações resistentes às principais classes disponíveis no mercado, podendo levar também a problemas como contaminação ambiental e resíduos em alimentos (leite e carne) (THULLNER, 1997; PEREIRA; LABRUNA, 2008; GARCIA et al., 2019).

A resistência a um produto consiste no aumento significativo do número de indivíduos dentro de uma população de parasitos, capazes de tolerar dose/concentrações de uma droga que se mostrou letal para a maioria dos indivíduos da mesma espécie (FAO, 2004). Diante deste cenário de resistência, é comum se verificar a utilização de compostos em concentração acima do recomendado pelos fabricantes e um aumento na frequência de tratamentos realizados na tentativa de controlar o carrapato, tornando a situação ainda mais crítica (FURLONG et al., 2007). O manejo inadequado de carrapaticidas, incluindo erros de aplicação, o uso constante de formulações em spray, alta frequência de tratamentos, início dos tratamentos somente após a observação de fêmeas ingurgitadas e intervalo incorreto entre os tratamentos, são fatores que contribuem para o surgimento de populações resistentes (VILELA et al., 2020).

Nas últimas décadas o fenômeno da resistência aos carrapaticidas tem se mostrado generalizado, e países como Brasil (FURLONG, 1999; CASTRO-JANER et al., 2010; FAZA et al., 2013; MENDES et al., 2013; LOPES et al., 2014; RECK et al.,

2014; VILELA et al., 2020), México (RODRÍGUEZ-VIVAS ET AL., 2012; HIGA et al., 2020) e Índia (SHARMA et al., 2012; SHYMA et al., 2015, GODARA et al., 2019) onde são encontrados diversos relatos da existência de populações resistentes de *R. microplus*.

Vários mecanismos podem estar envolvidos no processo de resistência a carrapaticidas em *R. microplus*, mas a resistência metabólica e a insensibilidade do sítio-alvo são os mais estudados (GUERRERO et al., 2012; KUMAR et al., 2020).

Estudos têm demonstrado a ocorrência de populações de *R. microplus* resistentes a um ou vários compostos carrapaticidas sintéticos (populações multiressistentes) (RECK et al., 2014; KLAFKE et al., 2016; RODRIGUEZ-VIVAS et al., 2018), o que tem evidenciando ainda mais a necessidade de implantação e desenvolvimento de medidas de controle estratégico para *R. microplus*, as quais podem incluir manejo de pastagens, utilização de plantas desfavoráveis ao carrapato, seleção de hospedeiros resistentes, utilização de formulações mistas (diferentes grupos químicos) e utilização de produtos naturais para controle do carrapato (RODRÍGUEZ-VIVAS et al., 2018).

2.3. Metabólitos secundários de plantas como alternativa para o controle do carrapato

Formulações carrapaticidas utilizando compostos de origem vegetal caracterizam-se como uma importante alternativa ao uso exclusive de compostos químicos sintéticos, pois têm apresentado uma alta toxicidade sobre carrapatos com o mínimo de efeitos adversos em animais e seres humanos (NEVES et al., 2011; PEIXOTO et al., 2015).

A atividade carrapaticida dessas substâncias tem sido atribuída principalmente aos óleos essenciais e extratos, os quais apresentam efeito sobre carrapatos dos gêneros *Amblyomma*, *Rhipicephalus, Dermacentor*, *Ixodes*, *Hyalomma* e Argas (GOMES et al., 2012; GARCIA et al., 2012; DE OLIVEIRA-CRUZ et al., 2013; KOC et al., 2013; DANTAS et al., 2016; MUYOBELA et al., 2016).

Grande parte destes óleos essenciais tiveram as suas composições químicas determinadas, identificando diversos metabólitos secundários (terpenos, estilbenos, cumarinas, ácidos, álcoois, compostos sulfurados e aldeídos) que foram associados aos efeitos carrapaticidas destes produtos vegetais (RIBEIRO et al., 2008; CETIN et al., 2010; GOMES et al., 2012).

De acordo com a pressão de sistemas naturais sobre as plantas, o metabolismo destes organismos produz uma série de moléculas chamadas de metabólitos secundários, que são substâncias envolvidas na proteção contra bactérias, vírus, fungos, herbívoros e outras plantas parasitas e concorrentes (WINK, 2008; SCHAFER; WINK, 2009).

Por conta das diversas atividades biológicas sobre microrganismos e artrópodes, assim como aparesentarem uma variação estrutural muito maior do que os obtidos por síntese química, os metabólitos secundários de plantas têm se caracterizado como uma alternativa promissora ao uso de produtos químicos sintéticos para o controle de carrapatos (CRUZ et al., 2013; ROSADO-AGUILAR et al., 2017).

De todos os metabólitos secundários vegetais já conhecidos, os que apresentam maior número de estudos sobre o potencial carrapaticida, são os monoterpenos timol e carvacrol. Já, quando se refere à espécie de carrapato, os estudos da atividade carrapaticida desses metabólitos têm se concentrado sobre as espécies *R. microplus* e *Rhipicephalus sanguineus*, fato este que se justifica por conta da grande importância econômica e para a saúde pública que estas duas espécies apresentam.

O timol ($C_{10}H_{14}O$) é um monoterpeno fenólico derivado do cimeno e quimicamente conhecido como 2-isopropil-5-metil-fenol (Figura 2). As principais fontes naturais do timol são as espécies do gênero *Thymus*, dentre elas destacam-se *Thymus glandulosus* (Bouchra et al., 2003), *Thymus hyemalis* (GOODNER et al., 2006), *Thymus vulgaris* (LEE et al., 2005), e *Thymus zygis* (MOLDÃO et al., 2000), assim como *Lippia sidoides* e *Oreganum vulgare.* Este monoterpeno é o que mais tem sido estudado para a determinação dos seus efeitos sobre carrapatos.

O carvacrol ($C_{10}H_{14}O$) é um monoterpeno fenólico isômero com timol (Figura 2) predominante em muitas plantas aromáticas, caracterizando-se como o constituinte majoritário dos óleos essenciais de diversas espécies de plantas da família Lamiaceae (*Lippia, Satureja, Thymbra, Thymus, Origanum* e *Corydothymus*) (D'ANTUONO et al., 2000; KINTZIOS 2002; DE VINCENZI et al., 2004; SÖKMEN et al., 2004).

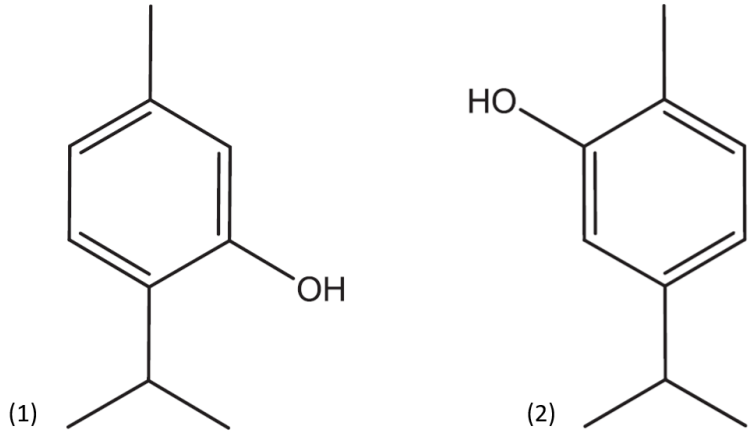

Figura 2: Estrutura dos monoterpenos timol (1) e carvacrol (2).

Os estudos que avaliam a atividade dos metabólitos secundários isolados sobre carrapatos, estão apresentados na Tabela 1, demonstrando que as investigações sobre essa temática são de grande relevância na busca por alternativas de controle dos carrapatos, mas que se caracteriza ainda como um vasto campo de exploração, principalmente no que se refere à elucidação dos mecanismos de ação sobre esses ectoparasitos.

Tabela 1. Metabólitos secundários de plantas que apresentam efeito sobre diferentes espécies, estágios e fases de carrapatos.

Metabólito Secundário	Carrapato	Estágio/Fase	Autor
Timol	*Rhipicephalus microplus*	Larva Ninfa ingurgitada Fêmea ingurgitada	Novelino et al. (2007); Monteiro et al. (2009); Monteiro et al. (2010); Scoralik et al. (2012); Hüe et al. (2014); Daemon et al. (2012b); De Oliveira Cruz et al. (2013); Matos et al. (2014a); Araújo et al. (2015); Costa-Júnior et al. (2016); Soares et al. (2016); Araújo et al. (2016); Novato et al. (2018); Lima et al. (2018); Cardoso et al. (2020); Penha et al. (2021); Tavares et al. (2022)
	Rhipicephalus sanguineus	Larva não ingurgitada Larva ingurgitada Ninfa não ingurgitada Ninfa ingurgitada Fêmea semi-ingurgitada Fêmea ingurgitada	Daemon et al. (2009); Daemon et al. (2012a); Senra et al. (2013b); Matos et al. (2014b); Araújo et al. (2016); Delmonte et al. (2017); Matos et al. (2019); Matos et al. (2020); Coelho et al. (2020); Monteiro et al. (2021)
	Rhipicephalus annulatus	Ovo Larva Fêmea ingurgitada	Arafa et al. (2020)
	Amblyomma cajennense	Larva não ingurgitada Larva ingurgitada Ninfa ingurgitada	Mendes et al. (2011); Senra et al. (2013b)
	Dermacentor nitens	Larva não ingurgitada	Daemon et al. (2012a); Novato et al. (2015)
	Amblyomma sculptum	Larva não ingurgitada	Novato et al. (2015); Vale et al. (2021)
	Ixodes ricinus	Ovos Larva não ingurgitada	Tabari et al. (2017)
Carvacrol	*Rhipicephalus microplus*	Larva Fêmea ingurgitada	De Oliveira-Cruz et al. (2013); Senra et al. (2013a); Concepción et al. (2013); Costa-Júnior et al. (2016); Ramírez et al. (2016); Soares et al. (2016); Araújo et al. (2016); Lima et al. (2017); Novato et al. (2018); Cardoso et al. (2020); Pereira Junior et al. (2020); Penha et al. (2021)
	Rhipicephalus sanguineus	Larva não ingurgitada Ninfa Fêmea semi-ingurgitada	Senra et al. (2013b); Araújo et al. (2016); De Souza et al. (2019); Lima-de-Souza et al. (2021)
	Rhipicephalus turanicus	Adulto	Koc et al. (2013)

	Amblyomma cajennense	Larva Ninfa	Senra et al. (2013b)
	Dermacentor nitens	Larva não ingurgitada	Senra et al. (2013a); Novato et al. (2015)
	Amblyomma sculptum	Larva não ingurgitada	Novato et al. (2015); Vale et al. (2021)
	Ixodes ricinus	Ovos Larva não ingurgitada	Tabari et al. (2017)
Eugenol	*Rhipicephalus microplus*	Larva Fêmea ingurgitada	Brown et al. (1998); Hüe et al. (2014); Araújo et al. (2016); Novato et al. (2018); Monteiro et al. (2012); Valente et al. (2013); Ferreira et al. (2017)
	Rhipicephalus sanguineus	Larva não ingurgitada Larva Ninfa Fêmea ingurgitada	Senra et al. (2013b); Araújo et al. (2016); Coelho et al. (2020); Monteiro et al. (2021)
	Amblyomma cajennense	Larva Ninfa	Senra et al. (2013b)
	Dermacentor nitens	Larva	Monteiro et al. (2012)
	Amblyomma sculptum	Larva não ingurgitada	Vale et al. (2021)
Mentol	*Rhipicephalus microplus*	Larva	Novelino et al. (2007)
Ácido Salicílico	*Rhipicephalus microplus*	Larva	Novelino et al. (2007); Concepción et al. (2013); Ramírez et al. (2016)
Salicilato de Metila	*Rhipicephalus microplus*	Larva	Novelino et al. (2007)
Cinamaldeído	*Rhipicephalus microplus*	Ovo Larva Fêmea ingurgitada	Senra et al. (2013a); Marchesini et al. (2020); Marchesini et al. (2021a); Marchesini et al. (2021b)
	Rhipicephalus sanguineus	Larva Ninfa	Senra et al. (2013b)
	Amblyomma cajennense	Larva Ninfa	Senra et al. (2013b)
	Amblyomma sculptum	Larva não ingurgitada	Novato et al. (2015)
	Dermacentor nitens	Larva não ingurgitada	Novato et al. (2015)

	Haemaphysalis longicornis	Larva Ninfa	Nwanade et al. (2021)
Linalool	*Rhipicephalus microplus*	Larva	Prates et al. (1998); Senra et al. (2013a)
	Ixodes ricinus	Ovos Larva não ingurgitada	Tabari et al. (2017)
Trans anetol	*Rhipicephalus microplus*	Larva	Senra et al. (2013a)
	Rhipicephalus annulatus	Larva Adulto	Aboelhadid et al. (2021)
Nerolidol	*Rhipicephalus microplus*	Larva Fêmea ingurgitada	Lage et al. (2015)
D-limoneno	*Rhipicephalus sanguineus*	Larva não ingurgitada	Prado-Rebolledo et al. (2017)
p-cymeno	*Rhipicephalus microplus*	Larva	Lima et al. (2018); Cardoso et al. (2020)
α-bisabolol	*Rhipicephalus microplus*	Ovo Larva Fêmea ingurgitada	Marchesini et al. (2020); Marchesini et al. (2021); Marchesini et al. (2021b)
γ-terpineno	*Rhipicephalus microplus*	Larva	Lima et al. (2018)
Citral	*Rhipicephalus microplus*	Larva Fêmea ingurgitada	Peixoto et al. (2015); Cardoso et al. (2020)
S-(+)-carvona R-(−)-carvona R-(+)-limoneno S-(−)-limoneno	*Rhipicephalus microplus*	Larva Fêmea ingurgitada	Peixoto et al. (2015)
1,8-Cineol	*Rhipicephalus microplus*	Larva Fêmea ingurgitada	Prates et al. (1998); Queiroz et al. (2020)
α-Pineno β-Pineno Hexanal Citronelol (+)-Cânfora Isopinocamfona	*Rhipicephalus microplus*	Larva	Prates et al. (1998)

2.4. Espécies reativas de oxigênio, desequilíbrio redox, enzimas antioxidantes e detoxificantes

Quando os organismos se encontram sob estresse oxidativo, geralmente interrompem alguns processos fisiológicos, como reprodução ou biossíntese, a fim de desenvolver respostas que previnam ou neutralizam as ações negativas das espécies reativas de oxigênio (EROs) (LUSHCHAK, 2014).

A respeito das EROs, podemos considerar que mais de 90% do oxigênio consumido por organismos vivos sob condições aeróbicas, é reduzido diretamente à água pela citocromo oxidase na cadeia transportadora de elétrons (CTE) via mecanismo de quatro elétrons sem liberação de EROs (OTT et al., 2007). Menos de 10% do oxigênio consumido é reduzido por vias sucessivas de 1 elétron (Figura 3), resultando na conversão do oxigênio molecular (O_2) em radical ânion superóxido $O_2^{\bullet -}$, o qual é espontaneamente convertido em peróxido de hidrogênio (H_2O_2). A molécula de H_2O_2 aceita mais um elétron e é dividida em radical hidroxila ($HO^{\bullet}$) e ânion hidroxila (OH^-). Por fim, $HO^{\bullet}$ interage com mais um elétron e um próton resultando na formação da molécula de água, assim como OH^- também interage com um próton gerando mais uma molécula de água. Desta forma $O_2^{\bullet -}$, H_2O_2 e $HO^{\bullet}$ são considerados, de maneira geral, espécies reativas de oxigênio. Não apenas esses, mas diversos peróxidos (lipídicos e proteicos), também são considerados EROs (LUSHCHAK, 2014).

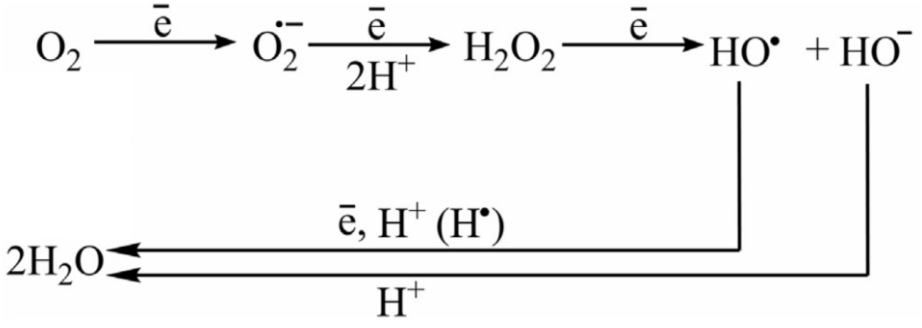

Figura 3: Redução do oxigênio molecular via esquema de um elétron (LUSHCHAK, 2014. Modificado).

Para manter níveis baixos de EROs, a fim de se tornarem menos prejudiciais, os organismos vivos possuem sistemas que atuam nesta regulação, ou seja, garantem o equilíbrio da produção e eliminação de EROs garatindo um nível dessas moléculas em estado estacionário. O desequilíbrio entre os processos de geração e eliminação de EROs perturba o metabolismo celular e sua regulação, danificando os constituintes celulares. A esta sistuação é dado o nome de desequilíbrio (desbalanço) redoz. Este estado de desequilíbrio pode ser estabelecido por meio de algumas circunstâncias: (1) aumento do nível de compostos endógenos e exógenos que entram na auto-oxidação juntamente com a produção de EROs; (2) esgotamento das reservas de antioxidantes de baixa massa molecular; (3) inativação de enzimas antioxidantes; (4) diminuição da produção de enzimas antioxidantes e antioxidantes de baixa massa molecular; e (5) combinações de dois ou mais dos fatores listados acima (LUSHCHAK, 2014).

Nestas condições alguns mecanismos proporcionam a sobrevivência sob estresse oxidative, os quais se baseiam principalmente na regulação positiva de enzimas antioxidantes. A sequência destes mecanismos se dá basicamente pela detecção de ROS, transdução de sinais através de vias específicas e regulação positiva de genes-alvo para aumentar o nível de seus produtos. Os sistemas de controle dos níveis de EROs podem ser classificados em dois grupos: antioxidantes de baixa massa molecular (massa molecular menor que um quilodalton), e antioxidantes de alta massa molecular (massa molecular superior a um quilodalton, mais comumente superior a dez quilodaltons). O grupo de antioxidantes de baixa massa molecular inclui as vitaminas C (ácido ascórbico) e E (tocoferol), carotenóides, antocianinas, polifenóis e ácido úrico. Os antioxidantes de alto peso molecular envolvem enzimas catalizadoras como: superóxido dismutase (SODs), catalase (CAT, glutationa peroxidase (GPx) e Glutationa-S-transferase (GSTs).

As SODs atuam na remoção catalítica de $O_2^{\bullet-}$, protonando-o e gerando como produto, oxigênio e H_2O_2 (MCCORD; FRIDOVICH, 1969). A dismutação do $O_2^{\bullet-}$ gera H_2O_2, E ele pode ser removido por dois tipos de enzimas: as catalases e as peroxidases. As catalases catalisam a decomposição direta do peróxido de

hidrogênio em oxigênio e água ($2H_2O_2 \leftrightarrows 2H_2O + O_2$). GPx remove o H_2O_2 de forma indireta, usando-o como substrato para a oxidação de glutationa reduzida (GSH), um tripeptídeo que contém um grupo tiol. Assim, há a reação entre o H_2O_2 e GSH com a formação de glutationa oxidase (GSSG) e H_2O. Algumas GSTs mostram uma atividade semelhante à da GPx e são chamadas de glutationa peroxidases sem selênio. Elas catalisam reações de peróxidos orgânicos com GSH para formar GSSG e álcoois (FELTON; SUMMERS, 1995; LYAKHOVICH et al., 2006).

Apesar de apresentarem papel importante na catalisação de peróxidos orgânicos, as GSTs têm função primordial na metabolização de xenobióticos (detoxificação), processo de importância crítica na manutenção da saúde do organismo. De maneita geral, três vias metabólicas desempenhem papéis nesse processo de detoxificação: carboxilesterases, monooxigenases e glutationa S-transferases (DUSCHER et al., 2014). Glutationa S-transferases (GSTs) são enzimas multifuncionais que catalisam adições de tiol da glutationa reduzida (GSH) a compostos orgânicos através de seus centros eletrofílicos. A formação de conjugados mais solúveis em água facilitaria sua eliminação (WILCE; PARKER, 1994).

3. OBJETIVOS

3.1. Objetivo Geral

Avaliar os efeitos de carvacrol e timol, livres ou em combinação com cipermetrina, sobre o carrapato *Rhipicephalus microplus*.

3.2. Objetivos Específicos

- Avaliar o efeito da combinação de cipermetrina e timol em carrapatos de duas populações com diferentes níveis de suscetibilidade à cipermetrina

(baixa e alta suscetibilidade), a partir dos parâmetros de determinação da eficácia e penetração cuticular dos compostos em fêmeas ingurgitadas.

- Determinar os efeitos do carvacrol e do timol na atividade enzimática de superóxido dismutase (SOD), catalase (CAT), glutationa S-transferase (GST) e glutationa peroxidase (GPX) em larvas de duas populações de *R. microplus* com diferentes graus de resistência a compostos sintéticos.

4. RESULTADOS

Os resultados da presente tese estão apresentados sob forma de capítulos, consistindo em artigos científicos nas seguintes situações:

- **Capítulo I** – Combination of cypermethrin and thymol for control of *Rhipicephalus microplus*: Efficacy evaluation and description of an action mechanism.
  - Situação: Publicado no periódico Ticks and Tick-borne Diseases (Qualis B1 em Medicina I; Fator de Impacto: 3.744).
    Doi: https://doi:10.1016/j.ttbdis.2021.101874

- **Capítulo II** – Effects of carvacrol and thymol on the antioxidant and detoxifying enzymes of *Rhipicephalus microplus* (Acari: Ixodidae).
  - Situação: Publicado no periódico Ticks and Tick-borne Diseases (Qualis B1 em Medicina I; Fator de Impacto: 3.744).
  - Doi: https://doi.org/10.1016/j.ttbdis.2022.101929

CAPÍTULO I

Combination of cypermethrin and thymol for control of *Rhipicephalus microplus*: Efficacy evaluation and description of an action mechanism.

Neste capítulo consta o artigo publicado no periódico Ticks and Tick-borne Diseases (Qualis B1 em Medicina I; Fator de Impacto: 3.744).
Doi: https://doi:10.1016/j.ttbdis.2021.101874

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Original article

Combination of cypermethrin and thymol for control of *Rhipicephalus microplus*: Efficacy evaluation and description of an action mechanism

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ABSTRACT

The cattle tick *Rhipicephalus microplus* is one of the most important ectoparasites in the tropical and subtropical regions of the world. Synthetic pyrethroids are widely used to control this tick, and the selection of resistant populations is a huge problem worldwide. The activity of thymol, a natural monoterpene, free or in combination with other compounds, has been demonstrated against different species of ticks. However, the mode of action is not fully understood. The present study aimed to evaluate the efficacy and the potential mode of action of the combination of cypermethrin and thymol on ticks from two populations with different levels of susceptibility to cypermethrin (low and high susceptibility). The isolated acaricidal activity of cypermethrin and thymol on larvae was carried out in different concentrations. The combination with different concentrations of cypermethrin and fixed concentrations of thymol (1300 μg/mL for the low susceptibility population; 690 μg/mL for the high susceptibility population) were performed. Adult engorged females were divided into five experimental groups (n = 20): 1) Control group untreated; 2) Control group: 2.0% (v/v) DMSO; 3) Thymol group: 1300 μg/mL thymol; 4) Cypermethrin group: 3700 μg/mL cypermethrin; 5) Association of cypermethrin (3700 μg/mL) + thymol (1300 μg/mL). A subgroup was used to study the efficacy of the reproductive parameters and another subgroup, with ten adults from each treatment, was used to quantify thymol and cypermethrin by HPLC chromatographic analysis. All compounds tested were effective on larvae from both populations, and the combination with thymol decreased the LC_{50} of cypermethrin (232.4 to 52.7 μg/mL) on the low-susceptibility population. The combination of thymol and cypermethrin was effective in both populations of *R. microplus* (reproductive performance of engorged females) when compared to the untreated control group, even with higher percent control values (pop. 1: 93.5 ± 5.6% and pop. 2: 92.7 ± 1.1%) than the group treated only with cypermethrin (pop. 1: 87.3 ± 7.3% and pop. 2: 83.5 ± 1.2%). From the HPLC analyzes, a higher concentration of cypermethrin (pop. 1: 30.3 ± 6.9 and pop. 2: 45.4 ± 17.7 ng/mg) was detected in the tissues of engorged females treated with the combination compared to analyte concentrations in groups treated with cypermethrin only (pop. 1: 12.4 ± 4.4 pop. 2: 25.5 ± 9.4 ng/mg). This was the first study to investigate the acaricidal efficacy of the combination of thymol + cypermethrin on *R. microplus* and demonstrate that the presence of thymol increases the concentration of cypermethrin in the internal tissues of engorged females through a possible mechanism for increasing the penetration of cypermethrin at the cuticular level.

1. Introduction

The cattle tick *Rhipicephalus microplus* is one of the most important ectoparasites in the tropical and subtropical regions of the world (Estrada-Peña et al., 2006; Marques et al., 2020). A substantial number of active compounds have been developed into products to control cattle ticks. However, few new drugs have been launched in the market in recent years (Selzer and Epe, 2021). On the other hand, reports of multidrug-resistant tick populations are increasingly common in endemic areas (Sagar et al., 2020 Valsoni et al., 2020; Vilela et al., 2020; Upadhaya et al., 2020).

Currently, synthetic pyrethroids are widely used in the control of

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cattle ticks and, consequently, resistance to this group constitutes one of the most severe problems worldwide (Rodriguez-Vivas et al., 2007; Andreotti et al., 2011; Lovis et al., 2012; Godara et al., 2019; Villar et al., 2020; Agwunobi et al., 2021). In Brazil, approximately 59% of all acaricide brands available on the market have at least one compound of synthetic pyrethroids (SINDAN, 2018). Cypermethrin is the compound present in the largest number of acaricides in the Brazilian market, and several reports describing cypermethrin resistance were published (Andreotti et al., 2011; Klafke et al., 2017; SINDAN, 2018). A combination of molecules has been identified as an essential strategy for the control of cattle ticks. Several acaricidal combinations are quite effective (Li et al., 2007; Prullage et al., 2011), as well as the combination of pyrethroids with the synergist piperonyl butoxide (PBO) (R.J.W. Li et al., 2010; Rodriguez-Vivas et al., 2013; Higa et al., 2019).

In this context, products derived from plants as extracts (Nasreen et al., 2020; Bortolucci et al., 2020), essential oils (Silva et al., 2020; Sousa et al., 2020), and pure molecules (Novato et al., 2019; Cardoso et al., 2020) showed acaricidal activity or acted as synergists, potentiating the effect of other molecules. The synergistic effect observed in the combination of monoterpenes and phenylpropanoids has also been characterized as a promising alternative for developing acaricidal products (Novato et al., 2019).

Thymol (5-methyl-2-isopropyl-1-phenol) is a volatile monoterpene present in the essential oil of plants of the families Lamiaceae and Verbenaceae (Rosado-Aguilar et al., 2017), and its activity has been demonstrated against different stages and species of ticks (Monteiro et al., 2009; Senra et al., 2013a, 2013b; Araújo et al., 2015). However, the high concentrations required to be effective make it challenging to use thymol alone in commercial formulations for the control of *R. microplus* (Delmonte et al., 2017; Novato et al., 2019; Coelho et al., 2020). The combined use of thymol with other molecules is a promising alternative in several pharmacological activities. Thymol has been increased the activity of different drugs with analgesic, anti-inflammatory (Stott et al., 1998; Chang et al., 2007; Arunkumar et al., 2015), anticancer (Gao and Singh, 1997, 1998; El-Kattan et al., 2015; 2001) and antihypertensive effect (Songkro et al., 2004).

The mechanisms involved in the synergistic effect of natural compounds can be related to: 1) effects at different target sites, 2) physicochemical effects on solubility and penetration, 3) inhibition of detoxifying system enzymes, and 4) neutralization of adverse effects (Wagner and Ulrich-Merzenich, 2011). However, the mechanisms for different molecules and combinations are not fully understood, requiring further studies in this area. In the present study, the objective was to evaluate the effect of the combination of cypermethrin and thymol in ticks from two populations with different levels of susceptibility to cypermethrin (low and high susceptibility), by determining the efficacy and cuticular penetration of the compounds in engorged females, as a potential mode of action of this combination of molecules.

2. Materials and methods

2.1. Ticks

This experiment was approved by the Ethics Committee on Animal Experimentation of Federal University of Maranhão, Brazil, under protocol number 23,115.005443/2017-51. *Rhipicephalus microplus* engorged females naturally detached were obtained from calves artificially infested with larvae from two populations with different susceptibility profiles to cypermethrin (low and high susceptibility). The low susceptibility population (Jaguar) comes from naturally infested cattle from a farm located in Eldorado do Sul, Rio Grande do Sul, Brazil, in 2011. The high susceptibility population (Porto Alegre) comes from a farm located in Rio Grande do Sul, along the border of Brazil and Uruguay, and was collected from naturally infested cattle in 1992 (Reck et al., 2014). Both tick populations were maintained for at least five years with the artificial infestation of calves in the Federal University of Maranhão (UFMA) facilities. The engorged females were washed with distilled water, dried on filter paper, and used directly in the adult immersion test or placed in Petri dishes and kept at a temperature of 27 °C (+/- 1 °C) with relative humidity (RH) ≥ 80% until complete oviposition. The eggs were collected and placed in conical bottom tubes of 15 mL closed with cotton to allow air and moisture. The eggs were then incubated at 27 °C (+/- 1 °C), with RH ≥ 80% until hatching. For larval immersion tests, non-fed larvae aged between 14 and 21 days were used.

2.2. Acaricidal bioassays

Cypermethrin with ≥93.2% purity (Tagros Chemicals Ltda, Chennai, India) and thymol with 99% purity (Sigma Chemical Co., St. Louis, MO, USA) was used to perform the acaricidal bioassays.

2.2.1. Larval immersion test

The larval immersion test was performed according to Klafke et al. (2006). Pure cypermethrin was tested at concentrations of 1500.0, 750.0, 375.0, 187.5, 93.7, 46.8, 23.4, 11.7, 5.8 and 2.9 μg/mL and thymol at 5000.0, 3500.0, 2450.0, 1715.0, 1.200,5, 840,35, 588,24, 411,77, 288,24 and 201,76 μg/mL. For combination tests, cypermethrin (same concentrations described above) was associated with fixed concentrations of 1300 and 690 μg/mL of thymol in tick larvae of population 1 (high susceptibility) and population 2 (low susceptibility), respectively. The fixed concentrations of thymol were selected according to the first round of experiment results with pure compound to cause larval mortality ≤13%. All dilutions were performed using 1% DMSO and 0.02% Triton X-100 solution. The larval immersion test was performed in three independent assays, each with four replicates. The control group was treated with solvent (1% DMSO and 0.02% Triton X-100).

Approximately 500 larvae were immersed for 10 min in each concentration and then transferred to dry on a filter paper base. Then, approximately 100 larvae were transferred to a clean, dry filter paper package (8.5 × 7.5 cm) closed with plastic clips. The packets were incubated at 27 ± 1 °C with RH ≥80% for 24 h. After incubation, dead and alive larvae were manually counted. Ticks without movement were considered dead.

The doses were initially transformed to log (X), and the percentage of mortality normalized; subsequently, nonlinear regression was performed to obtain the LC_{50} (50% lethal concentration) using GraphPad Prism 8.0.2 software (GraphPad Inc., San Diego, CA, USA). The significance of each comparison was determined when the calculated confidence intervals did not overlap (Roditakis et al. 2005).

2.2.2. Adult immersion test

Engorged females from two different populations of *R. microplus* (low and high susceptibility) were subjected to the adult immersion test (AIT) as described by Drummond et al. (1973). For each population of *R. microplus*, ticks with homogeneous body mass (n =80; $p > 0.05$) were divided into five experimental groups (n=10): 1) Control group untreated; 2) Control group was treated with 2.0% (v/v) DMSO solution; 3) Thymol group was treated with 1300 μg/mL thymol solution; 4) Cypermethrin group was treated with 3700 μg/mL cypermethrin solution; 5) Group was treated with the combination of 3700 μg/mL cypermethrin + 1300 μg/mL thymol solution. The cypermethrin concentration used in AIT was the LC50 determined by Ghosh et al. (2017) in a pyrethroid-resistant strain of *R. microplus*. The used thymol concentrations were based on the results of the larval immersion test. For each compound, stock solutions at a concentration of 10,000 μg/mL in DMSO P.A. were previously prepared, and the solutions for the treatment were dissolved in 2% DMSO for a final volume of 10 mL. Each group of ticks was immersed in their respective solutions for five minutes, washed, and dried on absorbent paper. The AIT was performed in three independent tests to assess the acaricidal efficacy and only one test to quantify cypermethrin and thymol using HPLC.

2.4. Assessment of acaricidal efficacy

For the evaluation of the acaricidal efficacy, 10 specimens of engorged females from each of the four groups mentioned above were placed in a B.O.D. (Biochemical oXygen demand) incubator with temperature of 27 ± 1 °C and RH ≥ 80%, for 15 days. The eggs were collected, weighed, transferred to conical bottom tubes of 15 mL and placed in the incubator at 27 ± 1 °C and RH ≥ 80%, for 25 days. The following reproductive parameters of engorged females were evaluated: Weight of females (FW) before treatment and Egg mass weight (EW). The following index were determined: Egg production index (EPI) = (EW/FW) x 100 (Bennett, 1974); Percentage of reduction in oviposition (% Rovip) = [(EWcontrol - EWtreatment)/EWcontrol] x 100; Percentage of hatching reduction (% Rhatch) = [(HRcontrol - HRtreatment)/HR-control] x 100 (Lopes et al., 2013); and Control percentage (% C) = [(ERcontrol - ERtreatment) / ERcontrol] x 100 (Drummond et al., 1973).

All averages obtained were analyzed statistically by Analysis of Variance (ANOVA), followed by Tukey's test ($p < 0.05$). Statistical analyzes were performed using GraphPad Prism 8.0.2 software (GraphPad Inc.).

2.5. HPLC chromatographic analysis

The ticks of the control and treated groups were processed and analyzed individually. After treatment, the ticks were washed with distilled water and immediately stored, individually, in 1 mL of acetonitrile at −20 °C. Each tick was macerated with a glass pestle in 1 mL of acetonitrile (original volume used for storage), stirred for 15 min, and centrifuged at 13,000 g for 10 min. The time between sample treatment and processing was ten days.

The quantification of thymol was performed according to the methodology described by Miró et al. (2020a), with adaptations. A volume of 500 µL of the supernatant was diluted in 500 µL of ultra-pure water. Then, 50 µL of the dilution was directly injected through the automatic sample injector (Shimadzu SIL 10A) into the HPLC system Shimadzu 10 (Shimadzu Corporation, Kyoto, Japan) equipped with a Kromasil C18 reverse phase column (5 µm, 250 mm x 4.60 mm, Eka Chemicals, USA) and UV detector (Shimadzu, SPD-10A UV detector) reading at 274 nm. The mobile phase consisted of a mixture of ultra-pure water (A)/acetonitrile (B) in an A/B ratio: 47/53 and a flow rate of 1.5 mL/min.

The quantification of cypermethrin was performed according to the methodology described by Chowdhury et al. (2013), with adaptations. The engorged females were processed as described previously except that the samples were not diluted in water. Then, 50 µL of the supernatant was directly injected into the same HPLC system mentioned above, except that the UV detector (Shimadzu, SPD-10A UV detector) was fixed at a wavelength of 204 nm. The mobile phase consisted of ultra-pure water (A)/acetonitrile (B) (20/80) and an isocratic flow of 1.5 mL/min.

Chromatographic peak areas of all the analytes were measured using the integrator software (LC Solution, Shimadzu Corporation) of the HPLC system. Validation of the analytical procedures for the quantification of thymol and cypermethrin in the tissues of *R. microplus* engorged females was performed before starting the analysis of the experimental samples. Briefly, known concentrations of each analyte were added to aliquots of untreated tick extracts to obtain calibration standards (thymol: 25 µg/mL and 400 µg/mL; cypermethrin: 1 µg/mL and 10 µg/mL) that were analyzed in triplicate by HPLC. For all thymol samples, 25 µL of carvacrol solution (3.2 µg/µL) was added as an internal standard. Calibration curves were also prepared for thymol (concentrations ranging from 0.1 to 25 µg/mL) and cypermethrin (concentrations ranging from 1 to 100 µg/mL) as standard in the mobile phase. Calibration curves were prepared using the least squares linear regression analysis of the analyte peak area ratios over the internal standard (thymol) or external standard (cypermethrin). Square correlation coefficients (R^2) were close to 1. The concentrations in the experimental samples were determined after interpolation of the peak area ratios of the analytes on the external standard calibration line.

The concentration values for each analyte were expressed as mean ± standard deviation (SD). The Mann-Whitney U test was used to evaluate the average concentrations of thymol analytes in engorged females in the population with high susceptibility; and thymol and cypermethrin of the population with low susceptibility (data showed non-normal distribution). The Student's *t*-test was used to evaluate the average concentrations of cypermethrin in the population with high susceptibility (data showed normal distribution). Differences were considered statistically significant at $p < 0.05$. All statistical analyzes were performed using GraphPad Prism 8.0.2 software (GraphPad Inc.).

3. Results

3.1. Effect on larvae

Cypermethrin and thymol showed acaricidal activity on larvae of both populations of ticks. The addition of thymol increased the toxicity of cypermethrin in the low-susceptibility population by 4.46-fold, resulting in a reduction in the LC_{50} from 232.4 to 52.7 µg/mL (Table 1). In the case of the highly susceptible ticks, the addition of thymol slightly decreased the toxicity of cypermethrin, with LC_{50} values ranging from 11.02 to 24.09 µg/mL.

3.2. Effect on engorged females

Thymol alone showed low efficacy in engorged females, however, cypermethrin alone had a percent control of 83.5 ± 1.2% and 87.3 ± 7.3% in populations with low and high susceptibility to cypermethrin, respectively (Table 2). The combination of thymol and cypermethrin increases the efficacy in both populations of *R. microplus*, with significant differences ($p = 0.0141$) in low susceptibility population (Table 2). The egg hatchability in engorged females with high susceptibility rate was also low (9.1 ± 4.6%) after the combination of cypermethrin and thymol. Additionally, in the low-susceptibility population, the combination of cypermethrin and thymol significantly reduced ($p = 0.0355$) egg production (22.1 ± 1.2) when compared to the cypermethrin group, resulting in a significant increase in the efficacy of combination compared to incubation with cypermethrin alone (Table 2).

Table 1
Lethal concentration of thymol, cypermethrin and its combination for 50% of *Rhipicephalus microplus* larvae with high and low susceptibility to synthetic pyrethroids.

Compound	Population 1 (high susceptibility)			Population 2 (low susceptibility)		
	LC_{50} (µg/mL)	CI 95%	R^2	LC_{50} (µg/mL)	CI 95%	R^2
Thymol (THY)*	1532.0	1439.0 – 1626.0	0.90	1940.0	1833.0 – 2052.0	0.91
Cypermethrin (CYP)**	11.02	8.833 – 1.347	0.90	232.4	192.6 – 280.1	0.85
CYP + THY***	24.09	19.04 – 30.36	0.92	52.77	51.66 – 53.94	0.99

LC_{50}: Lethal concentration (µg/mL) for 50% of individuals; SD: Standard deviation; CI: 95% confidence interval; R^2: Regression Correlation Coefficient.

* Concentrations of thymol: 5000.0, 3500.0, 2450.0, 1715.0, 1.200,5, 840,35, 588,24, 411,77, 288,24 and 201,76 µg/mL;.

** Concentrations of cypermethrin: 1500.0, 750.0, 375.0, 187.5, 93.7, 46.8, 23.4, 11.7, 5.8 and 2.9 µg/mL;.

*** Fixed concentrations: Thymol – 690 µg/mL in the population with high susceptibility to cypermethrin and 1300 µg/mL in the population with low susceptibility to cypermethrin. Values are significantly different when confidence intervals do not overlap.

Table 2
Effects of thymol, cypermethrin, and its combination in the reproductive parameters of *Rhipicephalus microplus* populations with high and low susceptibility to pyrethroids.

	Population 1 (high susceptibility)						Population 2 (low susceptibility)					
Compound	FW (g)	EW (g)	EPI	%Rovip	%Rhatch	C%	FW (g)	EW (g)	EPI	%Rovip	%Rhatch	C%
Untreated	2.53 ± 0.04[a]	1.05 ± 0.06[a]	41.4 ± 2.4[a]	–	–	–	2.60 ± 0.04[a]	1.12 ± 0.19[a]	43.0 ± 6.6[a]	–	–	–
Control - DMSO (2%)	2.44[a] ± 0.24	0.93[a] ± 0.17	38.1 ± 4.1[a]	–	–	–	2.44[a] ± 0.12[a]	0.97[a] ± 0.16[a]	40.0 ± 7.7[a]	–	–	–
Thymol (THY)	2.45 ± 0.18[a]	0.81 ± 0.09[a]	32.7 ± 1.5[a]	12.9 ± 7.9[a]	3.2 ± 17.2[a]	**17.1 ± 8.9[a]**	2.41 ± 0.06[a]	0.90 ± 0.16[a]	37.4 ± 7.1[a]	7.0 ± 10.4[a]	10.0 ± 6.2[a]	**15.3 ± 13.6[a]**
Cypermethrin (CYP)	2.51 ± 0.14[a]	0.76 ± 0.22[a]	29.9 ± 7.2[a]	20.1 ± 9.7[a]	83.6 ± 8.3[b]	**87.3 ± 7.3[b]**	2.43 ± 0.11[a]	0.76 ± 0.10[a]	31.2 ± 4.9[a]	21.8 ± 3.8[a]	78.9 ± 1.63[b]	**83.5 ± 1.2[b]**
CYP + THY	2.43 ± 0.21[a]	0.55 ± 0.43[a]	21.8 ± 16.6[a]	44.9 ± 41.8[a]	86.7 ± 5.2[b]	**93.5 ± 5.6[b]**	2.46 ± 0.14[a]	0.54 ± 0.06[b]	22.1 ± 1.2[b]	43.1 ± 9.4[b]	86.6 ± 3.9[c]	**92.7 ± 1.1[c]**

FW: Weight of engorged females before treatment; EW: Egg mass weight; EPI: Egg production index;%Rovip: Percentage of reduction in oviposition;%Rhatch: Percentage of hatching reduction; C%: Control percentage. Mean ± standard deviation. Mean with same letter in the same column does not differ significantly at $p < 0.05$.

3.3. *Quantification of thymol and cypermethrin in the tissues of engorged females*

A significantly higher concentrations of thymol was detected in the tissues of engorged females of both populations after the incubation with thymol alone (11.1 ± 5.5 and 13.9 ± 8 ng/mg in high and low susceptibility populations, respectively) compared to ticks treated with the combination of thymol and cypermethrin with p values = 0.0037 and 0.0003 for populations with high and low susceptibility, respectively (Fig. 1A and C). Significantly higher concentrations of cypermethrin were measured in the tissues of engorged females treated with the combination of cypermethrin + thymol (30.3 ± 6.9 ng/mg, $p = 0.0015$ and 45.4 ± 17.7 ng/mg, $p = 0.0317$ in high and low susceptibility populations to pyrethroids, respectively) than those observed in the engorged females treated with cypermethrin alone (12.4 ± 4.4 and 25.5 ± 9.4 ng/mg in populations with high and low susceptibility to pyrethroids, respectively) (Fig. 1B and D).

4. Discussion

The search for alternatives for controlling *R. microplus* is one of the biggest challenges for cattle production due to several reports of

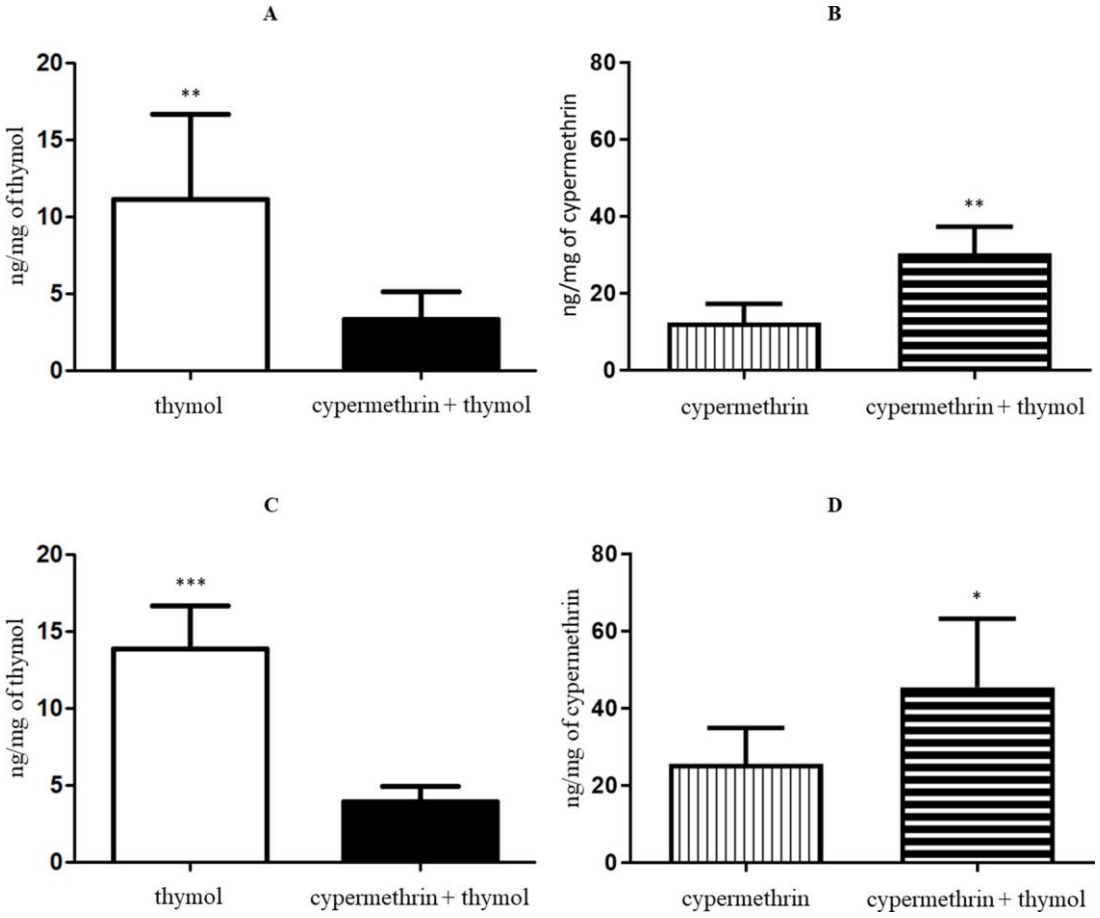

Fig. 1. Concentration of thymol and cypermethrin (ng/mg) in tissues of engorged females of *Rhipicephalus microplus*, with high (A and B) and low (C and D) susceptibility to synthetic pyrethroids. Ticks were treated with thymol (1300 μg/mL) and cypermethrin (3700 μg/mL) either alone or in combination. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

selecting multi-resistant populations. This study presents the first evidence of the acaricidal efficacy of the combination of thymol with a synthetic pyrethroid (cypermethrin) on *R. microplus*. Additionally, the current trial evaluated the potential mechanisms involved in the increment of activity after the combination treatment.

The acaricidal effect of thymol on different species of ticks has been previously studied (Mendes et al., 2011; Tabari et al., 2017; Lima et al., 2018; Matos et al., 2019). The current trial assessed the combination of this compound with cypermethrin for the control of *R. microplus* larvae demonstrating an increase of the toxicity of the pyrethroid (4.46-fold). The present study also demonstrated that the combination between these two compounds affected the reproductive parameters of engorged females and showed a higher percent control in both ticks' populations compared with cypermethrin alone.

The synergistic effect of thymol combination of with others secondary metabolites such as eugenol on immature stages of *Rhipicephalus sanguineus* s.l. (Araújo et al., 2016; Coelho et al., 2020), and carvacrol on *R. microplus*, *Amblyomma sculptum*, and *Dermacentor nitens* larvae (Araújo et al., 2016; Novato et al., 2015, 2019) proved to be efficient for tick control, with higher toxicity than the compounds tested alone (Novato et al., 2015). However, on ticks, thymol in combination with a pyrethroid was evaluated only once by Arafa et al. (2021) on *Rhipicephalus annulatus*, thus requiring further investigations such as the one carried out in this work.

The mechanism of synergy between natural compounds and synthetic acaricides/insecticides is not clearly understood. In general, the action of natural compounds and synthetic pyrethroids on arthropods involves some basic steps such as the penetration of the integument and the membranes of the target organ, binding in the active site, and detoxification mechanisms (Enan, 2001; Priestley et al., 2003; Rehman et al., 2014; Tak et al., 2015; Politi et al., 2017; Gaire et al., 2019). It is suggested that the synergism observed in the combination of these products may enhance the mode of neurotoxic action or other target sites of the organism (Suwannayod et al., 2019), decrease the metabolic detoxification of xenobiotics through the inhibition of the enzymatic system (cytochrome P450, monooxygenases, and carboxylesterases) (Scalerandi et al., 2018; Miró et al., 2020b) and may increase cuticular and cell membrane penetration of target organs (Delgado et al., 2004; Bakkali et al., 2008; Pei et al., 2009; Ahmad et al., 2011). Previous evidence shows that thymol has neurotoxic effects on arthropods, acting on the inhibition of the enzyme acetylcholinesterase (AChe) (Jankowska et al., 2018; Matos et al., 2019; Cardoso et al., 2020) and binding to GABA receptors, altering the functioning of synapses (Priestley et al., 2003; Tong and Coats, 2010). This monoterpene is also able to inhibit different enzymes involved in the detoxifying system (monooxygenase and cytochrome P450) (Miró et al., 2020b) and reduce the impermeability of membranes (Ahmad et al., 2011). These different mechanisms of action may be responsible for the synergistic effects observed in the current trial (Matos et al., 2019).

Essential oils and their constituents may increase the penetration of other molecules using different mechanisms of action based on the disintegration of the intercellular lipid structure, interaction with the intercellular domain of the protein, which induces its conformational modification and the increase in the drug partition (Herman and Herman, 2015). In fact, the stratum corneum treated with thymol shows an increase in the lipid acyl chains disorder, with a consequent rise in lipid fluidity and a greater partition and diffusion of the drug (Gao and Singh, 1997, 1998).

The higher concentrations of cypermethrin detected in the tissue of engorged females treated with the combination (cypermethrin + thymol) may justify the increase in larval mortality and the percent control of engorged females treated with the combination. Thymol is an apolar monoterpene capable of entering the cell membrane, increasing its permeability, both in prokaryotic and eukaryotic cells, facilitating the entrance of the other compound in the cytoplasm (Bakkali et al., 2008; Pei et al., 2009; Ahmad et al., 2011). This monoterpene proved to be a penetration enhancer for several drugs (Gao and Singh, 1997, 1998; Stott et al., 1998; El-Kattan et al., 2001; Songkro et al., 2004; Chang et al., 2007; Arunkumar et al., 2015). Hydrocarbons or nonpolar terpenes are considered suitable lipophilic drug enhancers in human skin (Williams and Barry, 1991). These properties attributed to the monoterpene thymol corroborate the results found in the present study, indicating a possible action of thymol on the penetration of cypermethrin in the tissue of engorged females. In this context, thymol as a synergist is a promising alternative for reducing its concentration by increasing the target organism's biological activity of an active compound as cypermethrin.

Reinforcing the synergistic activity of terpenes compounds in increasing cuticular penetrability of other compounds, Tak and Isman (2015) showed synergic insecticidal activity of monoterpenes 1.8-cineol and camphor on larvae of *Trichoplusia ni*, through the mechanism of increasing the penetrability of this compound. The combination of camphor and 1.8-cineole reduced the surface tension and increased solubility of camphor by 1.8-cineole, and the interaction of 1.8-cineole with the lipid layer of the arthropod cuticle justified the increase in the penetration of the molecule (Tak and Isman, 2015). The penetration of thymol in the cuticle increased with higher recovery in the hemolymph of *T. ni* larvae when used in combination with p-cymene (Tak and Isman, 2017).

Matos et al. (2014) reported that thymol caused deformations in the chorionic membrane of the ovaries of females of *R. sanguineus*. The rupture of the chorion would allow the direct contact of the thymol molecules with the oocyte membrane, promoting the entry of toxic compounds into the cytoplasm. The interaction of thymol with the membrane affects membrane permeability and results in the release of K^+ ions and ATP (R.J.W. Lambert et al., 2001; Xu et al., 2008). A similar effect was observed for the Gené organ (Matos et al., 2019) and synganglion (Matos et al., 2020). The hydroxyl group present in the structure of the thymol integrates within the polar head-groups of the lipid bilayer, causing damage to cells and tissues by alterations of the cell membrane (Nazzaro et al., 2013).

Contrary to that was observed for cypermethrin in this study, there was a decrease in the thymol concentrations measured in the tissue of engorged females treated with the combination of cypermethrin + thymol when compared to the group treated with thymol alone. Considering that the penetration of chemicals through the stratum corneum and the cuticle is a passive process (Lin et al., 2012) and highly dependent on the lipophilic property of the molecule, we suggest that the decrease in the amount of thymol is based on the higher concentration of cypermethrin in the mixture and its greater lipophilicity (logP of cypermethrin = 6.6; logP of thymol = 3.3). Therefore, cypermethrin penetrates and occupies more space than thymol in the tissue of engorged females.

The penetration enhancers of acaricides and insecticides have been little explored when compared to human drugs. Thus, studies on increased cuticular penetrability are necessary and relevant for tick control since one of the possible resistance mechanisms in ticks is a thicker cuticle layer, which could delay the penetration of toxic compounds. This cuticular resistance mechanism is already well established for insects (Ahmad et al., 2006; Lin et al., 2012) but is still unclear the importance in ticks.

Our results suggest that the combination between thymol and cypermethrin is significantly effective in controlling cattle tick *R. microplus*, acting on the mortality of larvae and the reproductive parameters of engorged females. The presence of thymol enhances the penetration of cypermethrin at the cuticular level, providing an increase in the concentration of cypermethrin in the tissues of engorged females, which may justify the increased efficacy. These results show that the combination of thymol and cypermethrin is a promising alternative for the control of *R. microplus*. Cuticular resistance in insects is well understood. However, in ticks, this mechanism is not well studied. For this reason, further studies are needed to assess the biochemical and

physiological mechanisms that explain the increased tissue concentration of cypermethrin in the presence of thymol in *R. microplus*, to understand whether cuticular resistance is a factor that can influence the penetration of molecules in ticks.

CRediT authorship contribution statement

Caio P Tavares: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Isabella C Sousa:** Methodology, Investigation, Writing – review & editing. **Matheus N Gomes:** Methodology, Investigation, Writing – review & editing. **Victoria Miró:** Methodology, Investigation, Formal analysis. **Guillermo Virkel:** Conceptualization, Methodology, Investigation, Formal analysis. **Adrian Lifschitz:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – review & editing, Visualization, Project administration, Funding acquisition. **Livio M Costa-Junior:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Visualization, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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References

Agwunobi, D.O., Yu, Z., Liu, J., 2021. A retrospective review on ixodid tick resistance against synthetic acaricides: implications and perspectives for future resistance prevention and mitigation. Pestic. Biochem. Physiol. 173, 104776 https://doi.org/10.1016/j.pestbp.2021.104776.

Ahmad, A., Khan, A., Akhtar, F., Yousuf, S., Xess, I., Khan, L.A., Manzoor, N., 2011. Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against Candida. Eur. J. Clin. Microbiol. Infect. Dis. 30, 41–50. https://doi.org/10.1007/s10096-010-1050-8.

Ahmad, M., Denholm, I., Bromilow, R.H, 2006. Delayed cuticular penetration and enhanced metabolism of deltamethrin in pyrethroid-resistant strains of *Helicoverpa armigera* from China and Pakistan. Pest Manag. Sci. 62, 805–810. https://doi.org/10.1002/ps.1225.

Andreotti, R., Guerrero, F.D., Soares, M.A., Barros, J.C., Miller, R.J., Léon, A.P.De, 2011. Acaricide resistance of *Rhipicephalus (Boophilus) microplus* in State of Mato Grosso do Sul, Brazil. Rev. Bras. Parasitol. Vet. 20, 127–133.

Arafa, W.M., Aboelhadid, S.M., Moawad, A., Shokeir, K.M., Ahmed, O., Pérez de León, A. A., 2021. Control of *Rhipicephalus annulatus* resistant to deltamethrin by spraying infested cattle with synergistic eucalyptus essential oil-thymol-deltamethrin combination. Vet. Parasitol. 290, 109346 https://doi.org/10.1016/j.vetpar.2021.109346.

Araújo, L.X., Novato, T.P.L., Zeringota, V., Matos, R.S., Senra, T.O.S., Maturano, R., Prata, M.C.A., Daemon, E., Monteiro, C.M.O., 2015. Acaricidal activity of thymol against larvae of *Rhipicephalus microplus* (Acari: ixodidae) under semi-natural conditions. Parasitol. Res. 114, 3271–3276. https://doi.org/10.1007/s00436-015-4547-3.

Araújo, L.X., Novato, T.P.L., Zeringota, V., Maturano, R., Melo, D., da Silva, B.C., Daemon, E., de Carvalho, M.G., Monteiro, C.M.O., 2016. Synergism of thymol, carvacrol and eugenol in larvae of the cattle tick, *Rhipicephalus microplus*, and brown dog tick, *Rhipicephalus sanguineus*. Med. Vet. Entomol. 30, 377–382. https://doi.org/10.1111/mve.12181.

Arunkumar, S., Ashok, P., Desai, B.G., Shivakumar, H.N., 2015. Effect of chemical penetration enhancer on transdermal iontophoretic delivery of diclofenac sodium under constant voltage. J. Drug Deliv. Sci. Technol. 30, 171–179. https://doi.org/10.1016/j.jddst.2015.10.007.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils - a review. Food Chem. Toxicol. 46, 446–475. https://doi.org/10.1016/j.fct.2007.09.106.

Bennett, G.F., 1974. Oviposition of *Boophilus microplus* (Canestrini) (Acarida: ixodidae). I. Influence of tick size on egg production. Acarol 16, 52–61. PMID: 4463680.

Bortolucci, W., Oliveira, H., Oliva, L.R., Gonçalves, J.E., Júnior, R.P., Colauto, N.B., Linde, G.A., Gazim, Z.C., 2020. Crude ethanolic extracts of different parts of *Gallesia integrifolia* (Phytolaccaceae) for the control of *Rhipicephalus microplus*. Int. J. Acarol. 46, 414–423. https://doi.org/10.1080/01647954.2020.1805003.

Cardoso, A.dos S., Santos, E.G.G., Lima, A.da S., Temeyer, K.B., Pérez de León, A.A., Costa, L.M., Soares, A.M.dos S., 2020. Terpenes on *Rhipicephalus (Boophilus) microplus*: acaricidal activity and acetylcholinesterase inhibition. Vet. Parasitol. 280, 109090 https://doi.org/10.1016/j.vetpar.2020.109090.

Chang, J.S., Tsai, Y.H., Wu, P.C., Huang, Y.Bin, 2007. The effect of mixed-solvent and terpenes on percutaneous absorption of meloxicam gel. Drug Dev. Ind. Pharm. 33, 984–989. https://doi.org/10.1080/03639040601150294.

Chowdhury, M.A.Z., Fakhruddin, A.N.M., Nazrul Islam, M., Moniruzzaman, M., Gan, S. H., Khorshed Alam, M., 2013. Detection of the residues of nineteen pesticides in fresh vegetable samples using gas chromatography-mass spectrometry. Food Control 34, 457–465. https://doi.org/10.1016/j.foodcont.2013.05.006.

Coelho, L., de Paula, L.G.F., Alves, S.das G.A., Sampaio, A.L.N., Bezerra, G.P., Vilela, F.M. P., Matos, R.da S., Zeringóta, V., Borges, L.M.F., Monteiro, C., 2020. Combination of thymol and eugenol for the control of *Rhipicephalus sanguineus* sensu lato: evaluation of synergism on immature stages and formulation development. Vet. Parasitol. 277, 108989 https://doi.org/10.1016/j.vetpar.2019.108989.

Delgado, B., Fernández, P.S., Palop, A., Periago, P.M., 2004. Effect of thymol and cymene on Bacillus cereus vegetative cells evaluated through the use of frequency distributions. Food Microbiol 21, 327–334. https://doi.org/10.1016/S0740-0020(03)00075-3.

Delmonte, C., Cruz, P.B., Zeringóta, V., de Mello, V., Ferreira, F., Amaral, M.P.H., Daemon, E., 2017. Evaluation of the acaricidal activity of thymol incorporated in two formulations for topical use against immature stages of Rhipicephalus sanguineus sensu lato (Latreille, 1806) (Acari: ixodidae). Parasitol. Res. 116 (11), 2957–2964. https://doi.org/10.1007/s00436-017-5604-x.

Drummond, R.O., Ernst, S.E., Trevino, J.L., Gladney, W.J., Graham, O.H., 1973. *Boophilus anulatus* and *Boophilus microplus*: laboratory test of insecticides. J. Econ. Entomol. 66, 130–133. https://doi.org/10.1093/jee/66.1.130.

El-Kattan, A.F., Asbill, C.S., Kim, N., Michniak, B.B., 2001. The effects of terpene enhancers on the percutaneous permeation of drugs with different lipophilicities. Int. J. Pharm. 215, 229–240. https://doi.org/10.1016/S0378-5173(00)00699-2.

Enan, E., 2001. Insecticidal activity of essential oils: octopaminergic sites of action. Comp. Biochem. Physiol. - C Toxicol. Pharmacol. 130, 325–337. https://doi.org/10.1016/S1532-0456(01)00255-1.

Estrada-Peña, A., Bouattour, A., Camicas, J.L., Guglielmone, A., Horak, I., Jongejan, F., Latif, A., Pegram, R., Walker, A.R., 2006. The known distribution and ecological preferences of the tick subgenus *Boophilus* (Acari: ixodidae) in Africa and Latin America. Exp. Appl. Acarol. 38, 219–235. https://doi.org/10.1007/s10493-006-0003-5.

Gaire, S., Scharf, M.E., Gondhalekar, A.D., 2019. Toxicity and neurophysiological impacts of plant essential oil components on bed bugs (Cimicidae: hemiptera). Sci. Rep. 9, 1–12. https://doi.org/10.1038/s41598-019-40275-5.

Gao, S., Singh, J., 1998. In vitro percutaneous absorption enhancement of a lipophilic drug tamoxifen by terpenes. J. Control. Release 51, 193–199. https://doi.org/10.1016/S0168-3659(97)00168-5.

Gao, S., Singh, J., 1997. Mechanism of transdermal transport of 5-fluorouracil by terpenes: carvone, 1,8-cineole and thymol. Int. J. Pharm. 154, 67–77. https://doi.org/10.1016/S0378-5173(97)00123-3.

Godara, R., Katoch, R., Rafiqi, S.I., Yadav, A., Nazim, K., Sharma, R., Singh, N.K., Katoch, M., 2019. Synthetic pyrethroid resistance in *Rhipicephalus (Boophilus) microplus* ticks from north-western Himalayas, India. Trop. Anim. Health Prod. 51, 1203–1208. https://doi.org/10.1007/s11250-019-01810-8.

Ghosh, S., Gupta, S., Ajith Kumar, K.G., Sharma, A.K., Kumar, S., Nagar, G., Kumar, R., Paul, S., Fular, A., Chigure, G., Nandi, A., Manjunathachar, H.V., Mohammad, A., Verma, M.R., Saravanan, B.C., Ray, D., 2017. Characterization and establishment of a reference deltamethrin and cypermethrin resistant tick line (IVRI-IV) of *Rhipicephalus (Boophilus) microplus*. Pestic. Biochem. Physiol. 138, 66–70. https://doi.org/10.1016/j.pestbp.2017.03.002.

Herman, A., Herman, A.P., 2015. Essential oils and their constituents as skin penetration enhancer for transdermal drug delivery: a review. J. Pharm. Pharmacol. 67, 473–485. https://doi.org/10.1111/jphp.12334.

Higa, L.D.O.S., Garcia, M.V., Rodrigues, V.D.S., Bonatte, P., Piña, F.T.B., Barros, J.C., Andreotti, R., 2019. Effects of cypermethrin, chlorpyrifos and piperonyl butoxide-based pour-on and spray acaricides on controlling the tick *Rhipicephalus microplus*. Syst. Appl. Acarol. 24, 278–286. https://doi.org/10.11158/saa.24.2.10.

Jankowska, M., Rogalska, J., Wyszkowska, J., Stankiewicz, M., 2018. Molecular targets for components of essential oils in the insect nervous system—A review. Molecules 23, 1–20. https://doi.org/10.3390/molecules23010034.

Klafke, G., Webster, A., Dall Agnol, B., Pradel, E., Silva, J., de La Canal, L.H., Becker, M., Osório, M.F., Mansson, M., Barreto, R., Scheffer, R., Souza, U.A., Corassini, V.B., dos Santos, J., Reck, J., Martins, J.R., 2017. Multiple resistance to acaricides in field populations of *Rhipicephalus microplus* from Rio Grande do Sul state, Southern Brazil. Ticks Tick. Borne. Dis. 8, 73–80. https://doi.org/10.1016/j.ttbdis.2016.09.019.

Klafke, G.M., Sabatini, G.A., de Albuquerque, T.A., Martins, J.R., Kemp, D.H., Miller, R. J., Schumaker, T.T.S., 2006. Larval immersion tests with ivermectin in populations of the cattle tick *Rhipicephalus (Boophilus) microplus* (Acari: ixodidae) from State of

Sao Paulo, Brazil. Vet. Parasitol. 142, 386 – 390. https://doi.org/10.1016/j.vetpar.2006.07.001.
Lambert, R.J.W., Skandamis, P.N., Coote, P.J., Nychas, G.J.E., 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Appl. Microbiol. 91, 453–462. https://doi.org/10.1046/j.1365-2672.2001.01428.x.
Li, A.Y., Chen, A.C., Davey, R.B., Miller, R.J., George, J.E., 2007. Acaricide resistance and synergism between permethrin and amitraz against susceptible and resistant strains of *Boophilus microplus* (Acari, Ixodidae). Pest. Manag. Sci. 63, 882 – 889. https://doi.org/10.1002/ps.1417.
Lima, A.S., Milhomem, M.N., Santos Monteiro, O., Arruda, A.C.P., de Castro, J.A.M., Fernandes, Y.M.L., Maia, J.G.S., Costa-Junior, L.M., 2018. Seasonal analysis and acaricidal activity of the thymol-type essential oil of *Ocimum gratissimum* and its major constituents against *Rhipicephalus microplus* (Acari: Ixodidae). Parasitol. Res. 117, 59–65. https://doi.org/10.1007/s00436-017-5662-0.
Lin, Y., Jin, T., Zeng, L., Lu, Y., 2012. Cuticular penetration of β-cypermethrin in insecticide-susceptible and resistant strains of *Bactrocera dorsalis*. Pestic. Biochem. Physiol. 103, 189–193. https://doi.org/10.1016/j.pestbp.2012.05.002.
Lopes, W.D.Z., Teixeira, W.F.P., de Matos, L.V.S., Felippelli, G., Cruz, B.C., Maciel, W.G., Buzzulini, C., Fávero, F.C., Soares, V.E., Oliveira, G.P.de, da Costa, A.J., 2013. Effects of macrocyclic lactones on the reproductive parameters of engorged *Rhipicephalus (Boophilus) microplus* females detached from experimentally infested cattle. Exp. Parasitol. 135, 72–78. https://doi.org/10.1016/j.exppara.2013.06.003.
Lovis, L., Guerrero, F.D., Miller, R.J., Bodine, D.M., Betschart, B., Sager, H., 2012. Distribution patterns of three sodium channel mutations associated with pyrethroid resistance in *Rhipicephalus (Boophilus) microplus* populations from North and South America, South Africa and Australia. Int. J. Parasitol. Drugs Drug Resist. 2, 216–224. https://doi.org/10.1016/j.ijpddr.2012.08.001.
Matos, R.S., Daemon, E., Monteiro, C.M.O., Sampieri, B.R., Marchesini, P.B.C., Delmonte, C., Camargo-Mathias, M.I., 2019. Thymol action on cells and tissues of the synganglia and salivary glands of *Rhipicephalus sanguineus* sensu lato females (Acari: Ixodidae). Ticks Tick. Borne. Dis. 10, 314–320. https://doi.org/10.1016/j.ttbdis.2018.11.003.
Matos, R.S., de Oliveira, P.R., Coelho, L., de Paula, L.G.F., Zeringota, V., Carvalho Silva, B., Monteiro, C., Daemon, E., Camargo-Mathias, M.I., 2020. Thymol: effects on reproductive biology and Gene's organ morphology in *Rhipicephalus sanguineus* sensu lato engorged females (Acari: Ixodidae). Ticks Tick Borne Dis. 11, 101308 https://doi.org/10.1016/j.ttbdis.2019.101308.
Matos, R.S., Daemon, E., Camargo-Mathias, M.I., Furquim, K.C.S., Sampieri, B.R., Remédio, R.N., Araújo, L.X., Novato, T.P.L., 2014. Histopathological study of ovaries of *Rhipicephalus sanguineus* (Acari: Ixodidae) exposed to different thymol concentrations. Parasitol. Res. 113, 4555–4565. https://doi.org/10.1007/s00436-014-4145-9.
Mendes, A.S., Daemon, E., Monteiro, C.M.O., Maturano, R., Brito, F.C., Massoni, T., 2011. Acaricidal activity of thymol on larvae and nymphs of *Amblyomma cajennense* (Acari: Ixodidae). Vet. Parasitol. 183, 136–139. https://doi.org/10.1016/j.vetpar.2011.07.007.
Miró, M.V., e Silva, C.R., Viviani, P., Luque, S., Lloberas, M., Costa-Júnior, L.M., Lanusse, C., Virkel, G., Lifschitz, A., 2020a. Combination of bioactive phytochemicals and synthetic anthelmintics: in vivo and in vitro assessment of the albendazole-thymol association. Vet. Parasitol. 281, 109121 https://doi.org/10.1016/j.vetpar.2020.109121.
Miró, V., Lifschitz, A., Viviani, P., Rocha, C., Lanusse, C., Costa, L., Virkel, G., 2020b. In vitro inhibition of the hepatic S-oxygenation of the anthelmintic albendazole by the natural monoterpene thymol in sheep. Xenobiotica 50, 408–414. https://doi.org/10.1080/00498254.2019.1644390.
Monteiro, C.M.O., Daemon, E., Clemente, M.A., Rosa, L.S., Maturano, R., 2009. Acaricidal efficacy of thymol on engorged nymphs and females of *Rhipicephalus sanguineus* (Latreille, 1808) (Acari: Ixodidae). Parasitol. Res. 105, 1093–1097. https://doi.org/10.1007/s00436-009-1530-x.
Nasreen, N., Niaz, S., Khan, A., Zaman, M.A., Ayaz, S., Naeem, H., Khan, N., Elgorban, A. M., 2020. The potential of *Allium sativum* and *Cannabis sativa* extracts for anti-tick activities against *Rhipicephalus (Boophilus) microplus*. Exp. Appl. Acarol. 82, 281–294. https://doi.org/10.1007/s10493-020-00540-z.
Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., De Feo, V., 2013. Effect of essential oils on pathogenic bacteria. Pharmaceuticals 6 (12), 1451–1474. https://doi.org/10.3390/ph6121451.
Novato, T.L.P., Marchesini, P., Muniz, N., Prata, M.C.de A., Furlong, J., Vilela, F.M.P., Daemon, E., Maturano, R., Monteiro, C., 2019. Evaluation of synergism and development of a formulation with thymol, carvacrol and eugenol for *Rhipicephalus microplus* control. Exp. Parasitol. 207, 107774 https://doi.org/10.1016/j.exppara.2019.107774.
Novato, T.P.L., Araújo, L.X., de Monteiro, C.M.O., Maturano, R., Senra, T.de O.S., da Silva Matos, R., Gomes, G.A., de Carvalho, M.G., Daemon, E., 2015. Evaluation of the combined effect of thymol, carvacrol and (E)-cinnamaldehyde on *Amblyomma sculptum* (Acari: Ixodidae) and *Dermacentor nitens* (Acari: Ixodidae) larvae. Vet. Parasitol. 212, 331–335. https://doi.org/10.1016/j.vetpar.2015.08.021.
Pei, R.S., Zhou, F., Ji, B.P., Xu, J., 2009. Evaluation of combined antibacterial effects of eugenol, cinnamaldehyde, thymol, and carvacrol against *E. coli* with an improved method. J. Food Sci. 74, 379–383. https://doi.org/10.1111/j.1750-3841.2009.01287.x.
Politi, F.A.S., Nascimento, J.D., da Silva, A.A., Moro, I.J., Garcia, M.L., Guido, R.V.C., Pietro, R.C.L.R., Godinho, A.F., Furlan, M., 2017. Insecticidal activity of an essential oil of *Tagetes patula* L. (Asteraceae) on common bed bug *Cimex lectularius* L. and molecular docking of major compounds at the catalytic site of ClAChE1. Parasitol. Res. 116, 415–424. https://doi.org/10.1007/s00436-016-5305-x.
Priestley, C.M., Williamson, E.M., Wafford, K.A., Sattelle, D.B., 2003. Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human GABA A receptors and a homo-oligomeric GABA receptor from *Drosophila melanogaster*. Br. J. Pharmacol. 140, 1363–1372. https://doi.org/10.1038/sj.bjp.0705542.
Prullage, J.B., Cawthorne, W.G., de Le Hir Fallois, L.P., Timmons, P.R., 2011. Synergy between fipronil and amitraz in a *Rhipicephalus sanguineus* tick residual contact test. Exp. Appl. Acarol. 54, 173–176. https://doi.org/10.1007/s10493-011-9424-x.
Rehman, H., Aziz, A.T., Saggu, S., Abbas, Z.K., Mohan, A., Ansari, A.A., 2014. Systematic review on pyrethroid toxicity with special reference to deltamethrin. J. Entomol. Zool. Stud. JEZS 2, 60–70.
Reck, J., Klafke, G.M., Webster, A., Dall'Agnol, B., Scheffer, R., Souza, U.A., Corassini, V. B., Vargas, R., dos Santos, J.S., Martins, J.R., 2014. First report of fluazuron resistance in *Rhipicephalus microplus*: a field tick population resistant to six classes of acaricides. Vet. Parasitol. 201, 128–136.
Roditakis, E., Roditakis, N.E., Tsagkarakou, A., 2005. Insecticide resistance in *Bemisia tabaci* (Homoptera: aleyrodidae) populations from Crete. Pest Manag Sci. 61, 577–582. https://doi.org/10.1002/ps.1029.
Rodriguez-Vivas, R.I., Li, A.Y., Ojeda-Chi, M.M., Trinidad-Martinez, I., Rosado-Aguilar, J. A., Miller, R.J., Perez de León, A.A., 2013. In vitro and in vivo evaluation of cypermethrin, amitraz, and piperonyl butoxide mixtures for the control of resistant *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) in the Mexican tropics. Vet. Parasitol. 197, 288–296. https://doi.org/10.1016/j.vetpar.2013.07.018.
Rodriguez-Vivas, R.I., Rivas, A.L., Chowell, G., Fragoso, S.H., Rosario, C.R., García, Z., Smith, S.D., Williams, J.J., Schwager, S.J., 2007. Spatial distribution of acaricide profiles (*Boophilus microplus* strains susceptible or resistant to acaricides) in southeastern Mexico. Vet. Parasitol. 146, 158–169. https://doi.org/10.1016/j.vetpar.2007.01.016.
Rosado-Aguilar, J.A., Arjona-Cambranes, K., Torres-Acosta, J.F.J., Rodríguez-Vivas, R.I., Bolio-González, M.E., Ortega-Pacheco, A., Alzina-López, A., Gutiérrez-Ruiz, E.J., Gutiérrez-Blanco, E., Aguilar-Caballero, A.J., 2017. Plant products and secondary metabolites with acaricide activity against ticks. Vet. Parasitol. 238, 66–76. https://doi.org/10.1016/j.vetpar.2017.03.023.
Sagar, S.V., Saini, K., Sharma, A.K., Kumar, Sachin, Kumar, R., Fular, A., Shakya, M., Upadhaya, D., Nagar, G., Shanmuganath, C., Samanta, S., Kumar, Suman, Ghosh, S., 2020. Acaricide resistance in *Rhipicephalus microplus* collected from selected districts of Madhya Pradesh, Uttar Pradesh and Punjab states of India. Trop. Anim. Health Prod. 52, 611–618. https://doi.org/10.1007/s11250-019-02048-0.
Scalerandi, E., Flores, G.A., Palacio, M., Defagó, M.T., Carpinella, M.C., Valladares, G., Bertoni, A., Palacios, S.M., 2018. Understanding synergistic toxicity of terpenes as insecticides: contribution of metabolic detoxification in *Musca domestica*. Front. Plant Sci. 871, 1–9. https://doi.org/10.3389/fpls.2018.01579.
Selzer, P.M., Epe, C., 2021. Antiparasitics in Animal Health: quo Vadis? Trends Parasitol 37, 77–89. https://doi.org/10.1016/j.pt.2020.09.004.
Senra, T.O.S., Calmon, F., Zeringóta, V., Monteiro, C.M.O., Maturano, R., Da Silva Matos, R., Melo, D., Gomes, G.A., De Carvalho, M.G., Daemon, E., 2013a. Investigation of activity of monoterpenes and phenylpropanoids against immature stages of *Amblyomma cajennense* and *Rhipicephalus sanguineus* (Acari: Ixodidae). Parasitol. Res. 112, 3471–3476. https://doi.org/10.1007/s00436-013-3527-8.
Senra, T.O.S., Zeringóta, V., Monteiro, C.M.O., Calmon, F., Maturano, R., Gomes, G.A., Faza, A., De Carvalho, M.G., Daemon, E., 2013b. Assessment of the acaricidal activity of carvacrol, (E)-cinnamaldehyde, trans-anethole, and linalool on larvae of *Rhipicephalus microplus* and *Dermacentor nitens* (Acari: Ixodidae). Parasitol. Res. 112, 1461–1466. https://doi.org/10.1007/s00436-013-3289-3.
Silva, L.C., Perinotto, W.M.S., Sá, F.A., Souza, M.A.A., Bitencourt, R.O.B., Sanavria, A., Santos, H.A., Marie-Magdeleine, C., Angelo, I.C., 2020. In vitro acaricidal activity of *Cymbopogon citratus, Cymbopogon nardus* and *Mentha arvensis* against *Rhipicephalus microplus* (Acari: Ixodidae). Exp. Parasitol. 216, 107937 https://doi.org/10.1016/j.exppara.2020.107937.
SINDAN - Sindicado Nacional da Indústria de Produtos para Saúde Animal. Compêndio de produtos veterinários, 2018. https://sistemas.sindan.org.br/cpvs/ (Accessed 13 December 2020).
Songkro, S., Becket, G., Rades, T., 2004. The effects of p-menthane monoterpenes and related compounds on the percutaneous absorption of propranolol hydrochloride across newborn pig skin: III. Correlation between penetration enhancing activity and physicochemical properties of terpenes determined by principal components analysis. J. Drug Deliv. Sci. Technol. 14, 223–228. https://doi.org/10.1016/s1773-2247(04)50104-1.
Sousa, E.O., Lima, A.D.S., Lopes, S.G., Costa-Junior, L.M., Da Costa, J.G.M., 2020. Chemical composition and acaricidal activity of *Lantana camara* L. and *Lantana montevidensis* Briq. essential oils on the tick *Rhipicephalus microplus*. J. Essent. Oil Res. 32, 316–322. https://doi.org/10.1080/10412905.2020.1752320.
Stott, P.W., Williams, A.C., Barry, B.W., 1998. Transdermal delivery from eutectic systems: enhanced permeation of a model drug, ibuprofen. J. Control. Release 50, 297–308. https://doi.org/10.1016/S0168-3659(97)00153-3.
Suwannayod, S., Sukontason, K.L., Pitasawat, B., Junkum, A., Limsopatham, K., Jones, M.K., Somboon, P., Leksomboon, R., Chareonviriyaphap, T., Tawatsin, A., Thavara, U., Sukontason, K., 2019. Synergistic toxicity of plant essential oils combined with pyrethroid insecticides against blow flies and the house fly. Insects 10. https://doi.org/10.3390/insects10060178.
Tabari, M.A., Youssefi, M.R., Maggi, F., Benelli, G., 2017. Toxic and repellent activity of selected monoterpenoids (thymol, carvacrol and linalool) against the castor bean tick, *Ixodes ricinus* (Acari: Ixodidae). Vet. Parasitol. 245, 86–91. https://doi.org/10.1016/j.vetpar.2017.08.012.
Tak, J.H., Isman, M.B., 2017. Enhanced cuticular penetration as the mechanism of synergy for the major constituents of thyme essential oil in the cabbage looper,

Trichoplusia ni. Ind. Crops Prod. 101, 29–35. https://doi.org/10.1016/j.indcrop.2017.03.003.

Tak, J.H., Isman, M.B., 2015. Enhanced cuticular penetration as the mechanism for synergy of insecticidal constituents of rosemary essential oil in *Trichoplusia ni*. Sci.Rep. 5, 1–10. https://doi.org/10.1038/srep12690.

Tong, F., Coats, J.R., 2010. Effects of monoterpenoid insecticides on [3H]-TBOB binding
in house fly GABA receptor and 36Cl- uptake in American cockroach ventral nervecord. Pestic. Biochem. Physiol. 98, 317–324. https://doi.org/10.1016/j.pestbp.2010.07.003.

Upadhaya, D., Kumar, B., Kumar, S., Sharma, A.K., Fular, A., Bisht, N., Srivastava, S., Boruah, R.R., Nagar, G., Shakya, M., Nath, T., Nandi, S.P., S, K., Ghosh, S., 2020.Characterization of acaricide resistance in *Rhipicephalus microplus* populations infesting cattle in northeastern India and assessment of local plant extracts for tickmanagement. Vet. Parasitol. 277, 109011 https://doi.org/10.1016/j. vetpar.2019.109011.

Valsoni, L.M., Freitas, M.G.de, Echeverria, J.T., Borges, D.G.L., Tutija, J., Borges, F.de A.,2020. Resistance to all chemical groups of acaricides in a single isolate of *Rhipicephalus microplus* in Mato Grosso do Sul, Brazil. Int. J. Acarol. 46, 276–280. https://doi.org/10.1080/01647954.2020.1765867.

Vilela, V.L.R., Feitosa, T.F., Bezerra, R.A., Klafke, G.M., Riet-Correa, F., 2020. Multipleacaricide-resistant *Rhipicephalus microplus* in the semi-arid region of Paraíba State, Brazil. Ticks Tick. Borne. Dis. 11, 101413 https://doi.org/10.1016/j. ttbdis.2020.101413.

Villar, D., Klafke, G.M., Rodríguez-Durán, A., Bossio, F., Miller, R., Pérez de León, A.A., Cortés-Vecino, J.A., Chaparro-Gutiérrez, J.J., 2020. Resistance profile and molecular characterization of pyrethroid resistance in a *Rhipicephalus microplus* strain from Colombia. Med. Vet. Entomol. 34, 105–115. https://doi.org/10.1111/mve.12418.

Wagner, H., 2011. Synergy research: approaching a new generation of phytopharmaceuticals. Fitoterapia 82, 34–37. https://doi.org/10.1016/j.fitote.2010.11.016.

Williams, A.C., Barry, B.W., 1991. Terpenes and the lipid-protein-partitioning theory ofskin penetration enhancement. Pharm. Res. 8, 17–24. https://doi.org/10.1023/a: 1015813803205.

CAPÍTULO II

Effects of carvacrol and thymol on the antioxidant and detoxifying enzymes of *Rhipicephalus microplus* (Acari: Ixodidae).

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Original article

Effects of carvacrol and thymol on the antioxidant and detoxifying enzymes of *Rhipicephalus microplus* (Acari: Ixodidae)

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ABSTRACT

The present study was conducted to evaluate the effects of carvacrol and thymol on the antioxidant and detoxifying enzymes of larvae from two populations of *R. microplus:* Jaguar (tick population resistant to six classes of acaricides) and Porto Alegre (susceptible tick population). Carvacrol and thymol were tested at concentrations ranging from 0.14 to 5.0 mg mL^{-1} in both populations to determine the LC_{50}. In addition, the LC_1, LC_{25}, and LC_{75} were estimated using the LC_{50} and HillSlope of each compound. Larvae of both populations of *R. microplus* were then treated with the LC_1, LC_{25}, LC_{50}, and LC_{75} of each monoterpene, and those that survived were processed to evaluate the effects of the compounds on the antioxidant and detoxifying systems of larvae; these effects were assessed by determining the activity of the enzymes, glutathione-S-transferase (GST), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX). Larvae from the Jaguar population treated with different lethal concentrations of carvacrol and thymol displayed a dose-dependent increase in CAT, GPX, SOD, and GST after treatment with LC_{25}. Further, larvae treated with the LC_{75} had the highest levels of enzyme activity for carvacrol (1.76 mg mL^{-1}) and thymol (1.32 mg mL^{-1}). CAT, GPX, SOD, and GST activity in Porto Alegre population larvae treated with carvacrol and thymol also increased significantly up to the LC_{50} of each monoterpene. However, at the LC_{75} of carvacrol and thymol, a decrease in the activity of all enzymes was observed for this tick population. These findings indicate that carvacrol and thymol induced increased activity of all evaluated enzymes at different lethal concentrations in *R. microplus* larvae from two populations. Such findings unveil the possible mechanisms of action of these natural acaricides.

1. Introduction

The cattle tick, *Rhipicephalus microplus*, is an ectoparasite widely distributed in tropical and subtropical regions that has a considerable economic impact on livestock (Estrada-Peña et al., 2006; Grisi et al., 2014; Marques et al., 2020). The occurrence and spread of *R. microplus* are mainly controlled using synthetic acaricides; however, the constant and sometimes indiscriminate use of these products has caused strong selective pressure, accelerating the emergence of populations resistant to the main acaricide classes available in the market (Valsoni et al., 2020; Vilela et al., 2020; Sagar et al., 2020; Upadhaya et al., 2020).

The mechanism of action of the main classes of synthetic acaricides is related to the inhibition or overactivation of target sites, such as for pyrethroids and formamidines, which act on sodium channels (Soderlund, 2012) and GABA-Cl^- receptors (Bloomquist, 2003), respectively. In addition, these compounds stimulate the production of reactive oxygen species (ROS), causing oxidative stress, which can result in oxidative damage in some tissues and increase the mortality of exposed organisms (Hyršl et al., 2007; Büyükgüzel, 2009). Lipid peroxidation and changes in the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), and glutathione peroxidase (GPX), are molecular mechanisms involved in the toxicity caused by endo- and xenobiotic compounds, including pesticides (Banerjee et al., 1999; Büyükgüzel et al., 2013). Therefore, the

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values of these parameters (peroxidation and enzymatic activity) are important for estimating the stress caused by xenobiotics and/or activation of detoxification mechanisms (Freitas et al., 2007).

One of the mechanisms of resistance to pesticides in arthropods is related to metabolic detoxification mediated mainly by GST enzymes, esterases, and monooxygenases (Hemingway et al., 2004; Rosario-Cruzet al., 2009). The GST, in particular, may be involved in resistance to ivermectin, deltamethrin, and coumaphos in *R. microplus* (Le Gall et al., 2018; Upadhaya et al., 2020; Kumar et al., 2021). Thus, at the metabolic level, the detoxification of acaricides may be related to different degreesof resistance and susceptibility to synthetic compounds in arthropods.

Carvacrol and thymol are monoterpenes present in the essential oilsof several species of aromatic plants (Moldão-Martins et al., 2000; De Vincenzi et al., 2004), and are promising alternatives for the control of *R. microplus* (Cardoso et al., 2020; Penha et al., 2021). The mechanismsof action of these two monoterpenes on ticks and insects are thought tobe related to tyramine receptor inhibition (Gross et al., 2017), acetylcholinesterase enzyme inhibition (Cardoso et al., 2020), and GABA receptor modulation (Tong and Coats, 2010). Thymol can also increase the concentration of cypermethrin in the internal tissues of engorged females of *R. microplus* by increasing the penetration of this pyrethroid at the cuticular level (Tavares et al., 2022). Carvacrol and thymol also can induce oxidative stress in bacteria, such as *Acinetobacter baumannii* and *Candida albicans* fungi (Khan et al., 2015; Montagu et al., 2016).

Reports on the mechanisms of action and physiological effects of carvacrol and thymol, especially in ticks, are still very incipient and scarce. Despite studies by Arafa et al. (2020) and Cardoso et al. (2020), who evaluated the effects of these monoterpenes on acetylcholinesterase activity, oxidative stress, and antioxidant defense biomarkers in *R. microplus*, additional studies are required to better understand the physiological effects of these potential natural acaricides. The objectiveof this study was to evaluate the effects of carvacrol and thymol on the enzymatic activity of superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), and glutathione peroxidase (GPX) in *R. microplus* populations with different degrees of resistance to synthetic compounds.

2. Materials and methods

2.1. Tick collection

Naturally detached engorged *R. microplus* females were obtained from calves artificially infested with larvae from two populations with different susceptibilities to synthetic acaricides. To conduct this study, susceptibility was first confirmed using the Adult Immersion Test (AIT). Porto Alegre is a reference tick population with high susceptibility to synthetic acaricides (Reck et al., 2009) while the Jaguar population is resistant to the six to the six classes of acaricides available for controlling *R. microplus* in Brazil, including pyrethroids, amidines, organophosphates, phenylpyrazoles, macrocyclic lactones, and growth inhibitors (Reck et al., 2014). The engorged females obtained were washed with distilled water, dried on filter paper, and used directly in the adult immersion test or placed in Petri dishes and kept at a temperature of 27 ± 1 °C with relative humidity (RH) ≥ 80% until complete oviposition. The eggs were collected and placed in 15 mL conical bottom tubes closed with cotton to allow air and moisture, and subsequently incubated at 271 °C with RH ≥ 80% until hatching. For larval immersion tests, unfed larvae between 14 and 21 d were used. This experiment was approved by the Ethics Committee on Animal Experimentation of the Federal University of Maranhão, Brazil, under protocol number 23, 115.005443/2017-51.

2.2. Larval immersion test (LIT)

LIT was performed according to the method described by Klafke et al. (2006). Carvacrol and thymol (both with 99% purity, Sigma-Aldrich, St. Louis, MO, USA) were tested at concentrations of 5.0, 3.0, 1.8, 1.08, 0.65, 0.39, 0.23, and 0.14 mg mL^{-1} with both populations (Porto Alegre and Jaguar). All dilutions were performed using 1% ethanol a nd 0.02% Triton X-100. The LIT was performed in four independent assays, each with four replicates, and the control group was treated with solvent (1% ethanol and 0.02% Triton X-100 solution).

Approximately 500 larvae were immersed for 10 min at each concentration, and then transferred to dry on filter paper. Thereafter, approximately 100 larvae were transferred to a clean, dry filter paper package (8.5 7.5 cm) closed with plastic clips. The packets wereplaced in a biochemical oxygen demand (BOD) incubator at a temperature of 27 ± 1 °C and RH ≥ 80% for 24 h. After incubation, dead and live larvae were counted manually. Tick larvae without movement after stimulation by CO_2, were considered dead.

2.3. Determination of enzymatic activity in larvae

2.3.1. Treatment and processing of larvae

Larvae of both populations of *R. microplus* were treated with LC_1, LC_{25}, LC_{50}, and LC_{75} of each monoterpene. Approximately 800 larvae were added to microcentrifuge tubes containing 1 mL of the lethal concentration of each monoterpene for larval treatment. The tubes were immediately closed, shaken for a few seconds, and left to rest for 10 min. After this period, the larvae were dried on filter paper, transferred to filter paper packages (8.5 7.5 cm), and closed with clips. The packages were placed in a B.O.D. incubator at 27 °C and RH 80% for 24 h, and larvae that survived the treatments were collected for further processing.

Surviving larvae were immediately macerated in PBS buffer (0.2 M NaH_2PO_4, 0.15 M NaCl, pH 6.7) and centrifuged (16,128 *g* × 20 min at 4 °C). The supernatant was removed and transferred to another microcentrifuge tube, where a mixture of enzyme inhibitors (1 μM pepstatin A, 10 μM E-64, 50 μM TPKC, and 5 μM EDTA) was added. The total protein content of all extracts was quantified using the Bradford method (Bradford, 1976). A negative control was established using the diluent used to prepare the concentrations for each treatment.

2.3.2. GST activity assay

The GST activity of the larval protein extracts obtained from the larvae was determined according to the method described by Habiget al. (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) and glutathione (GSH) (Sigma-Aldrich) as substrates. Ninety microliters of the mixing reaction (50 mM CDNB in methanol, 5 mM glutathione reduced in 100 mM Tris–HCl pH 7.5) and 10 μL of protein extract from treated larvae (100 mM Tris–HCl pH 7.5) were tested in a 96-well plate, according to Da Silva Vaz Jr. et al. (2004). The absorbance at 340 nm was measured for 15 min at 1 min intervals. As a negative control, 90 μL of the mixing reaction and 10 μL of Tris–HCl (100 mM, pH 7.5) were used. The concentration of the product was calculated using the molar extinction coefficient of 9.6 mM/cm for S-(2,4-dinitrophenyl glutathione) (Widersten and Mannervick, 1995). The activity is expressed as U/mg of protein.

2.3.3. SOD activity assay

SOD activity was determined based on the original method of McCord and Fridovich (1969) using the Ransod® kit (Randox, Crumlin, UK), with absorbance measured at 505 nm for 6 min at 1 min intervals ina 96-well plate, according to the manufacturer' s protocol. The assay used xanthine and xanthine oxidase to generate superoxide radicals, which react with 2-(4-iodophenyl) 3-(4-nitrophenol) 5-phenyl- tetrazolium chloride (INT) to form a red formazan dye. SOD activity wasthen measured by the degree of inhibition of the reaction and expressedas U/μg of protein.

2.3.4. GPX activity assay

GPX activity was assessed using the Randox Kit Ransel 504®

(Randox) in a 96-well plate by spectrophotometry with a technique based on the method of Paglia and Valentine (1967). The reaction was measured at 340 nm for 2 min and 33 s at 51 s-intervals. The GPX valueis expressed in U/g of protein.

2.3.5. CAT activity assay

The CAT assay was performed according to the method described by Aebi (1984). The assay reaction solution contained 560 µL of PBS buffer (pH=6.7), 30 µL of H_2O_2 (80 mM), and 10 µL of larval protein extract. The decomposition of H_2O_2 was followed by a decline in absorbance at 240 nm for 2 min at 10 s-intervals. CAT activity was calculated using the formula described by Cuéllar-Cruz et al. (2009). The specific activity of the enzyme is expressed as U/mg of protein.

2.4. Statistical analysis

To estimate the LC_{50} (50% lethal concentration) from LIT, the concentrations of carvacrol and thymol were initially transformed to log (X) and normalized, and nonlinear regression was performed using the GraphPad Prism 8.0.2 software (GraphPad Inc., San Diego, CA, USA). The significance of each comparison was determined when the calculated confidence intervals did not overlap. The LC_1, LC_{25}, and LC_{75} were estimated by using $EC_{anything}$ from EC_{50} (https://www.graphpad.com/quickcalcs/Ecanything1.cfm, Quick Calcs – GraphPad; EC: effective concentration) and entering the LC_{50} HillSlope of each compound for both populations of *R. microplus*.

All results for the determination of enzyme activity in larvae were expressed as mean ± standard deviation and were analyzed statistically by analysis of variance, followed by Tukey's test ($p < 0.05$). Statistical analyses were performed using GraphPad Prism 8.0.2 software (GraphPad Inc.).

3. Results

3.1. Effect on larval mortality

At the tested concentrations, carvacrol and thymol displayed acaricidal activity in both tick populations. Thymol showed significantly higher activity (CL_{50}) (Porto Alegre: 0.90 mg mL^{-1} and Jaguar: 1.32 mg mL^{-1}) than carvacrol (Porto Alegre: 1.42 mg mL^{-1} and Jaguar: 1.76 mg mL^{-1}) (non-IC overlap).

The lethal concentrations (LC_1, LC_{25}, LC_{50}, and LC_{75}) of the compounds for larvae of the two populations are listed in Table 1.

3.2. Effect of compounds on the antioxidant and detoxifying enzymes of larvae

Rhipicephalus microplus larvae from the Jaguar population showed a dose-dependent increase in CAT, GPX, SOD, and GST after treatment with the LC_{25} of carvacrol and thymol compared to the control. However, the SOD and GST enzymes from larvae treated with the LC_{50} of thymol (1.32 mg mL^{-1}) had activity of 0.99 0.01 U/µg and 8.23 ± 0.23 U/mg, respectively, revealing a decrease in activity compared with the activity of SOD (1.03 ± 0.01 U/µg) and GST (9.04 ± 0.06 U/mg) in larvae treated with LC_{25} (1.05 mg mL^{-1}) (Figs. 1 and 2). This decrease was not statistically significant for any enzyme ($P > 0.05$).

Larvae from this same population had the highest levels of enzyme activity for both monoterpenes when treated with the highest lethal concentration (LC_{75}) (Figs. 1 and 2). The enzymes that showed the highest activity were SOD and GPX. Compared with the control, SOD showed an activity increase of approximately 2.7-fold (1.92 ± 0.00 U/µg) and 1.9-fold (1.33 0.01 U/µg) in larvae treated with carvacrol and thymol, respectively. Further, GPX was increased by approximately 2.3-fold (398.44 21.54 U/g) and 2.2-fold (367.82 7.07 U/g) in larvae treated with carvacrol and thymol, respectively.

The activities of CAT, GPX, SOD, and GST in larvae of the Porto Alegre population treated with carvacrol and thymol increased significantly compared to those of the control, with a dose-dependent growth of activity from treatment with the lowest concentrations (LC_1) of carvacrol (1.06 mg mL^{-1}) and thymol (0.51 mg mL^{-1}). However, SOD, GPX, and GST in larvae treated with thymol LC_{25} (0.66 mg mL^{-1}) had a decrease in activity (SOD: 0.98 ± 0.01 U/µg; GPX: 221.64 ± 23.47 U/g; GST: 9.12 ± 0.17 U/mg) compared to treatment with thymol LC_1 (SOD: 1.28 0.01 U/µg; GPX: 282.94 7.07 U/g; GST: 11.52 0.43 U/mg); this variation was not statistically significant ($p > 0.05$). These three enzymes had increased activity in larvae treated with the LC_{50} of thymol (0.9 mg mL^{-1}). The increase in the activity of the four enzymes occurred until treatment with the LC_{50} of each of the monoterpenes. At thehighest concentration of carvacrol (LC_{75}: 1.99 mg mL^{-1}) and thymol (LC_{75}: 1.65 mg mL^{-1}), a decrease in the activity of all enzymes was observed. A significant reduction (p=0.0056) in CAT activity was observed in Porto Alegre larvae treated with the LC_{75} of carvacrol compared to the control larvae, with values of 60.8 2.77 U/mg and 73.29 1.78 U/mg, respectively, indicating a strong inhibition of the activity of this enzyme (Figs. 1 and 2).

The enzymatic activity of the control groups did not differ statistically for any enzyme when the two populations were compared.

4. Discussion

Biological compounds have become increasingly viable alternatives for pest control to satisfy the increasing consumer demand for biopesticides. Recently, the acaricidal effects of the monoterpenes, carvacrol and thymol, on different tick species were extensively reported (Silva Lima et al., 2018; Arafa et al., 2020; Cardoso et al., 2020; Coelhoet al., 2020), including studies in field and semi-field conditions (Arafaet al., 2021; Monteiro et al., 2021). However, the physiological and biochemical effects of these molecules on ticks need to be further explored to elucidate the modes of action and to better understand theirsafety and toxicity (Cardoso et al., 2020). In this study, we demonstrated for the first time the effect of carvacrol and thymol on important anti- oxidant and detoxifying enzymes in the tick, *R. microplus*.

The toxicity of the monoterpenes, thymol and carvacrol, was not only revealed by the larval mortality rates obtained in the two pop- ulations of *R. microplus*, but also by the alteration in the levels of enzymatic activity of the tick antioxidant and detoxifying systems. The physiological response of *R. microplus* larvae to treatments with carva- crol and thymol, based on the increased activity of the enzymes CAT,

Table 1
Lethal concentration (LC - mg mL^{-1}) of carvacrol and thymol for 1, 25, 50, and 75% of *Rhipicephalus microplus* larvae from Porto Alegre (susceptible) and Jaguar (resistant) populations.

Population	Compound	LC_1*	LC_{25}*	LC_{75}*	LC_{50}	HillSlope ± SE	CI 95%	R^2
Porto Alegre	Carvacrol	0.72	1.2	1.66	1.42	6.859 ± 0.645	1.366 - 1.470	0.98
	Thymol	0.25	0.66	1.21	0.9	3.651 ± 0.327	0.848 - 0.950	0.97
Jaguar	Carvacrol	1.06	1.56	1.99	1.76	9.059 ± 1.332	1.715 - 1.810	0.99
	Thymol	0.51	1.05	1.65	1.32	4.848 ± 0.426	1.261 - 1.371	0.98

*LC_1, LC_{25}, and LC_{75} were estimated using EC from EC50 (Quick Calcs – GraphPad) and the HillSlope value of LC50 for each population.
LC_{50}: lethal concentration (mg·mL^{-1}) for 50% of individuals; SE: standard error; CI: 95% confidence interval; R^2: regression correlation coefficient.

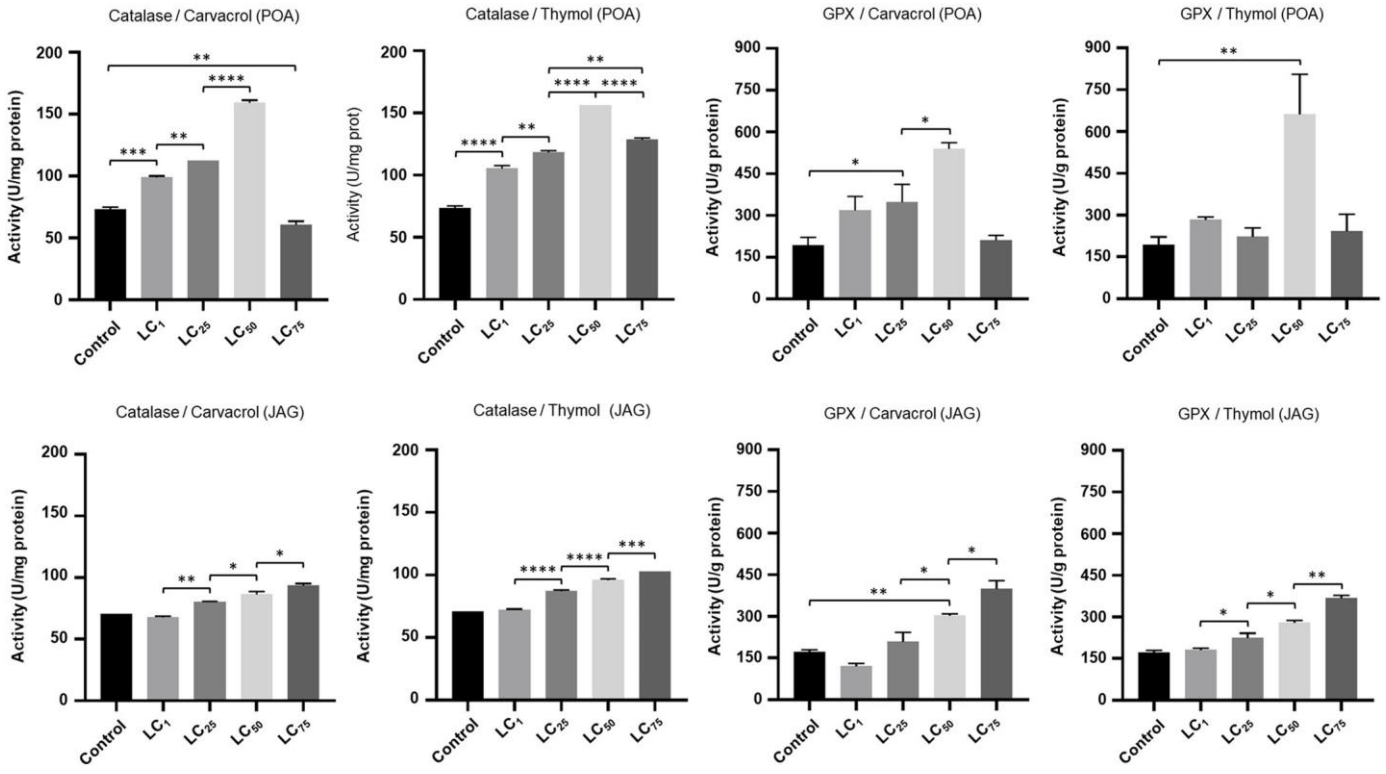

Fig. 1. Effect of different concentrations of carvacrol and thymol on the enzymatic activity of catalase and glutathione peroxidase (GPX) in *Rhipicephalus microplus* larvae from Porto Alegre (POA, susceptible) and Jaguar (resistant) populations. Statistical differences were determined between the averages of the activity of each enzyme and marked with asterisks (*–$p<0.05$, **–$p<0.01$, ***–$p<0.001$, ****–$p<0.0001$).
LC_1, LC_{25}, LC_{50}, and LC_{75} indicate the lethal concentrations of monoterpenes for the Porto Alegre population (carvacrol–0.72, 1.2, 1.42, 1.66 mg mL^{-1}; thymol–0.25, 0.66, 0.9, 1.21 mg mL^{-1}) and Jaguar population (carvacrol–1.06, 1.56, 1.76, 1.99 mg mL^{-1}; thymol–0.51, 1.05, 1.32, 1.65 mg mL^{-1}).

GPX, SOD, and GST, is a good indicator that these enzymes play a role in the toxicity of these two monoterpenes and that these compounds cause oxidative stress in *R. microplus* tissues.

Various xenobiotics stimulate the production of reactive oxygen species (ROS) (Adamski et al., 2003; Krishnan and Sehnal, 2006; Hyršl et al., 2007). Further, the increase in the activity of antioxidant enzymesin *R. microplus* larvae may be directly related to the increase in ROS dueto the toxic action of carvacrol and thymol. In *R. microplus* engorged females, the increase in catalase activity is directly related to the increase in ROS owing to the release of a large amount of heme group (pro-oxidant compound) resulting from the intracellular digestion of blood obtained in the feed. Thus, CAT plays an important role in the control of redox balance in *R. microplus* (Citelli et al., 2007). In eukaryotic cells, more than 90% of ROS are generated by the escape of electrons from the mitochondrial electron transport chain (ETC), which interacts with molecular oxygen to form the superoxide radical anion ($O_2^{\cdot -}$), which is spontaneously or enzymatically converted to hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$) (Skulachev, 2012). Carvacrol and thymol can destroy the mitochondrial membrane and promote the accumulation of ROS in organisms, such as fungi and protozoa (Monzote et al., 2018; Hou et al., 2020; Scariot et al., 2020), and human choriocarcinoma cell lines, where carvacrol increases ROS production as well as calcium ion concentrations in the mitochondrial matrix by disrupting mitochondrial membrane potential (MMP) (Lim et al., 2019).

Our results suggest that the dose-dependent increase in antioxidant enzymes in *R. microplus* larvae is a physiological response of the tick to the toxicity of monoterpenes to promote the pro-oxidative-antioxidant balance induced by tissue damage and increased generation of reac- tive oxygen species. Thymol can cause lipid peroxidation (determined by the production of malondialdehyde) in adult ticks of *Rhipicephalus annulatus*, as demonstrated by Arafa et al. (2020), reflecting the role of oxidative stress in the acaricidal effects of this monoterpene. Camphor, another monoterpene, has cytotoxic and apoptotic effects on the fungus, *Schizosaccharomyces pombe*, which is directly related to the excessive production of ROS (Agus et al., 2019). The effects of reactive oxygen species on pesticide toxicity are commonly observed (Adamski, 2007). Biological molecules, such as thymol and/or carvacrol, are known to cause tissue damage in several tick structures of the genus *Rhipicephalus*, such as changes in the ovary (Souza et al., 2019; Penha et al., 2021), morphohistochemical integument layer alterations (Souza et al., 2021), and changes in Gene' s organ (Matos et al., 2020), salivary glands, and synganglion (Matos et al., 2019).

Similar results were found in *Galleria mellonella* moth larvae treated with boric acid (a product used as an insecticide, acaricide, herbicide, and fungicide), which showed a dose-dependent increase in SOD, CAT, GST, and GPX activity in different organs (Büyükgüzel et al., 2013).

Carvacrol and thymol can modulate oxidative stress parameters in *Candida albicans*, with a dose-dependent increase in SOD and CAT activity at all concentrations tested. However, GPX activity in cells treated with different concentrations of carvacrol and thymol initially increased, and was accompanied by a significant decrease in its activityat the highest concentrations of monoterpenes (Khan et al., 2015).Thymol also affects the oxidative defense system of *Ephestia kuehniella* moth larvae, increasing the activity of SOD, GST, CAT, and peroxidase (POX) (Shahriari et al., 2018). These studies attributed the increased activity of these enzymes to the induction of oxidative stress caused by these toxic compounds, mainly through the generation of $O_2^{\cdot -}$ and H_2O_2.

SOD, CAT, and GPX are among the main antioxidant enzymes that eliminate ROS in cells. Briefly, toxic superoxide radicals ($O_2^{\cdot -}$) are converted to hydrogen peroxide (H_2O_2) and oxygen (O_2) by SODs. Subsequently, CATs and GPXs are activated to catalyze H_2O_2 into H_2O

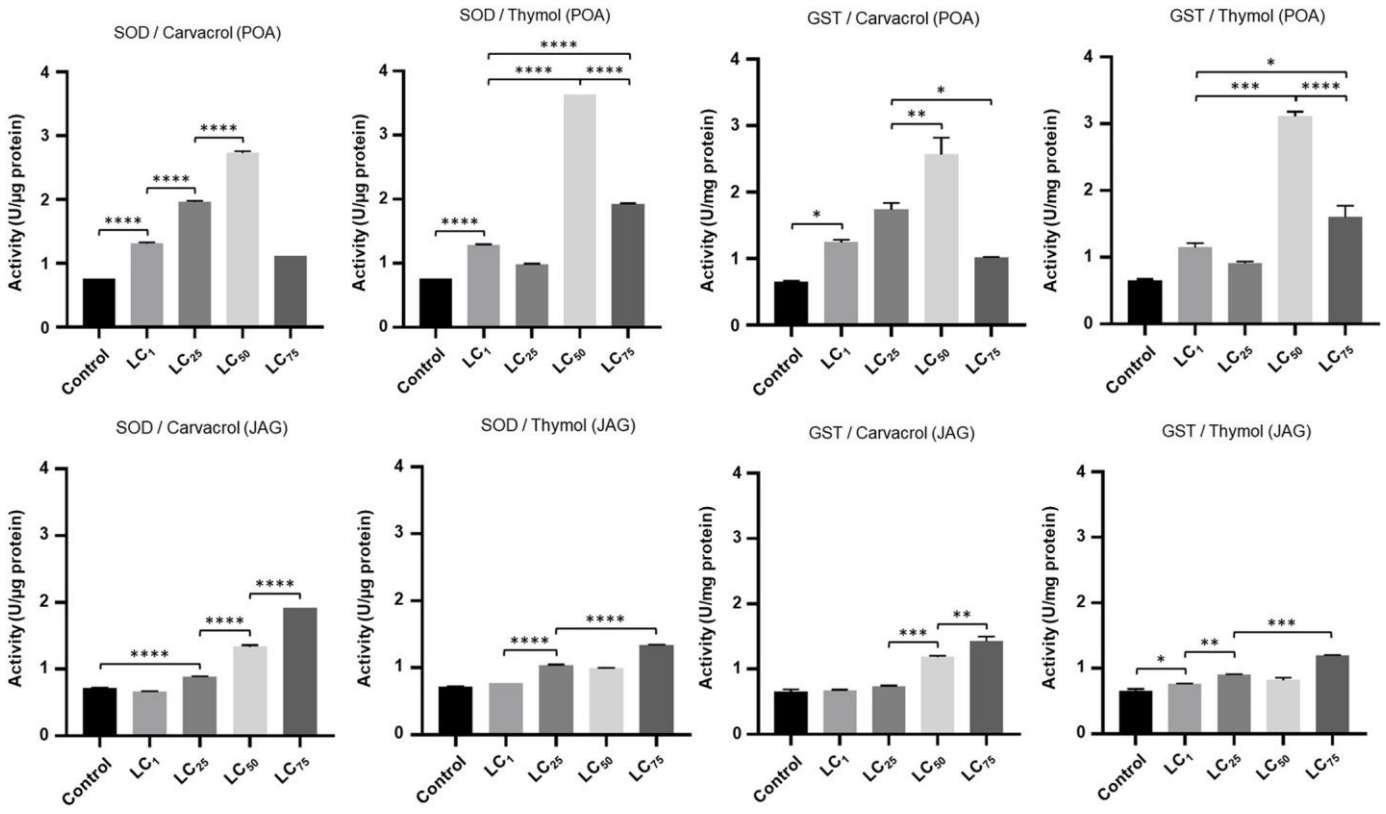

Fig. 2. Effect of different concentrations of carvacrol and thymol on the enzymatic activity of superoxide dismutase (SOD) and glutathione S-transferase (GST) in *Rhipicephalus microplus* larvae from Porto Alegre (POA, susceptible) and Jaguar (resistant) populations. Statistical differences were determined between the averages of the activity of each enzyme and marked with asterisks (*–$p<0.05$, **–$p<0.01$, ***–$p<0.001$, ****–$p<0.0001$).
LC_1, LC_{25}, LC_{50}, and LC_{75} indicate the lethal concentrations of monoterpenes for the Porto Alegre population (carvacrol–0.72, 1.2, 1.42, 1.66 mg mL^{-1}; thymol–0.25, 0.66, 0.9, 1.21 mg mL^{-1}) and Jaguar population (carvacrol–1.06, 1.56, 1.76, 1.99 mg mL^{-1}; thymol–0.51, 1.05, 1.32, 1.65 mg mL^{-1}).

and O_2 (Felton and Summers, 1995; Lyakhovich et al., 2006; Lushchak, 2014), and have been described as essential enzymes in tick metabolism, including reproduction and immune responses against infections (Citelliet al., 2007; Kumar et al., 2016; Kalil et al., 2017),

GSTs are a group of multifunctional enzymes involved in oxidative defense, whose activity is increased for the removal of hydroperoxides from damaged cells due to lipid peroxidation (LPO) (Enayati et al., 2005). GSTs also play a central role in the metabolism and detoxification of xenobiotics and other endogenous components (Wilce et al., 1994; Chelvanayagam et al., 2001), including tick resistance to acaricides (Li et al., 2004; Chevillon et al., 2007).

Similar to the results obtained in this study, a significant increase in the activity of GST was found in *Apis mellifera carnica* bees treated with sublethal concentrations (0.05, 0.5, and 1.0%) of carvacrol and thymol (Glavan et al., 2020). Elevated GST activity is indicative of intoxication (Berenbaum and Johnson, 2015), an effect that can be explained by oneof the mechanisms discussed above.

In studies performed with mice and rats fed thymol or carvacrol, GST activity was found to be elevated in liver cells (Samarghandian et al., 2017; Sasaki et al., 2005). GST activity was also found to be high in *Plutella xylostella* larvae (Kumrungsee et al., 2014) and *A. mellifera* bees (Loucif-Ayad et al., 2008), which were both treated with thymol.

GSTs play an important role in the detoxification of xenobiotics and physiological components in ticks (Huercha et al., 2020; Sabadin et al., 2021) and are directly involved in the detoxification of ticks (Hernandez et al., 2018). Therefore, GSTs are considered important markers of tick resistance, especially in *R. microplus* (Le Gall et al., 2018; Upadhayaet al., 2020; Fular et al., 2020, 2021).

A significant decrease in the activity of the four analyzed enzymes could be observed in the larvae of the Porto Alegre population following treatments with the LC_{75} of both monoterpenes compared to larvae treated with the LC_{50}. This decrease can be explained by the high concentration of exposure to the product, which enables the entry of a greater number of toxic substances into the tissues, damaging the cell structure and function by a rapid generation of ROS under stress, which would impede the expression of antioxidant enzymes (Calabrese, 2005). The same behavior was not observed in larvae of the Jaguar population, suggesting that this population may have been positively selected due to synthetic acaricide exposure and have a higher response of the antioxidant system to higher doses of carvacrol and thymol than larvae of the Porto Alegre population.

In a study carried out with *R. microplus*, the monoterpenes, camphor and eucalyptol, significantly and dose-dependently increased the GST activity in larvae and females at concentrations of 0.5, 1.0, and 2.0 mg mL^{-1}. However, at high concentrations (4.0, 8.0, and 16.0 mg mL^{-1}), GST activity was found to be significantly inhibited ($p<0.05$) (Yang et al., 2021). In *R. microplus* larvae, *Arisaema anurans* essential oil and its major constituents, asarone and cubenol, were found to increase GST activity at initial concentrations compared to the control larvae, followed by a marked decrease in enzyme activity as the concentrations increased (Jia et al., 2018). These results agree with the findings of the present study on the enzymatic activities in larvae of the Porto Alegre population, reinforcing the notion that higher concentrations of the compound can cause severe tissue damage in the tick, which inhibits the production of antioxidant and detoxifying enzymes (Calabrese, 2005).

Tick larvae from the Porto Alegre population appeared to have greater enzymatic activity in response to treatment with carvacrol and thymol than the Jaguar population. Although there are reports of metabolic resistance to synthetic ticks (Klafke et al., 2017; Le Gall et al., 2018), the difference in enzymatic activity between the two populations

in our study may not characterize these enzymes as markers of metabolic resistance, based on the conditions used in this study. The highest levelsof enzymatic activity in the Porto Alegre population might be related to the more intense damage suffered by the larvae of this population owing to the toxicity of monoterpenes, causing greater oxidative stress.

Furthermore, the lethal concentrations of carvacrol and thymol determined in this study were lower for the larvae of the Porto Alegre population, indicating that they are more susceptible to these monoterpenes than the larvae of the Jaguar population.

In conclusion, the findings of this study indicate that the monoterpenes, carvacrol and thymol, generated oxidative stress in *R. microplus* tick larvae from Jaguar and Porto Alegre populations, inducing an increase in the activity levels of CAT, SOD, GPX, and GST enzymes at different concentrations of the monoterpenes. These effects allowed us to understand the mechanisms involved in the toxicity of carvacrol and thymol in ticks. The increase in enzymatic activity might be an attempt to minimize the damage caused by monoterpenes, contributing to redox balance and detoxification. Overall, these enzymes could be targets for the development of new methods for tick control. To better understand the physiology of ticks exposed to carvacrol and thymol, further studies with other biochemical markers are needed to elucidate the mechanisms of action and reinforce the acaricidal potential of these monoterpenes.

CRediT authorship contribution statement

Caio P. Tavares: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Gabriela A. Sabadin:** Methodology, Investigation, Writing – review & editing. **Isabella C. Sousa:** Methodology, Investigation, Writing – review & editing. **Matheus N. Gomes:** Methodology, Investigation, Writing – review & editing. **Alexandra M.S. Soares:** Conceptualization, Methodology, Investigation, Formal analysis. **Caio M.O. Monteiro:** Formal analysis, Writing – review & editing. **Itabajara S Vaz:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – review & editing. **Livio M. Costa-Junior:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Visualization, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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References

Adamski, Z., 2007. Exposure to carbaryl leads to ultrastructural changes and alters activity of antioxidant enzymes in *Spodoptera exigua* (Lepidoptera: Noctuidae). Inverteb. Biol. 126, 191–201. https://doi.org/10.1111/j.1744-7410.2007.00089.x.

Adamski, Z., Ziemnicki, K., Fila, K., Žikič, R., Štajn, A., 2003. Effects of long-term exposure to fenitrothion on *Spodoptera exigua* and *Tenebrio molitor* larval development and antioxidant enzyme activity. Biol. Lett. 40, 43–52.

Aebi, H., 1984. Catalase in vitro, method. Enzymol. 105, 121–126.

Agus, H.H., Sengoz, C.O., Yilmaz, S., 2019. Oxidative stress-mediated apoptotic cell death induced by camphor in sod1-deficient *Schizosaccharomyces pombe*. Toxicol. Res. 8, 216–226. https://doi.org/10.1039/c8tx00279g.

Arafa, W.M., Aboelhadid, S.M., Moawad, A., Shokeir, K.M., Ahmed, O., 2020. Toxicity, repellency and anti-cholinesterase activities of thymol-eucalyptus combinations against phenotypically resistant *Rhipicephalus annulatus* ticks. Exp. Appl. Acarol. 81, 265–277. https://doi.org/10.1007/s10493-020-00506-1.

Arafa, W.M., Aboelhadid, S.M., Moawad, A., Shokeir, K.M., Ahmed, O., Pérez de León, A. A., 2021. Control of *Rhipicephalus annulatus* resistant to deltamethrin by spraying infested cattle with synergistic eucalyptus essential oil-thymol-deltamethrin combination. Vet. Parasitol. 290, 109346 https://doi.org/10.1016/j.vetpar.2021.109346.

Banerjee, B.D., Seth, V., Bhattacharya, A., Pasha, S.T., Chakraborty, A.K., 1999. Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. Toxicol. Lett. 107, 33–47. https://doi.org/10.1016/s0378-4274(99)00029-6.

Berenbaum, M.R., Johnson, R.M., 2015. Xenobiotic detoxification pathways in honey bees. Curr. Opin. Insect Sci. 10, 51–58. https://doi.org/10.1016/j.cois.2015.03.005.

Bloomquist, J.R., 2003. Chloride channels as tools for developing selective insecticides. Arch. Insect Biochem. Physiol. 54, 145–156. https://doi.org/10.1002/arch.10112.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1006/abio.1976.9999.

Büyükgüzel, E., 2009. Evidence of oxidative and antioxidative responses by *Galleria mellonella* larvae to malathion. J. Econ. Entomol. 102, 152–159. https://doi.org/10.1603/029.102.0122.

Büyükgüzel, E., Büyükgüzel, K., Snela, M., Erdem, M., Radtke, K., Ziemnicki, K., Adamski, Z., 2013. Effect of boric acid on antioxidant enzyme activity, lipid peroxidation, and ultrastructure of midgut and fat body of *Galleria mellonella*. Cell Biol. Toxicol. 29, 117–129. https://doi.org/10.1007/s10565-013-9240-7.

Calabrese, E.J., 2005. Paradigm lost, paradigm found–The reemergence of hormesis as a fundamental dose response model in the toxicological sciences. Environ. Pollut. 138, 379–411. https://doi.org/10.1016/j.envpol.2004.10.001.

Cardoso, A.D.S., Santos, E.G.G., Lima, A.D.S., Temeyer, K.B., Pérez de León, A.A., Costa-Junior, L.M., Soares, A.M.D.S., 2020. Terpenes on *Rhipicephalus (Boophilus) microplus*–Acaricidal activity and acetylcholinesterase inhibition. Vet. Parasitol. 280, 109090 https://doi.org/10.1016/j.vetpar.2020.109090.

Chelvanayagam, G., Parker, M.W., Board, P.G., 2001. Fly fishing for GSTs–A unified nomenclature for mammalian and insect glutathione transferases. Chem. Biol. Interact. 133, 256–260.

Chevillon, C., Ducornez, S., de Meeûs, T., Koffi, B.B., Gaïa, H., Delathière, J.M., Barré, N., 2007. Accumulation of acaricide resistance mechanisms in *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) populations from New Caledonia Island. Vet. Parasitol. 47, 276–288. https://doi.org/10.1016/j.vetpar.2007.05.003.

Citelli, M., Lara, F.A., da Silva Vaz Jr., I, Oliveira, P.L., 2007. Oxidative stress impairs heme detoxification in the midgut of the cattle tick, *Rhipicephalus (Boophilus) microplus*. Mol. Biochem. Parasitol. 151, 81–88. https://doi.org/10.1016/j.molbiopara.2006.10.008.

Coelho, L., de Paula, L.G.F., Alves, S.D.G.A., Sampaio, A.L.N., Bezerra, G.P., Vilela, F.M. P., Matos, R.D.S., Zeringóta, V., Borges, L.M.F., Monteiro, C., 2020. Combination of thymol and eugenol for the control of *Rhipicephalus sanguineus* sensu lato–Evaluation of synergism on immature stages and formulation development. Vet. Parasitol. 277, 108989 https://doi.org/10.1016/j.vetpar.2019.108989.

Cuéllar-Cruz, M., Castano, I., Arroyo-Helguera, O., De Las Penas, A., 2009. Oxidative stress response to menadione and cumene hydroperoxide in the opportunistic fungal pathogen *Candida glabrata*. Mem. Inst. Oswaldo Cruz. 104, 649–654. https://doi.org/10.1590/s0074-02762009000400020.

Da Silva Vaz Jr., I., Torino Lermen, T., Michelon, A., Ferreira, C.A.S., Freitas, D.R.J., Termignoni, C., Masuda, A., 2004. Effect of acaricides on the activity of a *Boophilus microplus* glutathione S-transferase. Vet. Parasitol. 119, 237–245. https://doi.org/10.1016/j.vetpar.2003.11.004.

De Vincenzi, M., Stammati, A., De Vincenzi, A., Silano, M., 2004. Constituents of aromatic plants–Carvacrol. Fitoterapia 75, 801–804. https://doi.org/10.1016/j.fitote.2004.05.002.

Enayati, A.A., Ranson, H., Hemingway, J., 2005. Insect glutathione transferases and insecticide resistance. Insect Mol. Biol. 14, 3–8. https://doi.org/10.1111/j.1365-2583.2004.00529.x.

Estrada-Peña, A., Bouattour, A., Camicas, J.L., Guglielmone, A., Horak, I., Jongejan, F., Latif, A., Pegram, R., Walker, A.R., 2006. The known distribution and ecological preferences of the tick subgenus *Boophilus* (Acari: Ixodidae) in Africa and Latin America. Exp. Appl. Acarol. 38, 219–235. https://doi.org/10.1007/s10493-006-0003-5.

Felton, G.W., Summers, C.B., 1995. Antioxidant systems in insects. Arch. Insect Biochem. Physiol. 29, 187–197. https://doi.org/10.1002/arch.940290208.

Freitas, D.R., Rosa, R.M., Moraes, J., Campos, E., Logullo, C., Da Silva Vaz Jr., I, Masuda, A., 2007. Relationship between glutathione S-transferase, catalase, oxygen consumption, lipid peroxidation and oxidative stress in eggs and larvae of *Boophilus microplus* (Acarina: Ixodidae). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 146, 688–694. https://doi.org/10.1016/j.cbpa.2006.04.032.

Fular, A., Gupta, S., Sharma, A.K., Kumar, S., Upadhaya, D., Shakya, M., Nagar, G., Ghosh, S., 2020. Standardization of tick specific biochemical tools for estimation of esterases, monooxygenases and glutathione S-transferase for characterization of acaricide resistance. Pestic. Biochem. Physiol. 164, 130–139. https://doi.org/10.1016/j.pestbp.2020.01.008.

Fular, A., Sharma, A.K., Upadhaya, D., Nandi, A., Nagar, G., Bisht, N., Shakya, M., Kumar, S., Kumar, S., Kumar, R., Ghosh, S., 2021. Evaluation of acaricidal resistance status of *Rhipicephalus microplus* ticks from the hilly state (Uttarakhand) of India and evaluation of efficacy of a natural formulation for the management of resistant ticks. Exp. Appl. Acarol. 85, 355–377. https://doi.org/10.1007/s10493-021-00677-5.

Glavan, G., Novak, S., Božič, J., Jemec Kokalj, A., 2020. Comparison of sublethal effects of natural acaricides carvacrol and thymol on honeybees. Pestic. Biochem. Physiol. 166, 104567 https://doi.org/10.1016/j.pestbp.2020.104567.

Grisi, L., Leite, J.R., Martins, S., Barros, A.T.M., Andreotti, R., Cançado, P.H.D., Léon, A. A.P., Pereira, J.B., Villela, H.S., 2014. Reassessment of the potential economic impact of cattle parasites in Brazil. Rev. Bras. Parasitol. 23, 150–156. https://doi.org/10.1590/s1984-29612014042.

Gross, A.D., Temeyer, K.B., Day, T.A., Pérez de León, A.A., Kimber, M.J., Coats, J.R., 2017. Interaction of plant essential oil terpenoids with the southern cattle tick tyramine receptor–A potential biopesticide target. Chem. Biol. Interact. 263, 1–6. https://doi.org/10.1016/j.cbi.2016.12.009.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139.

Hemingway, J., Hawkes, N.J., McCarroll, L., Ranson, H., 2004. The molecular basis of insecticide resistance in mosquitoes. Insect. Biochem. Mol. Biol. 34, 653–665. https://doi.org/10.1016/j.ibmb.2004.03.018.

Hernandez, E.P., Kusakisako, K., Talactac, M.R., Galay, R.L., Hatta, T., Matsuo, T., Fujisaki, K., Tsuji, N., Tanaka, T., 2018. Characterization and expression analysis of a newly identified glutathione Transferase of the hard tick *Haemaphysalis longicornis* during blood-feeding. Parasit. Vectors 11, 91. https://doi.org/10.1186/s13071-018-2667-1.

Hou, H., Zhang, X., Zhao, T., Zhou, L., 2020. Effects of *Origanum vulgare* essential oil and its two main components, carvacrol and thymol, on the plant pathogen *Botrytis cinerea*. PeerJ 8, 9626. https://doi.org/10.7717/peerj.9626.

Huercha, M.Y., Hao, Y., Li, M., Hu, Z., Song, R., Wei, L., Fan, S., Chen, S., Fan, X., Zhai, X., Guo, Q., Bayin, C., 2020. Sequence identification and expression profile of seven *Dermacentor marginatus* glutathione S-transferase genes. Exp. Appl. Acarol. 82, 295–308. https://doi.org/10.1007/s10493-020-00546-7.

Hyršl, P., Büyükgüzel, E., Büyükgüzel, K., 2007. The effects of boric acid-induced oXidative stress on antioXidant enzymes and survivorship in *Galleria mellonella*. Arch. Insect Biochem. Physiol. 66, 23–31. https://doi.org/10.1002/arch.20194.

Jia, M., He, Q., Wang, W., Dai, J., Zhu, L., 2018. Chemical composition and acaricidal activity of *Arisaema anurans* essential oil and its major constituents against *Rhipicephalus microplus* (Acari: Ixodidae). Vet. Parasitol. 261, 59–66. https://doi.org/10.1016/j.vetpar.2018.08.006.

Kalil, S.P., Rosa, R.D.D., Capelli-PeiXoto, J., Pohl, P.C., Oliveira, P.L., Fogaça, A.C., Daffre, S., 2017. Immune-related redoX metabolism of embryonic cells of the tick *Rhipicephalus microplus* (BME26) in response to infection with *Anaplasma marginale*. Parasit. Vectors 10, 613. https://doi.org/10.1186/s13071-017-2575-9.

Khan, A., Ahmad, A., Ahmad Khan, L., Padoa, C.J., van Vuuren, S., Manzoor, N., 2015. Effect of two monoterpene phenols on antioXidant defense system in *Candida albicans*. Microb. Pathog. 80, 50–56. https://doi.org/10.1016/j.micpath.2015.02.004.

Klafke, G.M., Sabatini, G.A., de Albuquerque, T.A., Martins, J.R., Kemp, D.H., Miller, R. J., Schumaker, T.T.S., 2006. Larval immersion tests with ivermectin in populations of the cattle tick *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) from State of Sao Paulo, Brazil. Vet. Parasitol. 142, 386–390. https://doi.org/10.1016/j.vetpar.2006.07.001.

Klafke, G.M., Webster, A., Dall Agnol, B., Pradel, E., Silva, J., de La Canal, L.H., Becker, M., Osório, M.F., Mansson, M., Barreto, R., Scheffer, R., Souza, U.A., Corassini, V.B., dos Santos, J., Reck, J., Martins, J.R., 2017. Multiple resistance to acaricides in field populations of *Rhipicephalus microplus* from Rio Grande do Sul state, Southern Brazil. Ticks Tick. Borne. Dis. 8, 73–80. https://doi.org/10.1016/j.ttbdis.2016.09.019.

Krishnan, N., Sehnal, F., 2006. Compartmentalization of oXidative stress and antioXidant defense in the larval gut of *Spodoptera littoralis*. Arch. Insect Biochem. Physiol. 63, 1–10. https://doi.org/10.1002/arch.20135.

Kumar, D., Budachetri, K., Meyers, V.C., Karim, S., 2016. Assessment of tick antioXidant responses to exogenous oXidative stressors and insight into the role of catalase in the reproductive fitness of the Gulf Coast tick, *Amblyomma maculatum*. Insect Mol. Biol. 25, 283–294. https://doi.org/10.1111/imb.12218.

Kumar, S., Sharma, A.K., Kumar, B., Shakya, M., Patel, J.A., Kumar, B., Bisht, N., Chigure, G.M., Singh, K., Kumar, R., Kumar, S., Srivastava, S., Rawat, P., Ghosh, S., 2021. Characterization of deltamethrin, cypermethrin, coumaphos and ivermectin resistance in populations of *Rhipicephalus microplus* in India and efficacy of an antitick natural formulation prepared from *Ageratum conyzoides*. Ticks Tick Borne Dis. 12, 101818 https://doi.org/10.1016/j.ttbdis.2021.101818.

Kumrungsee, N., Pluempanupat, W., Koul, O., Bullangpoti, V., 2014. ToXicity of essential oil compounds against diamondback moth, *Plutella xylostella*, and their impact on detoXification enzyme activities. J. Pest. Sci. 87, 721–729. https://doi.org/10.1007/s10340-014-0602-6.

Le Gall, V.L., Klafke, G.M., Torres, T.T., 2018. DetoXification mechanisms involved in ivermectin resistance in the cattle tick, *Rhipicephalus (Boophilus) microplus*. Sci. Rep. 8, 12401. https://doi.org/10.1038/s41598-018-30907-7.

Li, A.Y., Davey, R.B., Miller, R.J., George, J.E., 2004. Detection and characterization of amitraz resistance in the southern cattle tick, *Boophilus microplus* (Acari: Ixodidae). J. Med. Entomol. 41, 193–200. https://doi.org/10.1603/0022-2585-41.2.193.

Lim, W., Ham, J., Bazer, F.W., Song, G., 2019. Carvacrol induces mitochondria-mediated apoptosis via disruption of calcium homeostasis in human choriocarcinoma cells. J. Cell. Physiol. 234, 1803–1815. https://doi.org/10.1002/jcp.27054.

Loucif-Ayad, W., Aribi, N., Soltani, N., 2008. Evaluation of secondary effects of some acaricides on Apis mellifera intermissa (Hymenoptera, Apidae)–Acetylcholinesterase and glutathion S-transferase activities. Eur. J. Sci. Res. 21, 642–649.

Lushchak, V.I., 2014. Free radicals, reactive oXygen species, oXidative stress and its classification. Chem. Biol. Interact. 224, 164–175. https://doi.org/10.1016/j.cbi.2014.10.016.

Lyakhovich, V.V., Vavilin, V.A., Zenkov, N.K., Menshchikova, E.B., 2006. Active defense under oXidative stress. The antioXidant responsive element. Biochem. Mosc. 71, 962–974. https://doi.org/10.1134/s0006297906090033.

Marques, R., Krüger, R.F., Peterson, A.T., de Melo, L.F., Vicenzi, N., Jiménez-García, D., 2020. Climate change implications for the distribution of the babesiosis and anaplasmosis tick vector, *Rhipicephalus (Boophilus) microplus*. Vet. Res. 51 (81), 216–224. https://doi.org/10.1186/s13567-020-00802-z.

Matos, R.S., Daemon, E., de Oliveira Monteiro, C.M., Sampieri, B.R., Marchesini, P.B.C., Delmonte, C., Camargo-Mathias, M.I., 2019. Thymol action on cells and tissues of the synganglia and salivary glands of *Rhipicephalus sanguineus* sensu lato females (Acari: Ixodidae). Ticks Tick Borne Dis. 10, 314–320. https://doi.org/10.1016/j.ttbdis.2018.11.003.

Matos, R.S., Oliveira, P.R., Coelho, L., de Paula, L.G.F., Zeringota, V., Carvalho Silva, B., Monteiro, C., Daemon, E., Camargo-Mathias, M.I., 2020. Thymol–Effects on reproductive biology and Gene' s organ morphology in *Rhipicephalus sanguineus* sensu lato engorged females (Acari: Ixodidae). Ticks Tick Borne Dis. 11, 101308 https://doi.org/10.1016/j.ttbdis.2019.101308.

Mccord, J.M., I. Fridovich, I., 1969. The utility of superoXide dismutase in studying free radical reactions. I. Radicals generated by the interaction of sulfite, dimethyl sulfoXide, and oXygen. J. Biol. Chem. 244, 6056–6063.

Moldão-Martins, M., Palavra, A., Beirão da Costa, M., Bernardo-Gil, M., 2000. Supercritical CO_2 extraction of *Thymus zygis* L. subsp. *sylvestris* aroma. J. Supercrit. Fluids 18 (1), 25–34. https://doi.org/10.1016/S0896-8446(00)00047-4.

Montagu, A., Joly-Guillou, M.L., Rossines, E., Cayon, J., Kempf, M., Saulnier, P., 2016. Stress conditions induced by Carvacrol and Cinnamaldehyde on *Acinetobacter baumannii*. Front. Microbiol. 7, 1133. https://doi.org/10.3389/fmicb.2016.01133.

Monteiro, C., Ferreira, L.L., de Paula, L.G.F., de Oliveira Filho, J.G., de Oliveira Silva, F., Muniz, E.R., Menezes, K.M.F., de Camargo, F.R., de Oliveira Nonato, R., Martins, D. B., Marreto, R.N., Borges, L.M.F., 2021. Thymol and eugenol microemulsion for *Rhiphicephalus sanguineus* sensu lato control–Formulation development, field efficacy, and safety on dogs. Vet. Parasitol. 296, 109501 https://doi.org/10.1016/j.vetpar.2021.109501.

Monzote, L., Geroldinger, G., Tonner, M., Scull, R., De Sarkar, S., Bergmann, S., Bacher, M., Staniek, K., Chatterjee, M., Rosenau, T., Gille, L., 2018. Interaction of ascaridole, carvacrol, and caryophyllene oXide from essential oil of *Chenopodium ambrosioides* L. with mitochondria in *Leishmania* and other eukaryotes. Phytother. Res. 32, 1729–1740. https://doi.org/10.1002/ptr.6097.

Paglia, D.E., Valentine, W.N., 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutation peroXide. J. Lab. Clin. Med. 70, 158–169.

Penha, T., Costa, A.C.C., Lima, A.D.S., Camargo-Mathias, M.I., Blank, A.F., Abreu-Silva, A.L., Costa-Júnior, L.M., 2021. Effects of acaricidal essential oils from *Lippia sidoides* and *Lippia gracilis* and their main components on vitellogenesis in *Rhipicephalus microplus* (Canestrini, 1888) (Acari: Ixodidae). Vet. Parasitol. 299, 109584 https://doi.org/10.1016/j.vetpar.2021.109584.

Reck, J., Berger, M., Terra, R.M.S., Marks, F.S., da Silva Vaz, I., Guimarães, J.A., Termignoni, C., 2009. Systemic alterations of bovine hemostasis due to Rhipicephalus (Boophilus) microplus infestation. Res. Vet. Sci. 86, 56–62. https://doi.org/10.1016/j.rvsc.2008.05.007.

Reck, J., Klafke, G.M., Webster, A., Dall' Agnol, B., Scheffer, R., Souza, U.A., Corassini, V. B., Vargas, R., dos Santos, J.S., Martins, J.R., 2014. First report of fluazuron resistance in *Rhipicephalus microplus*–A field tick population resistant to siX classes of acaricides. Vet. Parasitol. 201, 128–136. https://doi.org/10.1016/j.vetpar.2014.01.012.

Rosario-Cruz, R., Almazan, C., Miller, R.J., Dominguez-Garcia, D.I., Hernandez-Ortiz, R., de la Fuente, J., 2009. Genetic basis and impact of tick acaricide resistance. Front. Biosci. 14, 2657–2665. https://doi.org/10.2741/3403.

Sabadin, G.A., Salomon, T.B., Leite, M.S., Benfato, M.S., Oliveira, P.L., da Silva Vaz Jr., I., 2021. An insight into the functional role of antioXidant and detoXification enzymes in adult *Rhipicephalus microplus* female ticks. Parasitol. Int. 81, 102274 https://doi.org/10.1016/j.parint.2020.102274.

Sagar, S.V., Saini, K., Sharma, A.K., Kumar, Sachin, Kumar, R., Fular, A., Shakya, M., Upadhaya, D., Nagar, G., Shanmuganath, C., Samanta, S., Kumar, Suman, Ghosh, S., 2020. Acaricide resistance in *Rhipicephalus microplus* collected from selected districts of Madhya Pradesh, Uttar Pradesh and Punjab states of India. Trop. Anim. Health Prod. 52, 611–618. https://doi.org/10.1007/s11250-019-02048-0.

Samarghandian, S., Nezhad, M.A., Farkhondeh, T., 2017. Preventive effect of carvacrol against oXidative damage in aged rat liver. Int. J. Vitam. Nutr. Res. 87, 59–65. https://doi.org/10.1024/0300-9831/a000393.

Sasaki, K., Wada, K., Tanaka, Y., Yoshimura, T., Matuoka, K., Anno, T., 2005. Thyme (Thymus vulgaris L.) leaves and its constituents increase the activities of Xenobioticmetabolizing enzymes in mouse liver. J. Med. Food 8, 184–189. https://doi.org/10.1089/jmf.2005.8.184.

Scariot, F.J., Foresti, L., Delamare, A.P.L., Echeverrigaray, A.P.L.S., 2020. Activity of monoterpenoids on the in vitro growth of two *Colletotrichum* species and the mode of action on *C. acutatum*. Pestic. Biochem. Physiol. 170, 104698 https://doi.org/10.1016/j.pestbp.2020.104698.

Shahriari, M., Zibaee, A., Sahebzadeh, N., Shamakhi, L., 2018. Effects of α-pinene, trans-anethole, and thymol as the essential oil constituents on antioXidant system and acetylcholine esterase of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Pestic. Biochem. Physiol. 150, 40–47. https://doi.org/10.1016/j.pestbp.2018.06.015.

Silva Lima, A., Milhomem, M.N., Santos Monteiro, O., Arruda, A.C.P., de Castro, J.A.M., Fernandes, Y.M.L., Maia, J.G.S., Costa-Junior, L.M., 2018. Seasonal analysis and acaricidal activity of the thymol-type essential oil of *Ocimum gratissimum* and its major constituents against *Rhipicephalus microplus* (Acari: Ixodidae). Parasitol. Res. 117, 59–65. https://doi.org/10.1007/s00436-017-5662-0.

Skulachev, V.P., 2012. Mitochondria-targeted antioXidants as promising drugs for treatment of age-related brain diseases. J. Alzheimers Dis. 28, 283–289. https://doi.org/10.3233/JAD-2011-111391.

Soderlund, D.M., 2012. Molecular mechanisms of pyrethroid insecticide neurotoxicity–Recent advances. Arch. Toxicol. 86, 165–181. https://doi.org/10.1007/s00204-011-0726-x.

Souza, J.R.L., Oliveira, P.R., Anholeto, L.A., Arnosti, A., Daemon, E., Remedio, R.N., Camargo-Mathias, M.I., 2019. Effects of carvacrol on oocyte development in semi-engorged *Rhipicephalus sanguineus* sensu lato females ticks (Acari: Ixodidae). Micron 116, 66–72. https://doi.org/10.1016/j.micron.2018.09.015.

Souza, J.R.L., Oliveira, P.R., Anholeto, L.A., Sodelli, L.F., Ferreira, A.R.F., Remedio, R.N., Camargo-Mathias, M.I., 2021. The bioactive compound carvacrol as a potential acaricide–An assessment of its effects on the integument of female *Rhipicephalus sanguineus* sensu lato ticks. Microsc. Res. Tech. https://doi.org/10.1002/jemt.240386.

Tavares, C.P., Sousa, I.C., Gomes, M.N., Miró, V., Virkel, G., Lifschitz, A., Costa-Junior, L. M., 2022. Combination of cypermethrin and thymol for control of *Rhipicephalus microplus*–Efficacy evaluation and description of an action mechanism. Ticks Tick Borne Dis. 13, 101874 https://doi.org/10.1016/j.ttbdis.2021.101874.

Tong, F., Coats, J.R., 2010. Effects of monoterpenoid insecticides on [3H]-TBOB binding in house fly GABA receptor and 36Cl⎯ uptake in *American cockroach* ventral nerve cord. Pestic. Biochem. Phys. 98 (3), 317–324. https://doi.org/10.1016/j.pestbp.2010.07.003.

Upadhaya, D., Kumar, B., Kumar, S., Sharma, A.K., Fular, A., Bisht, N., Srivastava, S., Boruah, R.R., Nagar, G., Shakya, M., Nath, T., Nandi, S.P., Karivaradharaja, S., Ghosh, S., 2020. Characterization of acaricide resistance in *Rhipicephalus microplus* populations infesting cattle in northeastern India and assessment of local plant extracts for tick management. Vet. Parasitol. 277, 109011 https://doi.org/10.1016/j.vetpar.2019.109011.

Valsoni, L.M., Freitas, M.G.de, Echeverria, J.T., Borges, D.G.L., Tutija, J., Borges, F.de A., 2020. Resistance to all chemical groups of acaricides in a single isolate of Rhipicephalus microplus in Mato Grosso do Sul, Brazil. Int. J. Acarol. 46, 276–280. https://doi.org/10.1080/01647954.2020.1765867.

Vilela, V.L.R., Feitosa, T.F., Bezerra, R.A., Klafke, G.M., Riet-Correa, F., 2020. Multiple acaricide-resistant Rhipicephalus microplus in the semi-arid region of Paraíba State, Brazil. Ticks Tick. Borne. Dis. 11, 101413 https://doi.org/10.1016/j.ttbdis.2020.101413.

Widersten, M., Mannervik, B., 1995. Glutathione transferases with novel active sites isolated by phage display from a library of random mutants. J. Mol. Biol. 250, 115–122. https://doi.org/10.1006/jmbi.1995.0362.

Wilce, M.C.J., Parker, M.W., 1994. Structure and function of glutathione S-transferases. Biochim. Biophys. Acta 1205, 1–18. https://doi.org/10.1016/0167-4838(94)90086-8.

Yang, P., Jia, M., Zhu, L., 2021. Acaricidal activity of the essential oil from *Senecio cannabifolius* and its constituents eucalyptol and camphor on engorged females and larvae of *Rhipicephalus microplus* (Acari: Ixodidae). Exp. Appl. Acarol. 83, 411–426. https://doi.org/10.1007/s10493-021-00590-x.

5. CONCLUSÕES

Neste estudo, foi demonstrado a eficácia carrapaticida da combinação de timol + cipermetrina em *R. microplus*, e que a presença de timol aumenta a concentração de cipermetrina nos tecidos internos de fêmeas ingurgitadas através de um possível mecanismo para aumentar a penetração de cipermetrina ao nível cuticular. Da mesma forma, demonstramos pela o efeito dos monoterpenos carvacrol e timol em importantes enzimas antioxidantes e detoxificantes das larvas de *R. microplus*, que constituem fênomenos importantes que podem explicar danos bioquímicos e fisiológicos proporcionados pela toxicidade dessas duas moléculas, contribuindo na elucidação de possíveis mecanismos de ação sobre o carrapato.

6. REFERÊNCIAS BIBLIOGRÁFICAS

ABBAS, R. Z. *et al*. Acaricide resistance in cattle ticks and approaches to its management: The state of play. Veterinary Parasitology, v. 203, n. 1–2, p. 6–20, 2014.

ABOELHADID SM, ARAFA WM, ABDEL-BAKI AS, SOKMEN A, AL-QURAISHY S, HASSAN AO, KAMEL AA. Acaricidal activity of Foeniculum vulgare against Rhipicephalus annulatus is mainly dependent on its constituent from trans-anethone. PLoS One. 2021 Dec 2;16(12):e0260172.

ARAFA WM, ABOELHADID SM, MOAWAD A, SHOKEIR KM, AHMED O. Toxicity, repellency and anti-cholinesterase activities of thymol-eucalyptus combinations against phenotypically resistant Rhipicephalus annulatus ticks. Exp Appl Acarol. 2020 Jun;81(2):265-277.

ARAFA, W.M., ABOELHADID, S.M., MOAWAD, A., SHOKEIR, K.M., AHMED, O., 2020. Toxicity, repellency and anti-cholinesterase activities of thymol-eucalyptus combinations against phenotypically resistant *Rhipicephalus annulatus* ticks. Exp. Appl. Acarol., 81, 265-277.

ARAÚJO, L. X. *et al*. Synergism of thymol, carvacrol and eugenol in larvae of the cattle tick, *Rhipicephalus microplus*, and brown dog tick, *Rhipicephalus sanguineus*. Medical and Veterinary Entomology, v. 30, n. 4, p. 377–382, 2016.

ARAÚJO, L. X., NOVATO, T. P. L., ZERINGOTA, V., MATOS, R. S., SENRA, T. O. S., MATURANO, R., MONTEIRO, C. M. O., 2015. Acaricidal activity of thymol against larvae of *Rhipicephalus microplus* (Acari: Ixodidae) under semi-natural conditions. Parasitology Research, 114(9), 3271–3276.

ARAÚJO, L. X., NOVATO, T. P. L., ZERINGOTA, V., MATURANO, R., MELO, D., DA SILVA, B. C., DAEMON, E., DE CARVALHO M. G., MONTEIRO, C. M. O., 2016. Synergism of thymol, carvacrol and eugenol in larvae of the cattle tick, *Rhipicephalus microplus*, and brown dog tick *Rhipicephalus sanguineus*. Medical and Veterinary Entomology, 30(4), 377–382.

BAFFI, M. A. *et al*. Esterase enzymes involved in pyrethroid and organophosphate resistance in a Brazilian population of *Riphicephallus (Boophilus) microplus* (Acari, Ixodidae). Molecular and biochemical parasitology, v. 160, n. 1, p. 70-73, 2008.

BROWN, H. A., MINOTT, D. A., INGRAM, C. W., & WILLIAMS, L. A. D., 1998. Biological activities of the extracts and constituents of pimento, *Pimenta dioica* L. against the southern cattle tick, *Boophilus microplus*. International Journal of Tropical Insect Science, 18(01), 9–16.

BURGER, T. D. *et al*. Phylogenetic analysis of mitochondrial genome sequences indicates that the cattle tick, *Rhipicephalus (Boophilus) microplus*, contains a cryptic species. Molecular Phylogenetics and Evolution, v. 76, p. 241-253, 2014.

CARDOSO, A.D.S., SANTOS, E.G.G., LIMA, A.D.S., TEMEYER, K.B., PÉREZ DE LEÓN, A.A., COSTA-JUNIOR, L.M., SOARES, A.M.D.S., 2020. Terpenes on *Rhipicephalus (Boophilus) microplus*: Acaricidal activity and acetylcholinesterase inhibition. Vet. Parasitol. 280, 109090.

CASTRO-JANER, E., MARTINS, J.R., MENDES, M.C., NAMINDOME, A., KLAFKE, G.M., SCHUMAKER, T.T.S., 2010. Diagnoses of fipronil resistance in Brazilian cattle ticks (*Rhipicephalus (Boophilus) microplus*) using in vitro larval bioassays. Vet. Parasitol. 173, 300–306.

CETIN, H., CILEK, J. E., OZ, E., AYDIN, L., DEVECI, O., & YANIKOGLU, A., 2010. Acaricidal activity of *Satureja thymbra* L. essential oil and its major components, carvacrol and γ-terpinene against adult *Hyalomma marginatum* (Acari: Ixodidae). Veterinary Parasitology, 170(3-4), 287–290.

COELHO L, DE PAULA LGF, ALVES SDGA, SAMPAIO ALN, BEZERRA GP, VILELA FMP, MATOS RDS, ZERINGÓTA V, BORGES LMF, MONTEIRO C. Combination of thymol and eugenol for the control of Rhipicephalus sanguineus sensu lato: Evaluation of synergism on immature stages and formulation development. Vet Parasitol. 2020 Jan;277:108989.

CONCEPCIÓN, R. L., FROYLÁN, I. V., HERMINIA I, P. M., NORBERTO, M. A., HÉCTOR J, S. Z., YENIEL, G. C., 2013. In vitro assessment of the acaricidal activity of computer-selected analogues of carvacrol and salicylic acid on *Rhipicephalus* (Boophilus) *microplus*. Experimental and Applied Acarology, 61(2), 251–257.

COSTA-JÚNIOR, L. M. *et al*. Acaricidal efficacies of *Lippia gracilis* essential oil and its phytochemicals against organophosphate-resistant and susceptible strains of *Rhipicephalus (Boophilus) microplus*. Veterinary Parasitology, v. 228, p. 60–64, 2016.

CRUZ, E.M.O., COSTA-JUNIOR, L.M., PINTO, J.A.O. ET AL., 2013. Acaricidal activity of *Lippia gracilis* essential oil and its major constituents on the tick *Rhipicephalus* (Boophilus) *microplus*. Veterinary Parasitology, 195, 198–202.

D'ANTUONO, L., 2000. Variability of Essential Oil Content and Composition of *Origanum vulgare* L. Populations from a North Mediterranean Area (Liguria Region, Northern Italy). Annals of Botany, 86(3), 471–478.

DAEMON, E., DE OLIVEIRA MONTEIRO, C. M., DOS SANTOS ROSA, L., APARECIDO CLEMENTE, M., ARCOVERDE, A., 2009. Evaluation of the acaricide activity of thymol on engorged and unengorged larvae of *Rhipicephalus sanguineus* (Latreille, 1808) (Acari: Ixodidae). Parasitology Research, 105(2), 495–497.

DAEMON, E., MATURANO, R., MONTEIRO, C. M. DE O., GOLDNER, M. S., MASSONI, T., 2012a. Acaricidal activity of hydroethanolic formulations of thymol against *Rhipicephalus sanguineus* (Acari: Ixodidae) and *Dermacentor nitens* (Acari: Ixodidae) larvae. Veterinary Parasitology, 186(3-4), 542–545.

DAEMON, E., MONTEIRO, C. M. O., MATURANO, R., SENRA, T. O. S., CALMON, F., FAZA, A., PRATA, M. C. A., GEORGOPOULOS, S. L., DE OLIVEIRA, L. F. C., 2012b. Spectroscopic evaluation of thymol dissolved by different methods and influence on acaricidal activity against larvae of *Rhipicephalus microplus* (Acari: Ixodidae). Parasitology Research, 111(5), 1901–1906

DANTAS, A. C. DOS S., ARAUJO, A. DE C., PACHECO, A. G. M., BRANCO, A., SANGIONI, L. A., ALMEIDA, J. R. G. DA S., & HORTA, M. C., 2016. Acaricidal activity of *Amburana cearensis* on the cattle tick *Rhipicephalus* (Boophilus) *microplus*. Ciência Rural, 46(3), 536–541.

DANTAS-TORRES, F. *et al*. Ticks (Ixodida: Argasidae, Ixodidae) of Brazil: updated species checklist and taxonomic keys. Ticks and tick-borne diseases, v. 10, n. 6, p. 101252, 2019.

DE OLIVEIRA-CRUZ, E. M., COSTA-JUNIOR, L. M., PINTO, J. A. O., SANTOS, D. DE A., ARAUJO, S. A. DE, ARRIGONI-BLANK, M. DE F., BACCI, L., BARRETO-ALVES, P., CAVALCANTI, S.C.H., BLANK, A. F., 2013. Acaricidal activity of *Lippia gracilis* essential oil and its major constituents on the tick *Rhipicephalus* (Boophilus) *microplus*. Veterinary Parasitology, 195(1-2), 198–202.

DE SOUZA CHAGAS, A. C. *et al*. Efficacy of 11 Brazilian essential oils on lethality of the cattle tick *Rhipicephalus (Boophilus) microplus*. Ticks and Tick-borne Diseases, v. 7, n. 3, p. 427-432, 2016.

DE SOUZA, J. R. L., OLIVEIRA, P. R. DE, ANHOLETO, L. A., ARNOSTI, A., DAEMON, E., REMEDIO, R. N., CAMARGO-MATHIAS, M. I., 2019. Effects of carvacrol on oocyte development in semi-engorged *Rhipicephalus sanguineus* sensu lato females ticks (Acari: Ixodidae). Micron, 116, 66–72.

DE VINCENZI, M., STAMMATI, A., DE VINCENZI, A., SILANO, M., 2004. Constituents of aromatic plants: carvacrol. Fitoterapia. 75, 801–804.

DELMONTE, C. *et al*. Evaluation of the acaricidal activity of thymol incorporated in two formulations for topical use against immature stages of *Rhipicephalus*

sanguineus sensu lato (Latreille, 1806) (Acari: Ixodidae). Parasitology research, v. 116, n. 11, p. 2957-2964, 2017.

ELLSE, L.; WALL, R. The use of essential oils in veterinary ectoparasite control: a review. Medical and Veterinary Entomology, v. 28, n. 3, p. 233-243, 2014.

ESTRADA-PEÑA, A. *et al*. The known distribution and ecological preferences of the tick subgenus *Boophilus* (Acari: Ixodidae) in Africa and Latin America. Experimental & applied acarology, v. 38, n. 2, p. 219-235, 2006.

FAO - FOOD AND AGRICULTURE ORGANISATION. Resistance management and integrated parasite control in ruminants: Guidelines. Roma: Food and Agriculture Organisation, Animal Production and Health Division, Roma, Itália, 2004. 53 p.

FAZA, A. P. *et al*. A new approach to characterization of the resistance of populations of *Rhipicephalus microplus* (Acari: Ixodidae) to organophosphate and pyrethroid in the state of Minas Gerais, Brazil. Experimental parasitology, v. 134, n. 4, p. 519-523, 2013.

FELTON, G. W., SUMMERS, C. B., 1995. Antioxidant systems in insects. Arch. Insect Biochem. Physiol. 29, 187–197.

FERREIRA, F. M., DELMONTE, C. C., NOVATO, T. L. P., MONTEIRO, C. M. O., DAEMON, E., VILELA, F. M. P., & AMARAL, M. P. H., 2017. Acaricidal activity of essential oil of *Syzygium aromaticum*, hydrolate and eugenol formulated or free on larvae and engorged females of *Rhipicephalus microplus*. Medical and Veterinary Entomology, 32(1), 41–47.

FURLONG, J. Diagnosis of the susceptibility of the cattle tick, *Boophilus microplus*, to acaracides in Minas Gerais state, Brazil. In: SEMINARIO INTERNACIONAL DE PARASITOLOGIA ANIMAL, 4., 1999, Puerto Vallarta, México. Proceedings... Puerto Vallarta: CONASAG, 1999. 41-46 p.

FURLONG, J. *et al*. O carrapato dos bovinos e a resistência: temos o que comemorar? A Hora Veterinária, Porto Alegre, v. 27, n. 159, p. 26-32, 2007.

GARCIA, M. V. *et al*. Biologia e importância do carrapato Rhipicephalus (Boophilus) microplus. *In*: ANDREOTTI, R. *et al*. (Ed.). Carrapatos na cadeia produtiva de bovinos. Brasília, DF: Embrapa, 2019. 240 p. il. color. p. 16-25.

GARCIA, M. V., MATIAS, J., BARROS, J. C., LIMA, D. P. DE, LOPES, R. DA S., & ANDREOTTI, R., 2012. Chemical identification of *Tagetes minuta* Linnaeus (Asteraceae) essential oil and its acaricidal effect on ticks. Revista Brasileira de Parasitologia Veterinária, 21(4), 405–411.

GODARA, R. *et al*. Synthetic pyrethroid resistance in *Rhipicephalus (Boophilus) microplus* ticks from north-western Himalayas, India. Tropical animal health and production, v. 51, n. 5, p. 1203-1208, 2019.

GOMES, G. A., MONTEIRO, C. M. DE O., SENRA, T. DE O. S., ZERINGOTA, V., CALMON, F., MATOS, R. DA S., DAEMON, E. R., GOIS, W., SANTIAGO, G. M., DE CARVALHO, M. G., 2012. Chemical composition and acaricidal activity of essential oil from *Lippia sidoides* on larvae of *Dermacentor nitens* (Acari: Ixodidae) and larvae and engorged females of *Rhipicephalus microplus* (Acari: Ixodidae). Parasitology Research, 111(6), 2423–2430.

GONZALES, J. C., *et al*. A vida livre do *Boophilus microplus*. *Arq. Fac. Vet*. UFRGS. RG. v. 3, p. 21-28, 1974.

GOODNER, K. L., MAHATTANATAWEE, K., PLOTTO, A., SOTOMAYOR, J. A., & JORDÁN, M. J., 2006. Aromatic profiles of *Thymus hyemalis* and Spanish *T. vulgaris* essential oils by GC–MS/GC–O. Industrial Crops and Products, 24(3), 264–268.

GRISI, L. *et al*. Reassessment of the potential economic impact of cattle parasites in Brazil. Revista Brasileira de Parasitologia Veterinária, v. 23, p. 150-156, 2014.

GROSS, A.D., TEMEYER, K.B., DAY, T.A., PÉREZ DE LEÓN, A.A., KIMBER, M.J., COATS, J.R. Interaction of plant essential oil terpenoids with the southern cattle tick tyramine receptor: A potential biopesticide target. Chemico-biological interactions, v. 263, p. 1-6, 2017.

GUERRERO, F. D. *et al*. Acaricide resistance mechanisms in *Rhipicephalus (Boophilus) microplus*. Revista Brasileira de Parasitologia Veterinária, v. 21, p. 1-6, 2012.

GUGLIELMONE, A. A. *et al*. The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world: a list of valid species names. 2010.

HIGA, L. O. S. *et al*. Evidence of acaricide resistance in different life stages of *Amblyomma mixtum* and *Rhipicephalus microplus* (Acari: Ixodidae) collected from the same farm in the state of Veracruz, Mexico. Preventive veterinary medicine, v. 174, p. 104837, 2020.

HIGA, L.D.O.S., GARCIA, M.V., RODRIGUES, V.D.S., BONATTE, P., PIˇNA, F.T.B., BARROS, J.C., ANDREOTTI, R., 2019. Effects of cypermethrin, chlorpyrifos and piperonyl butoxide-based pour-on and spray acaricides on controlling the tick *Rhipicephalus microplus*. Syst. Appl. Acarol. 24, 278–286.

HÜE, T., CAUQUIL, L., FOKOU, J. B. H., DONGMO, P. M. J., BAKARNGA-VIA, I., & MENUT, C., 2014. Acaricidal activity of five essential oils of *Ocimum* species on *Rhipicephalus* (Boophilus) *microplus* larvae. Parasitology Research, 114(1), 91–99.

KINTZIOS, S., 2002. Oregano: the genera *Origanum* and *Lippia* (medicinal and aromatic plants industrial profiles). New York: CRC Press. ISBN 9780415369435.

KLAFKE G. *et al*. Resistência múltipla a acaricidas em populações de *Rhipicephalus microplus* no estado do Rio Grande do Sul no campo. Doenças transmitidas por carrapatos do sul do Brasil*.* v. 8, n. 1, p. 73–80, 2016.

KLAFKE, G. *et al*. Multiple resistance to acaricides in field populations of *Rhipicephalus microplus* from Rio Grande do Sul state, Southern Brazil. Ticks and tick-borne diseases, v. 8, n. 1, p. 73-80, 2017. DOI: 10.1016/j.ttbdis.2016.09.019.

KLAFKE, G.M., WEBSTER, A., DALL AGNOL, B., PRADEL, E., SILVA, J., DE LA CANAL, L.H., BECKER, M., OSÓRIO, M.F., MANSSON, M., BARRETO, R., SCHEFFER, R., SOUZA, U.A., CORASSINI, V.B., DOS SANTOS, J., RECK, J., MARTINS, J.R., 2017. Multiple resistance to acaricides in field populations of Rhipicephalus microplus from Rio Grande do Sul state, Southern Brazil. Ticks Tick. Borne. Dis. 8, 73–80.

KOC, S., OZ, E., CINBILGEL, I., AYDIN, L., & CETIN, H., 2013. Acaricidal activity of *Origanum bilgeri* P.H. Davis (Lamiaceae) essential oil and its major component, carvacrol against adults *Rhipicephalus turanicus* (Acari: Ixodidae). Veterinary Parasitology, 193(1-3), 316–319.

KUMAR, R. *et al*. Voltage-gated sodium channel gene mutations and pyrethroid resistance in *Rhipicephalus microplus*. Ticks and tick-borne diseases, v. 11, n. 3, p. 101404, 2020.

LAGE, T. C. A., MONTANARI, R. M., FERNANDES, S. A., DE OLIVEIRA MONTEIRO, C. M., DE OLIVEIRA SOUZA SENRA, T., ZERINGOTA, V., MATOS, R. S., DAEMON, E., 2015. Chemical composition and acaricidal activity of the essential oil of *Baccharis dracunculifolia* De Candole (1836) and its constituents nerolidol and limonene on larvae and engorged females of *Rhipicephalus microplus* (Acari: Ixodidae). Experimental Parasitology, 148, 24–29.

LEE, S. J., UMANO, K., SHIBAMOTO, T., LEE, K. G., 2005. Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. Food Chemistry, 91(1), 131–137.

LI, A.Y., CHEN, A.C., DAVEY, R.B., MILLER, R.J., GEORGE, J.E., 2007. Acaricide resistance and synergism between permethrin and amitraz against susceptible and resistant strains of *Boophilus microplus* (Acari, Ixodidae). Pest. Manag. Sci. 63, 882–

889.

LIMA, A. S., MACIEL, A. P., MENDONÇA, C. DE J. S., COSTA JUNIOR, L. M., 2017. Use of encapsulated carvacrol with yeast cell walls to control resistant strains of Rhipicephalus microplus (Acari: Ixodidae). Industrial Crops and Products, 108, 190–194.

LIMA, A. S., MILHOMEM, M. N., SANTOS MONTEIRO, O., ARRUDA, A. C. P., DE CASTRO, J. A. M., FERNANDES, Y. M. L., MAIA, J. C. S., COSTA-JUNIOR, L. M., 2018. Seasonal analysis and acaricidal activity of the thymol-type essential oil of *Ocimum gratissimum* and its major constituents against *Rhipicephalus microplus* (Acari: Ixodidae). Parasitology Research, 117(1), 59–65.

LIMA-DE-SOUZA JR, DE OLIVEIRA PR, ANHOLETO LA, SODELLI LF, FERREIRA ARF, REMEDIO RN, CAMARGO-MATHIAS MI. The bioactive compound carvacrol as a potential acaricide: An assessment of its effects on the integument of female Rhipicephalus sanguineus sensu lato ticks. Microsc Res Tech. 2021 Dec 27.

LOPES, W. D. Z. *et al*. Efficacy of fipronil (1.0 mg/kg) against *Rhipicephalus (Boophilus) microplus* strains resistant to ivermectin (0.63 mg/kg). Preventive veterinary medicine, v. 115, n. 3-4, p. 88-93, 2014.

LUSHCHAK, V. I., 2014. Free radicals, reactive oxygen species, oxidative stress and its classification. Chem Biol Interact. 5(224), 164-75.

LYAKHOVICH, V. V., VAVILIN, V. A., ZENKOV, N. K., MENSHCHIKOVA, E. B., 2006. Active defense under oxidative stress. The antioxidant responsive element. Biochem. Mosc. 71, 962–974.

MARANGONI, C. *et al*. Utilização de óleos essenciais e extratos de plantas no controle de insetos. Revista de Ciências Ambientais, v. 6, n. 2, p. 95–112, 2012.

MARCHESINI P, LEMOS ASO, BITENCOURT ROB, FIOROTTI J, ANGELO IDC, FABRI RL, COSTA-JÚNIOR LM, LOPES WDZ, BITTENCOURT VREP, MONTEIRO C. Assessment of lipid profile in fat body and eggs of Rhipicephalus microplus engorged females exposed to (E)-cinnamaldehyde and α-bisabolol, potential acaricide compounds. Vet Parasitol. 2021a Dec;300:109596.

MARCHESINI P, NOVATO TP, CARDOSO SJ, DE AZEVEDO PRATA MC, DO NASCIMENTO RM, KLAFKE G, COSTA-JÚNIOR LM, MATURANO R, LOPES WDZ, BITTENCOURT VREP, MONTEIRO C. Acaricidal activity of (E)-cinnamaldehyde and α-bisabolol on populations of Rhipicephalus microplus (Acari: Ixodidae) with different resistance profiles. Vet Parasitol. 2020 Oct;286:109226.

MARCHESINI P, OLIVEIRA DR, GOMES GA, RODRIGUES THS, MATURANO R, FIDELIS QC, CATUNDA JÚNIOR FEA, CARVALHO MG, BITTENCOURT VREP, MONTEIRO CMO. Acaricidal activity of essential oils of Cinnamomum zeylanicum and Eremanthus erythropappus, major compounds and cinnamyl acetate in Rhipicephalus microplus. Rev Bras Parasitol Vet. 2021b Sep 6;30(3):e009221.

MARQUES, R. *et al*. Climate change implications for the distribution of the babesiosis and anaplasmosis tick vector, *Rhipicephalus (Boophilus) microplus*. Veterinary research, v. 51, n. 1, p. 1-10, 2020.

MASSARD, C. L.; FONSECA, A. H. Carrapatos e doenças transmitidas, comuns ao homem e aos animais. A Hora Veterinária, v. 135, n. 1, p. 15-23, 2004.

MATOS RS, DAEMON E, CAMARGO-MATHIAS MI, FURQUIM KC, SAMPIERI BR, REMÉDIO RN, ARAÚJO LX, NOVATO TP. Histopathological study of ovaries of Rhipicephalus sanguineus (Acari: Ixodidae) exposed to different thymol concentrations. Parasitol Res. 2014b Dec;113(12):4555-65.

MATOS RS, DE OLIVEIRA PR, COELHO L, DE PAULA LGF, ZERINGOTA V, CARVALHO SILVA B, MONTEIRO C, DAEMON E, CAMARGO-MATHIAS MI. Thymol: Effects on reproductive biology and Gene's organ morphology in Rhipicephalus sanguineus sensu lato engorged females (Acari: Ixodidae). Ticks Tick Borne Dis. 2020 Jan;11(1):101308. doi: 10.1016/j.ttbdis.2019.101308.

MATOS, R. S., DAEMON, E., DE OLIVEIRA MONTEIRO, C. M., SAMPIERI, B. R., MARCHESINI, P. B. C., DELMONTE, C., CAMARGO-MATHIAS, M. I., 2019. Thymol action on cells and tissues of the synganglia and salivary glands of *Rhipicephalus sanguineus* sensu lato females (Acari: Ixodidae). Ticks and Tick-Borne Diseases, 10, 314 - 320.

MCCORD, J. M., FRIDOVICH, I., 1969. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein), J. Biol. Chem. 244, 6049–6055.

MENDES, A. S., DAEMON, E., DE OLIVEIRA MONTEIRO, C. M., MATURANO, R., BRITO, F. C., MASSONI, T., 2011. Acaricidal activity of thymol on larvae and nymphs of *Amblyomma cajennense* (Acari: Ixodidae). Veterinary Parasitology, 183(1-2), 136–139.

MENDES, M. C. *et al*. Characterization of the pyrethroid resistance profile of *Rhipicephalus (Boophilus) microplus* populations from the states of Rio Grande do Sul and Mato Grosso do Sul, Brazil. Revista Brasileira de Parasitologia Veterinária, v. 22, p. 379-384, 2013.

MOLDÃO-MARTINS, M., PALAVRA, A., BEIRÃO DA COSTA, M., BERNARDO-GIL, M., 2000. Supercritical CO_2 extraction of *Thymus zygis* L. subsp. *sylvestris* aroma. J.

Supercritical Fluids., 18(1), 25–34.

MONTEIRO C, FERREIRA LL, DE PAULA LGF, DE OLIVEIRA FILHO JG, DE OLIVEIRA SILVA F, MUNIZ ER, MENEZES KMF, DE CAMARGO FR, DE OLIVEIRA NONATO R, MARTINS DB, MARRETO RN, BORGES LMF. Thymol and eugenol microemulsion for Rhiphicephalus sanguineus sensu lato control: Formulation development, field efficacy, and safety on dogs. Vet Parasitol. 2021 Aug;296:109501.

MONTEIRO, C. M. O., DAEMON, E., APARECIDO CLEMENTE, M., DOS SANTOS ROSA, L., MATURANO, R., 2009. Acaricidal efficacy of thymol on engorged nymphs and females of Rhipicephalus sanguineus (Latreille, 1808) (Acari: Ixodidae). Parasitology Research, 105(4), 1093–1097.

MONTEIRO, C. M. O., DAEMON, E., SILVA, A. M. R., MATURANO, R., AMARAL, C., 2010). Acaricide and ovicide activities of thymol on engorged females and eggs of *Rhipicephalus* (Boophilus) *microplus* (Acari: Ixodidae). Parasitology Research, 106(3), 615–619.

MONTEIRO, C. M., MATURANO, R., DAEMON, E., CATUNDA-JUNIOR, F. E. A., CALMON, F., DE SOUZA SENRA, T., SENRA, T. S., FAZA, A., CARVALHO, M. G., 2012. Acaricidal activity of eugenol on *Rhipicephalus microplus* (Acari: Ixodidae) and *Dermacentor nitens* (Acari: Ixodidae) larvae. Parasitology Research, 111(3), 1295–1300.

MUYOBELA, J., NKUNIKA, P. O. Y., & MWASE, E. T., 2016. *In vitro* acaricidal activity of *Bobgunnia madagascariensis* Desv. against *Amblyomma variegatum* (Fabricius) (Acari: Ixodidae). Tropical Animal Health and Production, 48(3), 625–631.

NAVA, S. *et al*. Ticks of the southern cone of America: diagnosis, distribution, and hosts with taxonomy, ecology and sanitary importance. Academic Press, 2017.

NEVES, I. I. DE A., DA CAMARA, C. A. G., DE OLIVIERA, J. C. S., & DE ALMEIDA, A. V., 2011. Acaricidal Activity and Essential Oil Composition of *Petiveria alliacea* L. from Pernambuco (Northeast Brazil). Journal of Essential Oil Research, 23(1), 23–26.

NOVATO, T. P. L., ARAÚJO, L. X., DE MONTEIRO, C. M. O., MATURANO, R., SENRA, T. DE O. S., DA SILVA MATOS, R., GOMES, G. A., CARVALHO, M. G., DAEMON, E., 2015. Evaluation of the combined effect of thymol, carvacrol and (E)-cinnamaldehyde on *Amblyomma sculptum* (Acari: Ixodidae) and *Dermacentor nitens* (Acari: Ixodidae) larvae. Veterinary Parasitology, 212(3-4), 331–335.

NOVATO, T., GOMES, G. A., ZERINGÓTA, V., FRANCO, C. T., DE OLIVEIRA, D. R., MELO, D., CARVALHO, M. G., DAEMON, E., DE OLIVEIRA MONTEIRO, C. M., 2018. In vitro assessment of the acaricidal activity of carvacrol, thymol, eugenol and

their acetylated derivatives on *Rhipicephalus microplus* (Acari: Ixodidae). Veterinary Parasitology, 260, 1–4.

NOVATO, T.L.P., MARCHESINI, P., MUNIZ, N., PRATA, M.C.A., FURLONG, J., VILELA, F.M.P., DAEMON, E., MATURANO, R., MONTEIRO, C., 2019. Evaluation of synergism and development of a formulation with thymol, carvacrol and eugenol for *Rhipicephalus microplus* control. Exp. Parasitol. 207, 107774.

NOVELINO, A. M. S., DAEMON, E., SOARES, G. L. G., 2007. Evaluation of the acaricide effect of thymol, menthol, salicylic acid, and methyl salicylate on *Boophilus microplus* (Canestrini 1887) (Acari: Ixodidae) larvae. Parasitology Research, 101(3), 809–811.

NWANADE CF, WANG M, WANG T, ZHANG X, WANG C, YU Z, LIU J. Acaricidal activity of Cinnamomum cassia (Chinese cinnamon) against the tick Haemaphysalis longicornis is linked to its content of (E)-cinnamaldehyde. Parasit Vectors. 2021 Jun 22;14(1):330. doi: 10.1186/s13071-021-04830-2.

PEIXOTO, M. G., COSTA-JÚNIOR, L. M., BLANK, A. F., LIMA, A. DA S., MENEZES, T. S. A., SANTOS, D. DE A., ALVES, P. B.; CAVALCANTI, S. C.; BACCI, L., ARRIGONI-BLANK, M. de F., 2015. Acaricidal activity of essential oils from *Lippia alba* genotypes and its major components carvone, limonene, and citral against *Rhipicephalus microplus*. Veterinary Parasitology, 210(1-2), 118–122.

PENHA, T., COSTA, A.C.C., LIMA, A.D.S., CAMARGO-MATHIAS, M.I., BLANK, A.F., ABREU-SILVA, A.L., COSTA-JÚNIOR, L.M., 2021. Effects of acaricidal essential oils from *Lippia sidoides* and *Lippia gracilis* and their main components on vitellogenesis in *Rhipicephalus microplus* (Canestrini, 1888) (Acari: Ixodidae). Vet. Parasitol. 299, 109584.

PEREIRA JUNIOR AM, CAMARGO-MATHIAS MI, DAEMON E, PECONICK AP, LIMA-SOUZA JR, OLIVEIRA PR, BRAGA AS, LARA LJ, REMEDIO RN. Efficacy of carvacrol on *Rhipicephalus (Boophilus) microplus* engorged female ticks (Canestrini, 1887) (Acari: Ixodidae): effects on mortality and reproduction. Nat Prod Res. 2020 Dec;34(23):3428-3431.

PEREIRA, M. C.; LABRUNA, M. B. *Rhipicephalus (Boophilus) microplus*. Chapter 3. In: CAMPOS PEREIRA, M. *et al*. Rhipicephalus (Boophilus) microplus: biologia, controle e resistência. Medicina Veterinária, São Paulo, 2008.169 p.

PETER, R. J. *et al*. Tick, fly and mosquito control - lessons from the past, solutions for the future. Veterinary Parasitology, v. 132, p. 205-215, 2005.

PRADO-REBOLLEDO, O. F., MOLINA-OCHOA, J., LEZAMA-GUTIÉRREZ, R., GARCÍA-MÁRQUEZ, L. J., MINCHACA-LLERENAS, Y. B., MORALES-BARRERA,

E., TELLEZ, G., HARGIS, B., SKODA, S. R., FOSTER, J. E., 2017. Effect of *Metarhizium anisopliae* (Ascomycete), Cypermethrin, and D-Limonene, Alone and Combined, on Larval Mortality of *Rhipicephalus sanguineus* (Acari: Ixodidae). Journal of Medical Entomology, 54(5), 1323–1327.

PRATES, H. T., LEITE, R. C., CRAVEIRO, A. A., OLIVEIRA, A. B., 1998. Identification of Some Chemical Components of the Essential Oil from Molasses Grass (*Melinis minutiflora* Beauv.) and their Activity Against Cattle-Tick (*Boophilus microplus*). Journal of the Brazilian Chemical Society, 9(2), 193–197.

PRULLAGE, J. *et al*. The combined mode of action of fipronil and amitraz on the motility of *Rhipicephalus sanguineus*. Veterinary Parasitology, v. 179, p. 302-310. 2011. DOI: 10.1016/j.vetpar.2011.03.041.

PRULLAGE, J.B., CAWTHORNE, W.G., DE LE HIR FALLOIS, L.P., TIMMONS, P.R., 2011. Synergy between fipronil and amitraz in a *Rhipicephalus sanguineus* tick residual contact test. Exp. Appl. Acarol. 54, 173–176.

QUEIROZ VT, CAMPOS NC, NUNES ET, COSTA AV, COELHO JD, TRIVILIN LO, DE MELO DCA, MORAIS PAB, MARTINS IVF. 1,8-cineole and castor oil in sodium lauryl ether sulphate disrupt reproduction and ovarian tissue of Rhipicephalus (Boophilus) microplus. Med Vet Entomol. 2020 Sep;34(3):316-326

RAMÍREZ, C., IBARRA, F., PÉREZ, H. I., MANJARREZ, N., SALGADO, H. J., ORTEGA, L., 2016. Assessment and determination of LC 50 of carvacrol and salicylic acid analogues with acaricide activity in larvae and adult ticks of *Rhipicephalus* (Boophilus) *microplus*. Parasite Epidemiology and Control, 1(2), 72–77.

RECK, J. *et al*. First report of fluazuron resistance in *Rhipicephalus microplus*: a field tick population resistant to six classes of acaricides. Veterinary Parasitology, v. 201, n. 1-2, p. 128-136, 2014.

RECK, J., KLAFKE, G.M., WEBSTER, A., DALL'AGNOL, B., SCHEFFER, R., SOUZA, U.A., CORASSINI, V.B., VARGAS, R., DOS SANTOS, J.S., DE SOUZA MARTINS, J.R., 2014. First report of fluazuron resistance in *Rhipicephalus microplus*: A field tick population resistant to six classes of acaricides. Vet. Parasitol. 201, 128–136.

RIBEIRO, V. L. S., ROLIM, V., BORDIGNON, S., HENRIQUES, A. T., DORNELES, G. G., LIMBERGER, R. P., & VON POSER, G., 2008. Chemical composition and larvicidal properties of the essential oils from *Drimys brasiliensis* Miers (Winteraceae) on the cattle tick *Rhipicephalus* (Boophilus) *microplus* and the brown dog tick *Rhipicephalus sanguineus*. Parasitology Research, 102(3), 531–535.

ROCHA, U. F. Biologia e controle biológico do carrapato *Boophilus microplus* (Canestrini). Jaboticabal: UNESP, p. 35, 1984.

RODRÍGUEZ-VIVAS, R. I. *et al*. Resistencia a los acaricidas en *Rhipicephalus (Boophilus) microplus*: situación actual y mecanismos de resistencia. Revista mexicana de ciencias pecuarias, v. 3, p. 9-24, 2012.

RODRÍGUEZ-VIVAS, R. I. et al. *Rhipicephalus (Boophilus) microplus* resistant to acaricides and ivermectin in cattle farms of Mexico. Revista Brasileira de Parasitologia Veterinária, v. 23, p. 113-122, 2014.

RODRIGUEZ-VIVAS, R. I. *et al*. Strategies for the control of *Rhipicephalus microplus* ticks in a world of conventional acaricide and macrocyclic lactone resistance. Parasitology research, v. 117, n. 1, p. 3-29, 2018.

RODRÍGUEZ-VIVAS, R. I. *et al*. Uso de lactonas macrocíclicas para el control de la garrapata *Rhipicephalus (Boophilus) microplus* en el ganado bovino. Archivos de medicina veterinaria, v. 42, n. 3, p. 115-123, 2010.

RODRIGUEZ-VIVAS, R.I., LI, A.Y., OJEDA-CHI, M.M., TRINIDAD-MARTINEZ, I., ROSADO-AGUILAR, J. A., MILLER, R.J., P´EREZ DE LE´ON, A.A., 2013. In vitro and in vivo evaluation of cypermethrin, amitraz, and piperonyl butoxide mixtures for the control of resistant *Rhipicephalus (Boophilus) microplus* (Acari: ixodidae) in the Mexican tropics. Vet. Parasitol. 197, 288–296.

ROSADO-AGUILAR, J.A., ARJONA-CAMBRANES, K., TORRES-ACOSTA, J.F.J., RODRÍGUEZ-VIVAS, R.I., BOLIO-GONZÁLEZ, M.E., ORTEGA-PACHECO, A., ALZINA-LÓPEZ, A., GUTIÉRREZ-RUIZ, E.J., GUTIÉRREZ-BLANCO, E., AGUILAR-CABALLERO, A.J., 2017. Plant products and secondary metabolites with acaricide activity against ticks. Vet. Parasitol. 238, 66–76.

SAGAR, S.V., SAINI, K., SHARMA, A.K., KUMAR, SACHIN, KUMAR, R., FULAR, A., SHAKYA, M., UPADHAYA, D., NAGAR, G., SHANMUGANATH, C., SAMANTA, S., KUMAR, SUMAN, GHOSH, S., 2020. Acaricide resistance in *Rhipicephalus microplus* collected from selected districts of Madhya Pradesh, Uttar Pradesh and Punjab states of India. Trop. Anim. Health Prod. 52, 611–618.

SCHAFER, H., & WINK, M., 2009. Medicinally important secondary metabolites in recombinant microorganisms or plants: Progress in alkaloid biosynthesis. Biotechnology Journal, 4(12), 1684–1703.

SCORALIK, M. G., DAEMON, E., DE OLIVEIRA MONTEIRO, C. M., MATURANO, R., 2012. Enhancing the acaricide effect of thymol on larvae of the cattle tick *Rhipicephalus microplus* (Acari: Ixodidae) by solubilization in ethanol. Parasitology Research, 110(2), 645–648.

SENRA, T. O. S., CALMON, F., ZERINGÓTA, V., MONTEIRO, C. M. O., MATURANO, R., DA SILVA MATOS, R., DAEMON, E. (2013b). Investigation of activity of monoterpenes and phenylpropanoids against immature stages of *Amblyomma cajennense* and *Rhipicephalus sanguineus* (Acari: Ixodidae). Parasitology Research, 112(10), 3471–3476.

SENRA, T. O. S., ZERINGÓTA, V., DE OLIVEIRA MONTEIRO, C. M., CALMON, F., MATURANO, R., GOMES, G. A., FAZA, A., CARVALHO, M. G., DAEMON, E., 2013a. Assessment of the acaricidal activity of carvacrol, (E)-cinnamaldehyde, trans-anethole, and linalool on larvae of *Rhipicephalus microplus* and *Dermacentor nitens* (Acari: Ixodidae). Parasitology Research, 112(4), 1461–1466.

SHARMA, A. K. *et al*. Deltamethrin and cypermethrin resistance status of *Rhipicephalus (Boophilus) microplus* collected from six agro-climatic regions of India. Veterinary Parasitology, v. 188, n. 3-4, p. 337-345, 2012.

SHYMA, K. P. *et al*. *In vitro* detection of acaricidal resistance status of *Rhipicephalus (Boophilus) microplus* against commercial preparation of deltamethrin, flumethrin, and fipronil from North Gujarat, India. Journal of parasitology research, v. 2015, 2015.

SILVA, L.C., PERINOTTO, W.M.S., S´A, F.A., SOUZA, M.A.A., BITENCOURT, R.O.B., SANAVRIA, A., SANTOS, H.A., MARIE-MAGDELEINE, C., ANGELO, I.C., 2020. In vitro acaricidal activity of *Cymbopogon citratus, Cymbopogon nardus* and *Mentha arvensis* against *Rhipicephalus microplus* (Acari: ixodidae). Exp. Parasitol. 216, 107937.

SOARES, A. M. DOS S., PENHA, T. A., ARAÚJO, S. A. DE, CRUZ, E. M. O., BLANK, A. F., COSTA-JUNIOR, L. M., 2016. Assessment of different *Lippia sidoides* genotypes regarding their acaricidal activity against *Rhipicephalus* (Boophilus) *microplus.* Revista Brasileira de Parasitologia Veterinária, 25(4), 401–406.

SÖKMEN, M., SERKEDJIEVA, J., DAFERERA, D., GULLUCE, M., POLISSIOU, M., TEPE, B., AKPULAT, H. A., SAHIN, F., SOKMEN, A., 2004. *In Vitro* Antioxidant, Antimicrobial, and Antiviral Activities of the Essential Oil and Various Extracts from Herbal Parts and Callus Cultures of *Origanum acutidens*. Journal of Agricultural and Food Chemistry, 52(11), 3309–3312.

SOUSA, E.O., LIMA, A.D.S., LOPES, S.G., COSTA-JUNIOR, L.M., DA COSTA, J.G.M., 2020. Chemical composition and acaricidal activity of *Lantana camara* L. and *Lantana montevidensis* Briq. essential oils on the tick *Rhipicephalus microplus*. J. Essent. Oil Res. 32, 316–322.

TABARI, M. A., YOUSSEFI, M. R., MAGGI, F., BENELLI, G., 2017. Toxic and repellent activity of selected monoterpenoids (thymol, carvacrol and linalool) against the castor bean tick, *Ixodes ricinus* (Acari: Ixodidae). Veterinary Parasitology, 245, 86–91.

TAVARES CP, SOUSA IC, GOMES MN, MIRÓ V, VIRKEL G, LIFSCHITZ A, COSTA-JUNIOR LM. Combination of cypermethrin and thymol for control of Rhipicephalus microplus: Efficacy evaluation and description of an action mechanism. Ticks Tick Borne Dis. 2022 Jan;13(1):101874.

THULLNER, F. Impact of pesticide resistance and network for global pesticide resistance management based on a regional structure. Revue Mondiale de Zootechnie (FAO); Revista Mundial de Zootecnia (FAO), 1997.

TONG, F., COATS, J. R., 2010. Effects of monoterpenoid insecticides on [3H]-TBOB binding in house fly GABA receptor and 36Cl− uptake in *American cockroach* ventral nerve cord. Pesticide Biochemistry and Physiology, 98(3), 317–324.

UPADHAYA, D., KUMAR, B., KUMAR, S., SHARMA, A.K., FULAR, A., BISHT, N., SRIVASTAVA, S., BORUAH, R.R., NAGAR, G., SHAKYA, M., NATH, T., NANDI, S.P., KARIVARADHARAJA, S., GHOSH S., 2020. Characterization of acaricide resistance in *Rhipicephalus microplus* populations infesting cattle in northeastern India and assessment of local plant extracts for tick management. Vet. Parasitol. 277, 109011.

VALE L, DE PAULA LGF, VIEIRA MS, ALVES SDGA, JUNIOR NRM, GOMES MDF, TEIXEIRA WFP, RIZZO PV, FREITAS FMC, FERREIRA LL, LOPES WDZ, MONTEIRO C. Binary combinations of thymol, carvacrol and eugenol for Amblyomma sculptum control: Evaluation of in vitro synergism and effectiveness under semi-field conditions. Ticks Tick Borne Dis. 2021 Nov;12(6):101816.

VALENTE, P. P., AMORIM, J. M., CASTILHO, R. O., LEITE, R. C., RIBEIRO, M. F. B., 2013. *In vitro* acaricidal efficacy of plant extracts from Brazilian flora and isolated substances against *Rhipicephalus microplus* (Acari: Ixodidae). Parasitology Research, 113(1), 417–423.

VALSONI, L.M., FREITAS, M.G. DE, ECHEVERRIA, J.T., BORGES, D.G.L., TUTIJA, J., BORGES, F. DE A., 2020. Resistance to all chemical groups of acaricides in a single isolate of Rhipicephalus microplus in Mato Grosso do Sul, Brazil. Int. J. Acarol. 46, 276–280.

VILELA, V.L.R., FEITOSA, T.F., BEZERRA, R.A., KLAFKE, G.M., RIET-CORREA, F., 2020. Multiple acaricide-resistant Rhipicephalus microplus in the semi-arid region of Paraíba State, Brazil. Ticks Tick. Borne. Dis. 11, 101413.

WAGNER, H., 2011. Synergy research: approaching a new generation of phytopharmaceuticals. Fitoterapia 82, 34–37.

WILCE, M. C. J., PARKER, M. W., 1994. Structure and function of glutathione S-transferases. Biochim Biophys Acta. 1205, 1–18.

WINK, M., 2008. Evolution of secondary plant metabolism. eLS.