UNIVERSIDADE FEDERAL DO MARANHÃO – UFMA
PROGRAMA DE PÓS-GRADUAÇÃO EM BIODIVERSIDADE
E BIOTECNOLOGIA DA REDE BIONORTE

ALTERAÇÕES MORFOLÓGICAS DOS OVÁRIOS DE *Rhipicephalus microplus* SUBMETIDOS AO TRATAMENTO COM *Lippia sidoides E Lippia gracilis*.

TATIANE ARANHA DA PENHA

São Luís - MA
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Tese de doutorado apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Biodiversidade e Biotecnologia da Rede BIONORTE, na Universidade Federal do Maranhão, como requisito parcial para a obtenção do Título de Doutor em Biotecnologia.

Orientador: Prof. Dr. Livio Martins Costa Júnior
Co-orientadora: Profa. Dra. Ana Lúcia Abreu Silva

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Penha, Tatiane Aranha da.
ALTERAÇÕES MORFOLÓGICAS DOS OVÁRIOS DE Rhipicephalus microplus SUBMETIDOS AO TRATAMENTO COM Lippia sidoides E Lippia gracilis / Tatiane Aranha da Penha. - 2016.
77 p.

Coorientador(a): Ana Lúcia Abreu-Silva.
Orientador(a): Livio Martins Costa-Júnior.
Tese (Doutorado) - Programa de Pós-graduação em Rede - Rede de Biodiversidade e Biotecnologia da Amazônia Legal/ccbs, Universidade Federal do Maranhão, São Luís, 2016.

1. Carrapaticida. 2. Carvacrol. 3. Lippia gracilis. 4. Lippia sidoides. 5. Timol. I. Abreu-Silva, Ana Lúcia. II. Costa-Júnior, Livio Martins. III. Título.

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Examinador 5 – externo

São Luís - MA
OUTUBRO/2016

Dedico este trabalho a minha família, por todo amor, apoio, orações e incentivo para que eu alcance minhas metas.

AGRADECIMENTOS

À Deus, por ter me dado saúde e perseverança para alcançar os meus objetivos.

À minha família, pelo amor, paciência, companheirismo e incentivo.

Aos meus parentes e amigos, por todo apoio e orações para que eu ultrapassasse os obstáculos.

Ao meu orientador Prof. Dr. Livio Martins Costa Júnior pela oportunidade, ensinamentos e confiança para execução desse trabalho.

À minha co-orientadora Prof. Dra. Ana Lúcia Abreu Silva pela amizade, pelo conhecimento transmitido, compreensão e oportunidade de crescimento desde a graduação até hoje.

À toda equipe dos Laboratórios de Parasitologia Animal/CCAA/UFMA e de Controle de Parasitos/CCBS/UFMA, especialmente Aldilene Lima pelo auxílio e apoio durante esses anos de doutorado.

À toda equipe do Laboratório de Anatomopatologia/UEMA, em especial ao Prof. Dr. Fábio Henrique Andrade, aos alunos Anderson Cássio Campelo, Higor Ferreira, Allana Barros e Renata Mondêgo por todo o auxílio e aprendizado para que o trabalho fosse executado.

Aos institutos Osvaldo Cruz e Adolf Lutz por permitirem a execução de algumas análises.

Ao Prof. Dr. Fernando Almeida Souza pela amizade e ajuda com as análises e correções.

Aos alunos da primeira turma de doutorado do programa BIONORTE/Maranhão.

À Universidade Federal do Maranhão pela oportunidade de crescimento.

Ao Programa de Pós-Graduação em Biodiversidade e Biotecnologia e a À Fundação de Amparo a Pesquisa do Estado do Maranhão (FAPEMA) pelo apoio financeiro.

À todos que direta ou indiretamente contribuíram para que este trabalho fosse concluído.

“Os que semeiam entre lágrimas ceifarão com alegria”

(Salmo 126, 5)

RESUMO

O carrapato *Rhipicephalus microplus*, encontrado em regiões tropicais e subtropicais, ocasionam mais de três bilhões de dólares à pecuária brasileira. Óleos essenciais de Verbenáceas do gênero *Lippia*, especialmente *Lippia sidoides* e *Lippia gracilis*, têm sido descritos com potencial atividade carrapaticida e interferindo na reprodução de diferentes espécies de carrapatos. O objetivo desse trabalho foi avaliar a atividade carrapaticida dos óleos essenciais de *L. sidoides* e *L. gracilis* e seus compostos majoritários timol e carvacrol, selecionando os genótipos que apresentam maiores atividades contra o carrapato bovino e caracterizar as alterações morfológicas dos ovários de fêmeas de *R. microplus* a partir das concentrações letais para 50 (CL_{50}) e 75% (CL_{75}) da população estudada. Após a seleção dos genótipos (LGRA-106, LGRA-201, LSDI-102 e LSDI-103, grupos de 10 fêmeas ingurgitadas foram submetidas ao teste de imersão em óleos essenciais em diferentes concentrações e para o controle foi utilizado DMSO 3%. Após sete dias, as fêmeas foram dissecadas em solução tampão fosfato e os ovários foram fixados em glutaraldeído 2,5% por um período de 24 horas. Os tecidos foram desidratados e incluídos em moldes plásticos de resina Leica para análise histológica. Os resultados permitiram a visualização e caracterização do ovário e dos diferentes estágios de desenvolvimento dos ovócitos. No grupo controle (DMSO 3%), não foram verificadas alterações nos tecidos. Nos grupos tratados com os óleos de *L. gracillis* e *L. sidoides* foram observadas vacuolizações de ovócitos II, III, IV e V, desintegração de membrana plasmática em ovócitos II, III, IV e V, com completa deformidade morfológica de ovócitos IV e V. Os compostos majoritários, timol e carvacrol parecem estar atuando ativamente nessas alterações. Os resultados obtidos mostraram atividade dos diferentes genótipos dos óleos essenciais e seus compostos majoritários em larvas e fêmeas de *R. microplus* e permitiu elucidar o mecanismo de ação dos mesmos na oogêse das fêmeas, levando a crer que os compostos testados são promissores para o desenvolvimento de produtos carrapaticidas.

Palavras-chave: timol, carvacrol, *Lippia sidoides*, *Lippia gracilis*, ovário, carrapaticida.

ABSTRACT

The tick *Rhipicephalus microplus*, found in tropical and subtropical regions, has caused great harm to the Brazilian livestock. The damage caused by this tick the Brazilian livestock exceeds three billion dollars. Verbenaceae *Lippia* genus, especially the species *L. sidoides* and *L. gracilis*, have been reported with potential activity in larvae and *R. microplus* females. Therefore, the aim of this study was to evaluate the acaricide activity of *L. sidoides* and *L. gracilis* essential oils and its major compounds thymol and carvacrol and characterize morphological changes in ovaries of *R. microplus* females from lethal concentrations 50 (LC_{50}) and 75% (LC_{75}) of the study population. After selection of the genotypes (LGRA-106, LGRA-201, LSDI-102 and LSDI-103), groups of 10 engorged females were subjected to the immersion test in essential oils at different concentrations and DMSO 3% was used for the control. After seven days, the ticks were dissected in phosphate buffer solution and the ovaries were fixed in 2.5% glutaraldehyde for a period of 24 hours. The tissues were dehydrated and embedded in plastic molds Leica resin for histological analysis. The results visualization and characterization of the ovary and the different stages of development of oocytes. In the control group, treated with 3% DMSO, changes in the tissues have not been verified. In groups treated with the *L. gracillis* oils and *L. sidoides* oils, vacuolization was observed in oocytes II, III, IV and V, cell membrane disintegration in oocytes II, III, IV and V, with complete morphological deformation of oocytes IV and V. The major compounds, thymol and carvacrol seem to be actively working on these changes. The results showed activity of the different genotypes of the essential oils and their major compounds in larvae and females of *R. microplus* and allowed to elucidate the mechanism of action of the same in the oogenesis of the females, leading to believe that the compounds tested are promising for the development of acaricide products.

Keywords: thymol, carvacrol, *Lippia sidoides*, *Lippia gracilis*, ovary, acaricide

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1. INTRODUÇÃO

O Brasil é o segundo país com maior produção de carne bovina do mundo, com rebanho praticamente concentrado nas regiões Sudeste, Sul e Centro-Oeste. Entretanto, devido à expansão da fronteira agrícola, o crescimento do rebanho bovino está alcançando estados da região Amazônica (IBGE, 2015), o que vem acompanhado de desmatamento. Desta maneira, o aumento de produtividade das criações animais é de extrema importância fazendo com que cresça a produção de alimentos e diminua o impacto sobre a floresta. Entretanto, alguns entraves como deficiências no manejo sanitário e nas tecnificações dos rebanhos são presentes.

O carrapato *Rhipicephalus microplus*, encontrado em regiões tropicais e subtropicais, vem causando prejuízos de mais de três bilhões de dólares ao ano à pecuária brasileira (GRISI et al., 2014), tais como: transmissão de parasitos do gênero *Babesia* e *Anaplasma*, redução do ganho de peso, da produção de bezerros e de leite, e da depreciação do valor do couro (RECK et al., 2014). O uso de produtos químicos para o controle de carrapatos ainda é a medida mais adotada pelos produtores, o qual apresenta altos custos, causa poluição ambiental, propicia o surgimento de populações resistentes e alguns induzem toxicidade para o hospedeiro vertebrado. Associados a estes fatores, estão o manejo inadequado dos produtos, não planejamento dos esquemas de tratamento e a falta de monitoramento da concentração das drogas (FURLONG, 2005).

A fitoterapia tem sido recomendada como uma medida alternativa de controle do parasitismo por ectoparasitas, especialmente o carrapato *R. microplus* (CABARET et al., 2002). Além de suprimento sustentável, esta medida apresenta baixo custo, alta biodegradabilidade, uma grande biodiversidade da flora nacional e fácil aceitação pela população (PRANCE, 1991; DEWITT, 1994).

Os produtos naturais podem ser utilizados como extratos, sendo obtidos com diferentes solventes variando a polaridade, óleos fixos e óleos essenciais. Óleos essenciais são constituídos de compostos voláteis que estão associados à sobrevivência dos vegetais, auxiliando na defesa contra microorganismos e predadores, e também na repelência de insetos. Em geral, o gênero *Lippia*, apresenta composição química constante com alguns compostos que são encontrados em varias espécies como timol, carvacrol, 1,8 cineol, α-hamuleno, p-cimeno entre outros, com atividades farmacológicas antimalárica, antiviral e citostática (CALVACANTI, 2006) e que podem servir para o controle de insetos e carrapatos.

Vários estudos têm se dedicado a avaliar o efeito de substâncias na eficiência reprodutiva de fêmeas de carrapatos, impedindo a postura de ovos viáveis à continuação do ciclo de vida. Esses produtos têm provocado alterações morfológicas no sistema reprodutor de fêmeas, impedindo ou inibindo o funcionamento do mesmo (OLIVEIRA et al., 2008;

ROMA et al., 2010; ARNOSTI et al., 2011; SOUSA et al., 2013; PRADO-OCHOA et al., 2014). Os óleos essenciais de *L. sidoides*, *L. gracilis* e seus compostos majoritários, timol e carvacrol têm apresentado atividade contra larvas e fêmeas de *R. microplus*, atuando na diminuição da eficiência reprodutiva (GOMES et al., 2012; CRUZ et al., 2013; GOMES et al., 2014; MONTEIRO et al., 2014; COSTA-JÚNIOR et al., 2016). Análises histológicas e ultraestruturais dos órgãos reprodutores do carrapato bovino podem auxiliar a elucidar o mecanismo de ação desses óleos.

2. REVISÃO DE LITERATURA

2.1 O carrapato *Rhipicephalus microplus*

O carrapato *R. microplus* é um parasito hematófago comumente conhecido como carrapato-do-boi. Esta espécie está distribuída geograficamente nas regiões tropicais e subtropicais, pertence ao Filo Arthropoda, Classe Arachnida, Ordem Ixodida, Família Ixodidae, Gênero *Rhipicephalus* sendo a única espécie do subgênero *Boophilus* conhecida no Brasil (PEREIRA et al., 2008).

O ciclo evolutivo desse parasito requer somente um hospedeiro com uma fase parasitária e outra fase não parasitária, integrando hospedeiro, parasita e ambiente (PEREIRA et al., 2008). A fase não parasitária inicia quando a fêmea ingurgitada, ao cair do hospedeiro, procura um local ideal para realizar a postura dos ovos, os quais, em condições ótimas de temperatura (28°C) e umidade relativa (≥ 85%), eclodem em torno de 18 dias. Após eclosão, as larvas ficam quiescentes e, posteriormente, tornam-se ativas, formando uma aglomeração na ponta ou haste do capim, o que facilita o encontro com seu hospedeiro. O reconhecimento do hospedeiro pelas larvas se dá por órgãos sensoriais, entre eles o órgão de Haller, localizado no primeiro par de patas. As larvas de *R. microplus* são atraídas por gases expirados e secreções da pele dos bovinos (SONESHINE et al., 2013).

A fase parasitária tem duração média de 21 dias. Inicia-se quando a larva infestante sobe no hospedeiro onde passa a se alimentar por hematofagia. A larva sofre muda em sete dias e passa a ser ninfa. Esta, por sua vez, sofre muda em aproximadamente sete dias, passando a ser adulto com dimorfismo sexual. Após o acasalamento, as fêmeas continuam a hematofagia até tornarem-se completamente ingurgitadas, sendo denominadas teleóginas (PEREIRA et al., 2008; SONESHINE et al., 2013). O processo de hematofagia pode provocar vários danos ao hospedeiro, principalmente se o mesmo estiver com alto grau de infestação (SONESHINE, 2013). Assim como os demais ixodídeos, o carrapato bovino *R. microplus*, consegue concentrar o sangue no processo de hematofagia,

eliminando a água por transpiração corporal, glândulas salivares e pelos restos fecais (PEREIRA et al., 2008). As fêmeas podem aumentar até 200 vezes seu tamanho ao ingerir o sangue do hospedeiro, levando a postura entre 2.000 e 3.000 ovos. Devido a esse fato, estudos vêm sendo realizados visando conhecer aspectos morfológicos e ultraestruturais dos órgãos reprodutores dos carrapatos (DENARDI et al. 2004; SAITO et al., 2005; OLIVEIRA et al., 2005; OLIVEIRA et al., 2007).

2.1.1 Sistema reprodutor de *R. microplus*

O sistema reprodutor dos carrapatos é composto por um ovário situado no terço posterior do seu corpo, um par de ovidutos, a vagina e um par de glândulas tubulares acessórias (SONESHINE, 2013; CAMARGO-MATHIAS, 2013). Os ovidutos são pareados e cruzados, localizados nas extremidades dos ovários e se fundem sob o receptáculo seminal para formarem o útero, o qual se abre na vagina cervical (SONESHINE, 2013).

Em fêmeas de *R. microplus*, alimentadas por 48 horas, os ovidutos variam entre 75 a 140 µm de diâmetro. Um tubo de conexão muscular revestido por epitélio é responsável pela comunicação do útero com a vagina cervical. Na junção da vagina cervical com a vagina vestibular são derramadas as secreções das glândulas tubulares acessórias. O epitélio glandular delimita a vagina vestibular, onde está situada a glândula acessória lobular, presente apenas em carrapatos ixodídeos. Particularmente em *R. microplus*, a vagina cervical é muito pequena, onde desembocam o útero e o receptáculo seminal, formando um ângulo menor que 90°, deixando o receptáculo completamente acima do útero, facilitando o caminho dos espermióforos até o ovário (LA VEGA et al, 2012).

Estudos ultramorfológicos revelaram o ovário de *R. microplus* como sendo um órgão tubular único em forma de ferradura (figura 1). Envolvendo o lúmen existe uma parede de células epiteliais pequenas e de núcleos arredondados. O ovário é do tipo panoístico, não apresentando células nutridoras. Neste tipo de ovário, as ovogônias darão origem a possíveis ovócitos. Ao contrário da maior parte dos artrópodes, os ovários de *R. microplus* não são segmentados em zonas diferentes de desenvolvimento (SAITO et al., 2005).

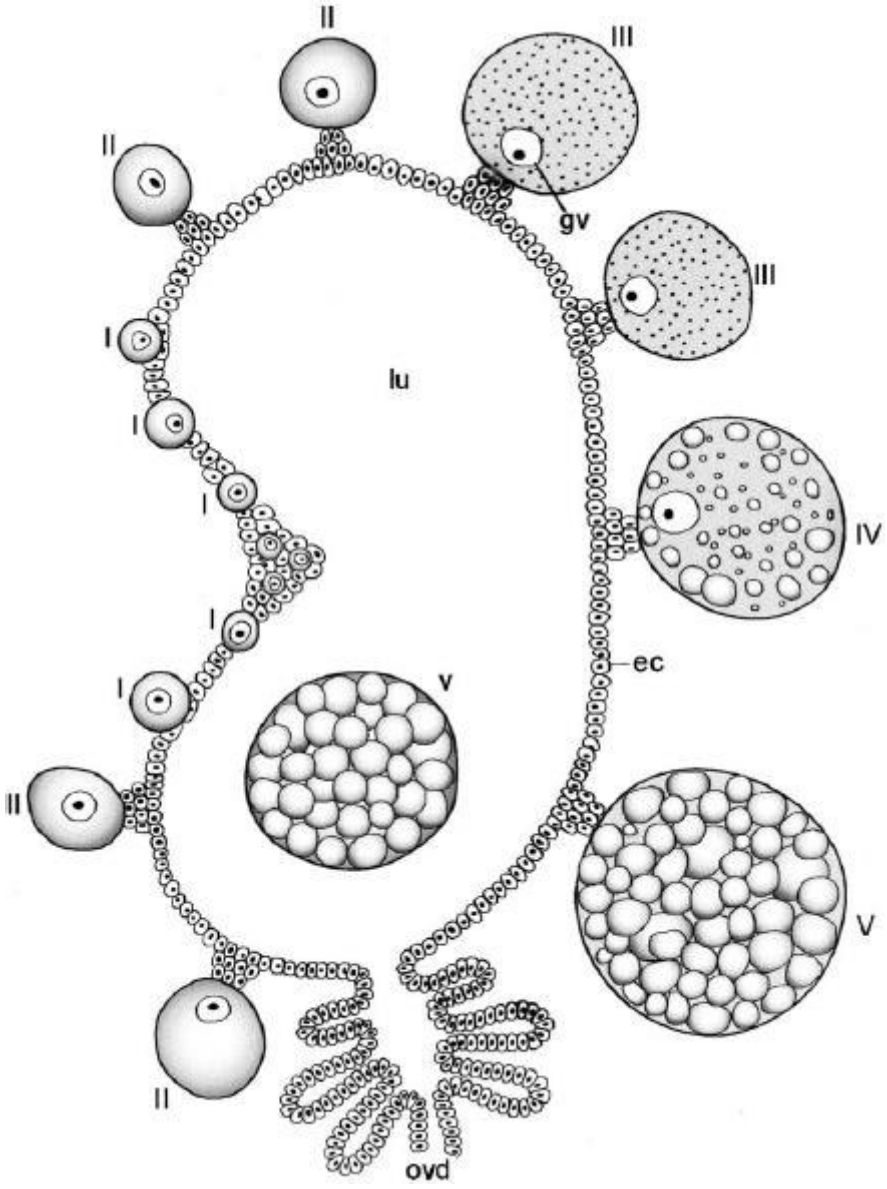

Figura 1: Ovário de fêmea de *Rhipicephalus microplus*. ovd: oviduto, lu: lúmen, ec: células epiteliais, gv: vesícula germinal, I-ovócito I, II- ovócito II, III- ovócito III, IV- ovócito IV, V- ovócito V (SAITO et al., 2005).

A proliferação de células da parede ovariana em que os ovócitos estão se desenvolvendo leva a formação do pedicelo que é responsável por fixar os diferentes ovócitos à parede do ovário. Através de estudos ultraestruturais, foi possível observar especializações da membrana plasmática que apresentam invaginações aumentando o contato na interface entre as células do pedicelo e os ovócitos, o que permite afirmar que o pedicelo está envolvido na produção de elementos que são incorporados aos ovócitos, atuando de maneira semelhante as células nutridoras (SAITO et al., 2005).

Após a maturação, esses ovócitos são liberados no lúmen para que, posteriormente sejam expulsos para o exterior. A ovogênese se processa da região distal, onde estão os ovócitos menos desenvolvidos para a proximal, onde estão os ovócitos mais

desenvolvidos. (SAITO et al., 2005; SONESHINE et al., 2013). Apesar de possuírem desenvolvimento simultâneo a vitelogênese ocorre em tempos diferentes, o que permite que estas células não sejam fecundadas simultaneamente (DENARDI et al., 2004; OLIVEIRA et al., 2007). Os estágios dos ovócitos de *R. microplus* apresentam diferenças morfológicas significativas e são classificados levando em consideração os mesmos critérios utilizados para a classificação de *Amblyomma cajennense* por Denardi et al. (2004): aparência do citoplasma, presença de grânulos de vitelo, presença de vesícula germinal e presença de cório. Assim, é possível a visualização de seis estágios bem definidos de ovócitos em *R. microplus* (SAITO et al., 2005).

Análises histoquímicas dos ovócitos de *R. microplus* mostraram que a membrana plasmática, assim como o citoplasma são constituídos por proteínas, principalmente a vitelogenina, que é a proteína encontrada em maior quantidade em ovos de carrapatos e constitui 11% da proteína total na hemolinfa (SONESHINE, 2013). A medida que as fêmeas realizam o repasto sanguíneo, a concentração de vitelogenina aumenta na hemolinfa e sua regulação ocorre através da absorção pelos ovócitos (SEIXAS et al., 2010). A distribuição da vitelogenina não é uniforme dentro dos ovócitos, assim, em ovócitos I e II, as proteínas são homogeneamente distribuídas, em ovócitos III aparecem como grânulos finos, em ovócitos IV, grânulos mais densos e em ovócitos V aparecem como grânulos vitelogênicos. Também foi possível observar cório presente nos ovócitos III a VI (SAITO et al., 2005; SOUSA et al., 2013; CAMARGO-MATHIAS, 2013; PRADO-OCHOA et al., 2014). Em *R. microplus*, assim como em outros ixodídeos, a vitelogênese se dá pela produção endógena de proteínas e lipídeos até o alcance do estágio III de desenvolvimento e, posteriormente, elementos provenientes da hemolinfa são incorporados através de pinocitose, já que é possível observar pequenas vesículas de vitelo na periferia dos ovócitos e a presença de grânulos densos centralmente. Nos ovócitos em estágio de desenvolvimento V, ocorre diminuição das invaginações da membrana plasmática em tamanho e número até o seu completo desaparecimento, confirmando a participação dessas estruturas na incorporação de elementos exógenos para produção de vitelo no interior dos ovócitos (DENARDI et al., 2004; SAITO et al., 2005; OLIVEIRA et al., 2007; SOUSA et al., 2013; CAMARGO-MATHIAS, 2013).

Os estudos histológicos e ultraestruturais auxiliam no entendimento de possíveis mecanismos de ação dos fármacos e, consequentemente, no desenvolvimento de medidas de controle do carrapato bovino. Devido à seleção de populações de carrapatos resistentes aos produtos carrapaticidas, tem-se buscado os compostos naturais como estratégias de controle alternativo.

2.2 Controle do carrapato bovino

Entre os métodos utilizados para o controle do carrapato bovino estão, o químico, com produtos injetáveis ou tópicos (ANDREOTTI et al., 2012; PRADO-OCHOA et al., 2014), o uso de predadores e parasitos (CAMARGO et al., 2016), ferormônios (DEBRUYNE e GUERIN, 1994), a rotação de pastagens e o cruzamento genético do hospedeiro para aumentar a resistência a infestação pelo parasito (LI et al., 2005; AGUILAR-TIPACAMÚ et al., 2011).

O fator que deve determinar a aplicação de um produto carrapaticida é a quantidade elevada de fêmeas parasitando os animais. Os produtos são aplicados várias vezes durante o ano e há troca frequente e indiscriminada do medicamento, não havendo controle estratégico do parasito. Isso permite a seleção acelerada dos indivíduos tolerantes aos carrapaticidas, tornando a população resistente (FURLONG, 2005). A resistência comprovada é irreversível e uso de um mesmo princípio ativo não terá efeito para controle futuro. A resistência surge através de mutações genéticas em uma população de carrapatos que podem causar modificações no local de ação, aumento do metabolismo ou sequestro do acaricida, e ainda redução da capacidade de penetração do acaricida através das camadas protetoras do carrapato (GUERRERO et al., 2012).

2.3 Métodos alternativos de controle

Os pesquisadores têm dado importância ao uso de plantas com atividade terapêutica. O nordeste brasileiro é caracterizado por apresentar clima semi-árido predominante e possuir como principal bioma a vegetação caatinga. A família Verbenaceae está distribuída nas regiões tropicais e subtropicais com aproximadamente 175 gêneros e 2.300 espécies, entre elas estão: *Lippia sidoides* e *L. gracilis*. O gênero *Lippia* é composto por arbustos, ervas e pequenas árvores, geralmente aromáticas e presentes na América Central e do Sul, além da África Central (LORENZI e MATOS, 2008).

2.3.1 *Lippia sidoides*

L. sidoides conhecida popularmente por “alecrim-pimenta” no Brasil, é uma espécie vegetal aromática que apresenta um óleo essencial rico em timol ou carvacrol, os quais são destacados pelas propriedades bactericida, fungicida, moluscicida, inseticida e acaricida (MATOS, 2000; MATOS, 2002; CARVALHO et al., 2003; CAVALCANTI et al, 2004; BOTELHO et al., 2007). Apesar da ocorrência desta espécie vegetal na caatinga do

Nordeste Brasileiro, passou a ser cultivada em vários estados brasileiros devido a suas características fitoterápicas (MATOS e OLIVEIRA, 1998).

Os óleos essenciais de *L. sidoides* apresentam diversidade química e variabilidade intraespecífica, podendo estar relacionadas às diferentes atividades farmacológicas do óleo. No nordeste brasileiro, o timol é o composto encontrado em maior concentração (FONTENELLE et al., 2007; CAVALCANTI et al., 2010; MARCO et al., 2012; FARIAS-JÚNIOR et al., 2012; MOTA et al., 2012; LIMA et al., 2013; VERAS et al., 2014). Em Minas Gerais, o composto majoritário identificado foi o carvacrol (LIMA et al., 2011; GUIMARÃES et al., 2014), 1,8-cineol (MORAIS et al. 2012; GUIMARÃES et al., 2014), isoborneol e acetato de bornila (MORAIS et al., 2012).

L. sidoides apresentou CL_{50} de 63 ppm quando testada contra larvas de *Aedes aegypti*. Essa atividade foi atribuída ao timol, constituinte de 80% da concentração do óleo testado (CAVALCANTI et al, 2004). Lima et al. (2011) detectaram atividade inseticida do óleo essencial e seus principais compostos químicos carvacrol, 1,8-cineol e timol contra o coleóptero *Tenebrio molitor.* O timol foi o composto que apresentou menor toxicidade. Nesse estudo, também foi observado efeito sinérgico entre misturas binárias e terciárias desses compostos, em que foi verificado que o timol foi o principal sinergista nas misturas binárias, aumentando a toxicidade do carvacrol e 1,8-cineol.

Diferentes genótipos de *L. sidoides* e seus compostos majoritários exibiram uma potente atividade acaricida contra o ácaro-aranha *Tetranychus urticae*. Dos quatro genótipos testados, apenas um apresentava concentração de carvacrol superior à de timol, entretanto, não houve diferença estatística significativa entre as CL_{50} dos acessos que variaram entre 0,011 a 0,014. Entre os quimiotipos testados o timol foi o mais eficaz (CL_{50}= 0,001 µ/L) (CAVALCANTI et al., 2010). Gomes et al. (2012) observaram efeito carrapaticida dessa planta contra larvas de *R. microplus* e *Dermacentor nitens* com CL_{50} de 11,1 e 5,6 µL/mL, respectivamente. Testes do óleo essencial de *L. sidoides* contra larvas e ninfas de *R. microplus* e *A. cajennense* também mostraram eficiência com CL_{90} entre 11,6 e 18,5 mg/ml (GOMES et al., 2014). Monteiro et al. (2014) avaliaram o efeito da associação entre o óleo de *L. sidoides* e nematoides entomopatogênicos (*Heterorhabditis bacteriophora* HP88 e *Heterorhabditis indica* LPP1) na eficiência reprodutiva de fêmeas de *R. microplus* e obtiveram porcentagem de controle entre 95 e 100%.

2.3.2 *Lippia gracilis*

L. gracilis, popularmente conhecida como “alecrim-de-tabuleiro” ou “alecrim-da-chapada”, é um arbusto caducifólio, típico da região semi-árida do nordeste brasileiro, capaz de alcançar até dois metros de altura (LORENZI e MATOS, 2008). As folhas e flores de *L.*

gracilis são as principais fontes de extração de óleo essencial, o qual apresenta atividade antimicrobiana (ALBUQUERQUE et al., 2006; NETO et al., 2010), anti-inflamatória (GUILHON et al., 2011), analgésica (MENDES et al., 2010), inseticida (SILVA et al., 2008) e carrapaticida (CRUZ et al., 2013; COSTA-JÚNIOR et al., 2016). Estudos relataram que a composição química de plantas do gênero *Lippia* são limitados em caracterizar os componentes voláteis, entretanto, outros componentes já foram citados como flavonoides, naftoquinonas e iridoides.

O óleo essencial de *L. gracilis* possui variações em sua composição química que podem ser influenciados pelo tempo de colheita, diversidade genética e localização geográfica (SANTOS et al., 2015; SANTOS et al. 2016), o que pode ser observado em diferentes estados do Brasil. No Ceará, o óleo essencial extraído apresentou como componentes majoritários o timol (30,6%), carvacrol (11,8%) e ρ-cimeno (10,7%) (LEMOS et al., 1992). No estado do Piauí, foram encontrados carvacrol (47,7%), ρ-cimeno (19,2%), metil timol (6,2%) e timol (4,8%) como principais componentes (MATOS et al., 1999). Em Sergipe, os principais compostos encontrados foram timol (24%), ρ-cimeno (15,9%), metil timol (11,7%) e γ-terpineno (10,9%) (TELES et al., 2010). No Maranhão, o timol foi o composto obtido em maior quantidade, constituindo 73,5% do óleo extraído de folhas e 70,1% na extração de hastes finas (FRANCO et al., 2014). Cruz et al. (2013) avaliaram quatro genótipos diferentes de *L. gracilis*, sendo 3 do estado do Sergipe (LGRA-106, LGRA-108 e LGRA-109) e 1 da Bahia (LGRA-201) e observaram diferenças na composição química entre os mesmos, tendo o carvacrol em maior concentração na maioria dos genótipos estudados: LGRA-106 (59,3% de timol), LGRA-108 (47,1% de carvacrol), LGRA-109 (49,0% de carvacrol) e LGRA-201(35,3% de carvacrol).

Silva et al. (2008) observaram efeito larvicida contra o mosquito *A. aegypti*, com CL_{50} de 1,99 ppm de óleo essencial. O carvacrol foi o composto majoritário e incriminado como composto ativo, sua ação também foi observada com CL_{50} 2,10 ppm. Dias et al. (2015), também avaliaram o efeito inseticida do óleo essencial de *L. gracilis* (CL_{50}= 282mg/L), entretanto, não encontraram carvacrol entre os componentes químicos, tendo como composto majoritário o 1,8-cineol (56,2%).

Diferentes genótipos de *L. gracilis* (LGRA-106, LGRA-108, LGRA-109 e LGRA-201) também apresentaram diferenças em atividades contra o carrapato *R. microplus*. O genótipo LGRA-201 foi o mais eficiente contra larvas do carrapato, enquanto o genótipo LGRA-106 obteve maior eficiência contra fêmeas de *R. microplus*, sugerindo o efeito sinérgico entre os componentes majoritários timol e carvacrol (CRUZ et al., 2013). Chagas et al. (2016) também observaram efeito carrapaticida contra larvas de *R. microplus* (CL_{50}= 3,2 mg/ml) entretanto, em fêmeas, a mortalidade observada não foi suficiente para determinar a CL_{50}. As atividades dos óleos essenciais de *L gracilis* e seus componentes

principais, timol e carvacrol, foram avaliadas em cepas de *R. microplus* resistentes e sensíveis a organofosforados. As ações de *L. gracilis* e carvacrol foram maiores em cepas resistentes enquanto o timol apresentou similar toxicidade em ambas as cepas (COSTA-JÚNIOR et al., 2016).

A busca por novas medidas de controle, em especial o uso de produtos naturais tem sido alvo de pesquisas nos últimos anos. Os óleos essenciais de *L. sidoides* e *L. gracilis* têm apresentado atividade carrapaticida, portanto, conhecer os seus mecanismos de ação é relevante para o desenvolvimento de novos produtos. Análises morfológicas podem auxiliar na elucidação dos possíveis mecanismos em que esses óleos podem estar envolvidos.

REFERÊNCIAS

AGUILAR-TIPACAMÚ, G.; ROSARIO-CRUZ, R.; MILLER, R.J.; GUERRERO, F.D.; RODRIGUEZ-VIVAS, R.I.; GARCÍA-VÁZQUEZ, Z. Phenotype changes inherited by crossing pyrethroid susceptible and resistant genotypes from the cattle tick *Riphicephalus* (*Boophilus*) *microplus*. **Experimental and Applied Acarology**, v.54, n.3, p.301-311, 2011.

ALBUQUERQUE, C. C.; CAMARA, T. R.; MARIANO, R. L. R.; WILLADINO, L.; JÚNIOR, C. M.; ULISSES, C. Antimicrobial Action of the Essential Oil of *Lippia gracilis* Schauer. Brazilian **Archives of Biology and Technology**, v.49, p.527-535, 2006.

ANDREOTTI, R.; CUNHA, R.C.; SOARES, M.A.; GUERRERO, F.D.; LEITE, F.P.L.; PÉREZ DE LEÓN, A.A. Protective immunity against tick infestation in cattle vaccinated with recombinant trypsin inhibitor of *Rhipicephalus microplus*. **Vaccine**, v.30, p. 6678– 6685, 2012.

ARNOSTI, A.; BRIENZA, P.D.; FURQUIM, K.C.; CHIERICE, G.O.; BECHARA, G.H.; CAL-LIGARIS, I.B.; CAMARGO-MATHIAS, M.I. Effects of ricinoleic acidesters from castor oil of *Ricinus communis* on the vitellogenesis of *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) ticks. **Experimental Parasitology**, v.127, p.575–580, 2011.

BOTELHO, M.A.; NOGUEIRA, N.A.P.; BASTOS, G.M.,; FONSECA, S.G.C.; LEMOS, .L.G.; MATOS, F.J.A.; MONTENEGRO, D.; HEUKELBACH, J.; R.A.O, V.S.; BRITO, G.A.C. Antimicrobial activity of the essential oil from *Lippia sidoides*, carvacrol and tymol against oral pathogens. **Brazilian Journal of Medical Biology Research**, v.40, 349–356, 2007.

CABARET, J.; BOUILHOL,M.; MAGE,C. Managing helminths of ruminants in organic farming. **Veterinary Research**, v.33, p.625–640, 2002.

CAMARGO, M.G.; NOGUEIRA, M.R.; MARCIANO, A.F.; PERINOTTO, W.M.; COUTINHO-RODRIGUES, C.J.; SCOTT, F.B.; ANGELO, I.C.; PRATA, M.C.; BITTENCOURT, V.R. Metarhizium anisopliae for controlling *Rhipicephalus microplus* ticks under field conditions. **Veterinary Parasitology**, v.223, p.38-42, 2016.

CAMARGO-MATHIAS, M.I. Guia básico de morfologia interna de carrapatos ixodídeos. Editora UNESP, p.84-107, 2013.

CARVALHO, A.F.U.; MELO, V.M.M.; CRAVEIRO, A.A.; MACHADO, M.I.L.; BANTIN, M.B.; RABELO, E.F. Larvicidal activity of the essential oil from *Lippia sidoides* Cham. *Against Aedes aegypti* Linn. **Memórias do Instituto Oswaldo Cruz**, v.98, p.569–571, 2003.

CAVALCANTI, E.S.; MORAIS, S.M.; LIMA, M.A.; SANTANA, E.W. Larvicidal activity of essential oils from Brazilian plants against *Aedes aegypti* L. **Memórias do Instituto Oswaldo Cruz**, v.99, n.5, p.541-4, 2004.

CAVALCANTI, V. O.; Atividade micobacteriana do óleo essencial de Lippia gracilis Schauer. Dissertação. Recife, UFPE, 2006.

CAVALCANTI, S.C.H.; NICULAU, E.S.; BLANK, A.F.; CÂMARA, C.A.G.; ARAÚJO, I.N.; ALVES, P.B. Composition and acaricidal activity of *Lippia sidoides* essential oil against two-spotted spider mite (*Tetranychus urticae* Koch). **Bioresource Technology**, v.101, p. 829–832, 2010.

CHAGAS, A.C.S.; OLIVEIRA, M.C.S.; GIGLIOTI, R.; SANTANA, R.C.M.; BIZZO, H.R.; GAMA, P.E.; CHAVES, F.C.M. 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.

COSTA-JÚNIOR, L.M.; MILLER, R.J.; ALVES, P.B.; BLANK, A.F.; LI, A.Y.; PÉREZ DE LÉON, A.A.; 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.; COSTA-JÚNIOR, L.M.; PINTO, J.A.; SANTOS, D.A.; ARAUJO, S.A.; ARRIGONI-BLANK, M.F.; BACCI, L.; ALVES, P.B.; CAVALCANTI, S.C.; BLANK, A.F. Acaricidal activity of *Lippia gracilis* essential oil and its major constituents on the tick *Rhipicephalus* (*Boophilus*) *microplus*. **Veterinary Parasitology**, v.195, n.1-2, p.198-202, 2013.

DEBRUYNE, M.; GUERIN, P.M. Isolation of 2,6-Dichlorophenol from the cattle tick *Boophilus microplus*. Receptor cell responses but no evidence for a behavioral response. **Journal of Insect Physiology**, v. 40, p. 143-154. 1994.

DENARDI, S.E.; BECHARA, G.H.; OLIVEIRA, P.R.; NUNES, E.T.; SAITO, K.C.; CAMARGO-MATHIAS, M.I. Morphological characterization of the ovary and vitellogenesis dynamics in the *Amblyomma cajennense* (Acari: Ixodidae). **Veterinary Parasitology,** v. 125, p.379-395, 2004.

DEWITT, S.H. Molecular Diversity Strategies. **Pharmaceuticals News,** v.1, p.11-14, 1994.

DIAS, C.N.; ALVES, L.P.L.; RODRIGUES, K.A.F.; BRITO, M.C.A.; ROSA, C.S.; AMARAL, F.M.M.; MONTEIRO, O.S.; ANDRADE, E.H.A.; MAIA, J.G.S.; MORAES, D.F.C. Chemical Composition and Larvicidal Activity of Essential Oils Extracted from Brazilian Legal Amazon Plants against *Aedes aegypti* L. (Diptera: Culicidae). **Evidence-Based Complementary and Alternative Medicine**, 2015.

FARIAS-JÚNIOR, P.A.; RIOS, M.C.; MOURA, T.A.; ALMEIDA, R.P.; BLANK, A.F.; FERNANDES, R.P.M.; SCHER, R. Leishmanicidal activity of carvacrol-rich essencial oil drom *Lippia sidoides* Cham. **Biologycal Research,** v.45, p.399-402, 2012.

FONTENELLE, R.O.S.; MORAIS, S.M.; BRITO, E.H.S. KERNTOPF, M.R.; BRILHANTE, R.S.N.; CORDEIRO, R. A.; TOME, A. R.; QUEIROZ, M.G.R.; NASCIMENTO, N.R.F.; SIDRIM, J.J.C.; ROCHA, M.F.G. Chemical composition, toxicological aspects and antifungal activity of essential oil from *Lippia sidoides* Cham. **Journal of Antimicrobial Chemotherapy**, v.59, p.934-940, 2007.

FRANCO, C.S.; RIBEIRO, A.F.; CARVALHO, N.C.C.; MONTEIRO, O.S.; SILVA, J.K.R.; ANDRADE, E.H.; MAIA, J.G.S. Composition and antioxidante and antifungical activities of essential oil from *Lippia gracilis* Schauer. **African Journal of Biotecnology**, v.13, n.30, p.3107-3113, 2014.

FURLONG, J. Carapato: problemas e soluções. Juiz de Fora: Embrapa Gado de Leite, 65p., 2005.

GOMES, A. Controle do carrapato do boi: um problema para quem cria raças européias. Gado de Corte Divulga, Campo Grande: Embrapa, n.31, ago.1998.

GOMES, G.A.; OLIVEIRA-MONTEIRO, C.M.; JULIÃO, L.S.; MATURANO, R.; SENRA, T.O.S; ZERINGOTA, V.; CALMON, F.; MATOS, R.S.; DAEMON, E.; CARVALHO, G.C. Acaricidal activity of essential oil from *Lippia sidoides* on unengorged larvae and nymphs of

Rhipicephalus sanguineus (Acari: Ixodidae) and *Amblyomma cajennense* (Acari: Ixodidae). **Experimental Parasitology,** v.137, p.41-45, 2014.

GOMES, G.A., OLIVEIRA-MONTEIRO, C.M.; SENRA, T.O.S.; ZERINGOTA, V.; CALMON, F.; MATOS, R.S.; DAEMON, E.; GOIS, R.W.S.; SANTIAGO, G.M.P.; CARAVALHO, G.C. 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**, v.111, p.2423-2430, 2012.

GRILLO, J.M. El problema de la resistencia a los acaricidas en los programas de control de la garrapata. **Boletín de la Oficina Sanitaria Panamericana**., v.21, n. 3, p. 246-250, 1976.

GRISI, L.; LEITE, R.C.; MARTINS, J.R.S.; BARROS, A.T.M.; ANDREOTTI, R.; CANÇADO, P.H.D.; PÉREZ DE LEÓN, A.A.; PEREIRA, J.B.; VILLELA, H. S. Reassessment of the potential economic impact of cattle parasites in Brazil. **Brazialian Journal of Veterinary Parasitology**, v.23, n.2, p.150-156, 2014.

GUERRERO, F.D.; LOVIS, L.; MARTINS, J.R. Acaricide resistance mechanisms in *Rhipicephalus (Boophilus) microplus*. **Brazialian Journal of Veterinary Parasitology**, v. 21, n. 1, p. 1-6, 2012.

GUILHON, C. C.; RAYMUNDO, L. J. R. P.; ALVIANO, D. S.; ARIE F. BLANK, A. F.; ARRIGONI-BLANK, M. F.; MATHEUS, M. E.; CAVALCANTI, S. C. H.; ALVIANO, C. S.; FERNANDES, P. D. Characterization of the anti-inflammatory and antinociceptive activities and the mechanism of the action of *Lippia gracilis* essential oil. **Journal of Ethnopharmacology**, v.135, p.406-413, 2011.

GUIMARÃES, L.G.; CARDOSO, M.G.; SOUZA, R.M.; ZACARONI, A.B.; SANTOS, G.R. Essential oil of Lippia sidoides native to Minas Gerais: Composition, secretory structures and antibacterial activity. **Revista Ciência Agronômica**, v. 45, n. 2, p. 267-275, 2014.

IBGE, Diretoria de Pesquisas, Coordenação de Agropecuária, Pesquisa da Pecuária Municipal 2014-2015.

LA VEGA, R.; DÍAZ, G.; GALÁN, M.; FERNÁNDEZ, C. Anatomy and histology of the female reproductive system of *Boophilus microplus* (acari: ixodidae). **Revista de Salud Animal**, v. 34, n.1, p.1-10, 2012.

LEMOS, E.L.G.; MONTE, F.J.Q.; MATOS, F.J.A.; ALENCAR, J.W.; CRAVEIRO, A.A.; BARBOSA, R.C.S.B.; LIMA, E.O. Chemical composition and antimicrobial activity of essential oils from Brazilian plants. **Fitoterapia**, v.63, p.266-268, 1992.

LI, A.Y.; DAVEY, R.B.; MILLER, R.J.; GEORGE, J.E. Mode of inheritance of amitraz resistance in a Brazilian strain of the southern cattle tick, *Boophilus microplus* (Acari: Ixodidae). **Experimantal and Applied Acarology**, v.37, n.3-4, p.183-98, 2005.

LIMA, G. P. G.; SOUZA, T. M.; FREIRE, G. P.; FARIAS, D. F.; CUNHA, A. P.; RICARDO, N. M. P.S.; MORAIS, S. M.; CARVALHO, A. F. U. Further insecticidal activities of essential oils from *Lippia sidoides* and *Croton* species against *Aedes aegypti* L. **Parasitology Research**, v.112, p.1953–1958, 2013.

LIMA, R.K.; CARDOSO, M.G.; MORAES, J.C.; CARVALHO, S.M.; RODRIGUES, V.G.; GUIMARÃES, L.G.L. Chemical composition and fumigant effect of essential oil of Lippia sidoides cham. and monoterpenes against Tenebrio Molitor (L.) (Coleoptera: Tenebrionidae). **Ciência e Agrotecnologia**., v.35, n.4, p.664-671, 2011.

LIPA, J.J. Microbial control of mites and ticks. In: BURGES, H.D., HUSSEY, N.W. (Eds). Microbial control of insects and mites. 2°edição. London: Academic, p. 357-374, 1971.

LORENZI, H.; MATOS, F.J.A. Plantas medicinais no Brasil: nativas e exóticas. 2.ed. Nova Odessa: **Plantarum**, 544p, 2008.

MARCO, C. A. et al. Chemical composition and allelopathyc activity of essential oil of Lippia sidoides Cham. Chilean **Journal of Agricultural Research**, v. 72, n. 1, p. 157-160, 2012.

MATOS, F.J.A. Farmácias vivas: sistema de utilização de plantas medicinais projetado para pequenas comunidades. 4. ed. rev. ampliada. Fortaleza: UFC, p.267, 2002.

MATOS, F.J.A. Plantas medicinais – guia de seleção e emprego de plantas usadas em fitoterapia no nordeste do Brasil. Fortaleza: Imp. Universitária, UFC, p. 344, 2000.

MATOS, F.J.A.; MACHADO, M.I.: CRAVEIRO, A.A.; ALENCAR, J.W. SILVA, M.G. Medicinal plants northeast Brazil containing thymol and carvacrol - *Lippia sidoides* Cham. and *L. gracillis* H. B. K (Verbenaceae). **Journal of Essential Oil Research**, v.11, p.666-8,1999.

MATOS, F.J.A.; OLIVEIRA, F. *Lippia sidoides* Cham. – Farmacognosia, química e farmacologia. **Revista Brasileira de Farmácia**, v. 79, p. 84-7, 1998.

MORAIS, S. R.; OLIVEIRA, T.L.; BARA, M.T.; CONCEIÇÃO, E.C.; REZENDE, M.H.; FERRI, P.H.; PAULA, J.R. Chemical constituents of essential oil from *Lippia sidoides* Cham. (Verbenaceae) leaves grown in Hidrolândia, Goiás, Brazil. **International Journal of Analytical Chemistry**, v.2012, p.1-4, 2012.

MOTA, M.L.; LOBO, L.T.; COSTA, J.M.; COSTA, L.S.; ROCHA, H.A.; ROCHA E SILVA, L.F.; POHLIT, A.M.; NETO, V.F. In vitro and in vivo antimalarial activity of essential oils and chemical components from three medicinal plants found in Northeastern Brazil. **Planta Medica**, v. 78, p. 658-664, 2012.

MENDES, S. S.; BOMFIM, R. R.; JESUS, H. C. R.; ALVES, P. B.; BLANK, A. F.; ESTEVAM, C. S.; ANTONIOLLI, A. R.; THOMAZZI, S. M. Evaluation of the analgesic and anti-inflammatory effects of the essential oil of *Lippia gracilis* leaves. **Journal of Ethnopharmacology**, v.129, p.391-397, 2010.

MONTEIRO, C.M.A.; ARAÚJO, L.X.; GOMES, G.A.; SENRA, T.O.S.; CALMON, F.; DAEMON, E.; CARVALHO, M.G.; BITTENCOURT, V.R.E.P.; FURLONG, J.; PRATA, M.C.A. Entomopathogenic nematodes associated with essential oil of Lippia sidoides for control of Rhipicephalus microplus (Acari: Ixodidae). **Parasitolology Research**, v.113, p.189–195, 2014.

NETO, R. M.; MATOS, F. J. de A.; ANDRADE, V. S. A.; MELO, M. C. N.; CARVALHO, C. B. M.; GUIMARÃES, S. B.; PESSOA, O. D. L.; SILVA, S. L.;.SILVA, S.F.R; VASCONCELOS, P.R.L. The essential oil from *Lippia gracilis* Schauer, Verbenaceae, in diabetic rats. **Revista Brasileira de Farmacognosia**, v.20 n.2, p.261-266, 2010.

OLIVEIRA, P.R.; BECHARA, G.H.; DENARDI, S.E.; NUNES, E.T.; CAMARGO-MATHIAS, M.I. Morphological characterization of the ovary and oocytes vitellogenesis of the tick *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae). **Experimental Parasitology**, v.110, n.2, p. 146-156, 2005.

OLIVEIRA, P.R.; CAMARGO-MATHIAS, M.I.; BECHARA, G.H.B. Vitellogenesis in the tick *Amblyomma triste* (Koch, 1844) (Acari: Ixodidae) Role for pedicel cells. **Veterinary Parasitology**, v.143, p.134–139, 2007.

OLIVEIRA, P.R.; BECHARA, G.H.B.; CAMARGO-MATHIAS, M.I. Evaluation of cytotoxic effects of fipronil on ovaries of semi-engorged *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) tick female. **Food and Chemical Toxicology**, v.46, p.2459–2465, 2008.

PEREIRA, M.C.; LABRUNA, M.B.; SZABÓ, M.P.J.; KLAFKE, G.M. (Org.). *Rhipicephalus* (*Boophilus*) *microplus*: Biologia, Controle e Resistência. 1 ed. São Paulo, SP: MedVet Livros, v. 1, 169p., 2008.

PRADO-OCHOA, M.G.; RAMÍREZ-NOGUERA, P.; DÍAZ-TORRES, R.; GARRIDO-FARIÑA, G.I.; VÁZQUEZ-VALADEZ, V.H.; VELÁZQUEZ-SÁNCHEZ, A.M.; MUÑOZ-GUZMÁN, M.A.; ANGELES, E.; ALBA-HURTADO, F. The action of two ethyl carbamates on acetylcholinesterase and reproductive organs of *Rhipicephalus microplus*. **Veterinary Parasitology**, v.199, n.3-4, p.215-24, 2014.

PRANCE, G.T. What is ethnobotany today? **Journal of Ethnopharmacology** v.32, p.209-16, 1991.

RECK**,** JOSÉ; MARKS, FERNANDA S.; RODRIGUES, ROGÉRIO O.; SOUZA, UGO A.; WEBSTER, ANELISE; LEITE, ROMÁRIO C.; GONZALES, JOÃO CARLOS; KLAFKE, GUILHERME M.; MARTINS, JOÃO RICARDO. Does *Rhipicephalus microplus* tick infestation increase the risk for myiasis caused by *Cochliomyia hominivorax* in cattle**?. Preventive Veterinary Medicine**, v. 113, p. 59-62, 2014.

ROMA, G.C.; FURQUIM, K.C.; BECHARA, G.H.; CAMARGO-MATHIAS, M.I. Permethrin-induced morphological changes in oocytes of *Rhipicephalus sanguineus* (Acari: Ixodidae) semi-engorged females. **Food and Chemical Toxicology**, v.48, p.825–830, 2010.

SAITO, K.C., BECHARA, G.H., OLIVEIRA, P.R., NUNES, E.T., DENARDI, S.E., CAMARGO-MATHIAS, M.I. Morphological, histological, and ultrastructural studies of the ovary of the tick *Boophilus microplus* (Canestrini, 1887) (Acari: Ixodidae). **Veterinary Parasitology**, v.129, 299–311, 2005.

SANTOS, C.P.; PINHEIRO, J.B.; ZUCCHI, M.I.; BAJAY, M.M.; CAMPOS, J.B.; ARRIGONI-BLANK, M.F.; PINTO, J.A.O.; BLANK, A.F. Genetic diversity of Lippia sidoides Cham. and *L. gracilis* Schauer germplasm. **Genetics and Molecular Research**, v.15, n.3, 2016.

SANTOS, C.P.; PINTO, J.A.O.; SANTOS, C.A.; CRUZ, E.M.O.; ARRIGONI-BLANK, M.F.; ANDRADE, T.M.; SANTOS, D.A.; ALVES, P.B.; BLANK, A.F. Harvest time and geographical origin affect the essential oil of *Lippia gracilis* Schauer. **Industrial and Crops Products**, v.79, p. 205–210, 2015.

SEIXAS, A.; OLDIGES, D.P.; VAZ, I.S.J.R.; TERMIGNONI, C. Endocrinologia e controle da vitelogênese em carrapatos. **Acta Science Veterinary**, v.38, n.2, p.95-111, 2010.

SILVA, W.J.; DÓRIA, G.A.A.; MARIA, R.T.; NUNES, R.S.; CARVALHO, G.A.; BLANK, A.F.; ALVES, P.B.; MARÇAL, R.M.; CAVALCANTI, S.C.H. Effects of essential oils on *Aedes aegypti* larvae: Alternatives to environmentally safe insecticides. **Bioresource Technology**, v.99, p.3251-3255, 2008.

SONENSHINE, D.E. Biology of ticks, v. 1. 2Ed. Oxford University, New York, pp. 280–303, 2013.

SOUSA, L.A.; ROCHA, T.L.; SABÓIA-MORAIS, S.M.; BORGES, L.M. Ovary histology and quantification of hemolymph proteins of *Rhipicephalus* (*Boophilus*) *microplus* treated with *Melia azedarach.* **Brazilian Journal of Veterinary Parasitology**, v.22, n.3, p.339-345, 2013.

TELES, T.V.; BOMFIM, R.R.; ALVES, P.B.; BLANK, A.F.; JESUS, H.C.R.; QUINTANS-JR, L.J.; SERAFINI, M.R.; BONJARDIM, L.R.; ARAÚJO, A.A.S. Composition and evaluation of the lethality of *Lippia gracilis* essential oil to adults of *Biomphalaria glabrata* and larvae of *Artemia salina*. **African Journal of Biotechnology**, v.9, n.22, 2010.

VERAS, H.N.H.; RODRIGUES, F.F.G.; BOTELHO, M.A.; MENEZES, I.R.A.; COUTINHO, H.D.M.; COSTA, J.G.M. Antimicrobial Effect of *Lippia sidoides* and Thymol on *Enterococcus faecalis* Biofilm of the Bacterium Isolated from Root Canals. **The Scientific World Journal**, v.2014, 2014.

3. RESULTADOS

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

- ✓ Capítulo 1: Biological activities of *Lippia gracilis* essential oils, a review.
  Situação: Artigo a ser submetido no periódico *BMC Complementary and Alternative Medicine*.

- ✓ Capítulo 2: Assessment of different *Lippia sidoides* genotypes regarding their acaricidal activity against *Rhipicephalus* (*Boophilus*) *microplus*.
  Situação: Publicado no periódico *Brazilian Journal of Veterinary Parasitology*, doi: http://dx.doi.org/10.1590/S1984-29612016087.

- ✓ Capítulo 3: Histopathology of ovary of *Riphicephalus microplus* female treated with *Lippia sidoides* and *Lippia gracilis* essential oils and its main components.
  Situação: Artigo ser submetido na *Industrial Crops and Products*.

3.1 Capítulo 1

Chemical composition and biological activities of *Lippia gracilis* essential oils, a review.

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Abstract

Brazil is among the countries that sought to regulate the production and use of natural products, as it features extreme weather conditions which enable the adaptation of many plants species. Many Verbenaceae species are endemic and have particular morphological adaptations including *Lippia gracilis*. Its leaves and flowers are the main sources of essential oil extraction. The chemical composition and pharmacological activity of *L. gracilis* were revised and compared. The main activities were described antimicrobial, acaricidal, insecticidal, cytotoxic, anti-inflammatory and antinociceptive. The chemical composition of *L. gracilis* essential oil varies in different parts of Brazil and the major components were described thymol, carvacrol and ρ-cimene, wich were attributed as active components of *L. gracilis* plant by the authors. Despite published research, the mechanisms of action of *L. gracilis* specie are still unknow.

Keywords: *Lippia gracilis*, natural products, biological activities, essential oils

Background

Global trade in essential oils has increased exponentially since the 1990s. Brazil is among the countries that sought to regulate the production and use of natural products, as

it features extreme weather conditions which enable the adaptation of many plants species. Brazilian northeast presents a predominantly semi-arid climate and savanna vegetation as its main biome, with approximately 1.539 million square kilometers. The Verbenaceae family, distributed in tropical and subtropical regions, consists of approximately 175 genera and 2300 species, including *Lippia* spp. The *Lippia* genus consists of 200 species of shrubs, herbs and small trees, usually aromatic and present in Central and South America, as well as Central Africa [1]. Due to weather conditions in Brazil, many Verbenaceae species are endemic and have particular morphological adaptations [2].

Lippia gracilis, popularly known as "alecrim-do-tabuleiro" or "alecrim-da-chapada" is a deciduous shrub, typical of Northeastern Brazil semi-arid region, up to 2m high [1]. Natural products derived from *L. gracilis* can be obtained from extracts prepared with different solvents, which vary in polarity, for the preparation of fixed and essential oils. Essential oils are volatile compounds associated with the survival of the plant, assisting in defense against microorganisms and predators, as well as insect repellent. These compounds are sometimes made up of terpenes, and eventually phenylpropanoid and others molecules [3, 4].

L. gracilis leaves and flowers are the main sources of essential oil extraction, which has biological activities [5, 6, 7, 8, 9]. Due to its potent therapeutic effects, the knowledge of the ethnopharmacological properties, in addition to the chemical components and possible mechanisms of action is necessary. This review aims to discuss the chemical composition of *L. gracilis* and describe its main pharmacological activities described in literature.

Chemical composition

The availability of water and the harvest season of the different *L. gracilis* genotypes were analyzed and, despite different environmental conditions, was observed stability in the chemical composition of the oils studied, which showed little variation in yields and content [10]. The major components found in the plant reflect the biophysical and biological characteristics of its essential oils. An effect of the main chemotype may be dependent on its concentration when tested alone. However, one cannot rule out the effect of the components that are at lower levels, which may be modulating the effect of the main constituents [8].

Different genotypes of the same plant species may have different biological activities [11] The chemical composition of *L. gracilis* essential oil varies as the genetic diversity and geographical location. Thymol (figure 1a) and carvacrol (figure 1b) are usually the constituents found in greater quantity [12, 13, 14, 15]. Cruz et al. [16] evaluated four

different genotypes of *L. gracilis* and found differences in chemical composition between them, and carvacrol was present in higher concentrations in most genotypes: LGRA-106 (59.26% thymol), LGRA -108 (47.1% carvacrol), LGRA-109 (48.99% of carvacrol) and LGRA-201 (35.28% carvacrol).

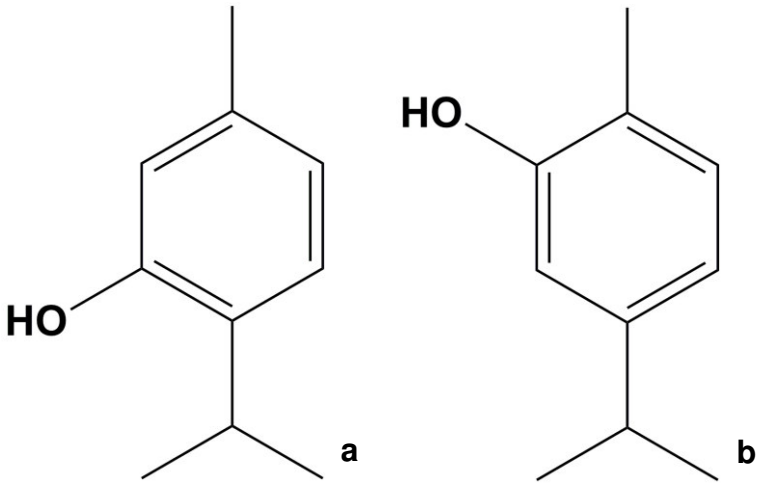

Figure 1: Chemical structure of thymol (a) and carvacrol (b).

Biological activity and action mechanism

Due high chemical variability of *L. gracilis* essential oil leaves, several studies have shown different mechanisms of action (Table 1).

1 Antibacterial activity

Studies using *L. gracilis* essential oil proved their antibacterial effect on antibiotic-resistant bacteria *Listeria monocytogenes*, *Staphylococcus aureus* and *S. epidermidis*. *S. aureus* was the most sensitive to oil activity with Minimal Inibitory Concentration (MIC) 9µl/mL [17]. The essential oil of *L. gracilis* had significant synergistic effect with antibiotics and decreased the minimum inhibitory concentration of aminoglycosides, demonstrating the ability to modulate bacterial resistance and act as an adjuvant against multiresistant microorganisms [18]. *L. gracilis* essential oil activity was evident against *Salmonella* spp. (MIC=30µL/mL) isolated from tilapia (*Oreochromis niloticus*)

[19]. Studies have demonstrated the activity of the main component of *L. gracilis* oil, carvacrol, acting as an inhibitor of flagellin synthesis, a protein responsible for the flagella formation in bacteria, and thus preventing bacterial motility [20, 21, 22]. Albuquerque et al [5] evaluated *L. gracilis* antimicrobial activity against fungal in plant tissue cultures in laboratories and endophytic bacteria isoled of helicons cultived *in vitro*. The inhibition percentage of fungi growth was between 89.4 and 100% and all the studied bacteria had inhibited growth in minimal inhibitory concentration (MIC = 420µL/L). The chemicals implicated as responsible for this effect were carvacrol (41.7%) and thymol (10.1%) [5]. Inhibition of fungal growth by *L. gracilis* action can be related to the activity of its carvacrol and thymol chemotypes that prevent the biosynthesis of ergosterol and thus destroy the integrity of the fungal membrane. This action was described in genus *Candida* [23].

2 Fungicide activity

Genotypes of *L. gracilis* essential oil rich in thymol showed antidermatophytic activity [24]. Franco et al. [15] showed antifungal effect of essential oil from leaves and thin stems against *Cladosporium sphaerospermum* and *C. cladosporoides* (MIC=5 µl/L) and thymol was fouded as main component. *L. gracilis* essential oil, at the three tested concentrations, was effective in controlling the fungus *Monosporasccus cannonballus*, showing inhibition percentages of 100% [25].

3 Inseticide activity

The larvicidal effect of *L. gracilis* essential oil was observed against *Aedes aegypti* with LC50 of 1.99 ppm and carvacrol was the active ingredient, since it was found in greater quantity and its LC50 was 2.10 ppm [6]. Dias et al. [26], also evaluated the insecticidal effect of *L. gracilis* essential oil (LC50 = 282mg/L), however, they have not found carvacrol between the chemical components, with the active chemotype 1,8-cineole (56.16%). The inhibitory action of terpenes in acethylcoline esterase of *Aedes albopictus* larvae was detected. Carvacrol presented high inhibitory activity and thymol presented moderate activity [27]. Acethylcoline esterase is an enzyme which hydrolyses the neurotransmitter acetylcholine preventing its excess in the synaptic cleft. Both components may be associated with accumulation of acetylcholine in the nervous system of *Aedes albopictus* nerve leading to hyperactivity and collapse of the nervous system.

4 Acaricide activity

Different genotypes of *L. gracilis* (LGRA-106, LGRA-108, LGRA-109 and LGRA-201) also showed differences in activity against *Rhipicephalus microplus* tick. LGRA-201 genotype was the most effective against tick larvae, while LGRA-106 genotype had a higher efficiency against *R. microplus* females, suggesting a synergistic effect between the main components thymol and carvacrol [16]. The activities of essential oils of *L. gracilis* and its main components, thymol and carvacrol, were evaluated in susceptible and organophosphate resistant strains of *R. microplus*. The *L. gracilis* actions and carvacrol were higher in resistant strains as thymol showed similar toxicity in both strains [28]. Chagas et al. [29] also observed acaricide effect against *R. microplus* larvae (LC50 = 3.21mg/mL) however, in females, the mortality rate was not enough to determine the LC 50. In our findings we observed the action of *L. gracilis*, thymol and carvacrol essential oils in vitellogenesis of *R. microplus* females, preventing the completion of their life cycle (unpublished data).

5 Cytotoxic activity

Cytotoxic effect in vitro and in vivo of *L. gracilis* essential oil in tumor cells was evaluated and it was observed G1 arrest in HepG2 cells and induction of DNA fragmentation without affecting cell membrane integrity [30]. This study was able to show apoptosis and a remarkable activation of caspase-3 and it was concluded that the oil induced caspase-dependent apoptosis in HepG2 cells (human hepatocellular carcinoma). *L. gracilis* showed cytotoxic effect against normal and tumor cell lines (3T3 and MRC-5, HeLa, MCF-7 and B16) with LC50 31.25 to 62.50. This effect was mainly attributed to thymol, found in high concentration in oil, capable of disrupt the cell membrane and increases its fluidity and permeability [31]. The cytotoxicity of essential oil was evaluated in line of human pulmonary mucoepidermoid carcinoma cells (NCI-H292) and it was observed low toxicity [32].

6 Anti-inflamatory and anti-nociceptive activity

L. gracilis essential oil was able to reduce carrageenin-induced paw edema in rats, by inhibiting one or more intracellular signaling pathways, including inflammatory mediators. Leukocyte migration induced by intraperitoneal injection of carrageenin was inhibited by essential oil, suggesting the inhibition of inflammatory mediators' synthesis, whose involvement in cell migration is well established [8]. Guilhon et al. [9] found a reduction in second phase of nociceptive response, indicating an anti-inflammatory effect of this plant, suggesting that the mechanism underlying the anti-inflammatory response produced would be due to the inhibition of mediator formation or function of the receptor.

Moreover, they confirmed inhibition of inflammatory responses by blocking the pathway of NO and the oil was able to attenuate the hyperalgesia by blocking the opioid and cholinergic systems. This work detected carvacrol in higher concentrations, characterizing it as responsible for the essential oil action mechanism. The role of thymol in modulating leukocyte influx into the carrageenan lesion area was also suggested in histological analysis, once there was a reduction in the inflammatory content of the treated samples. Furthermore, the residual perivascular inflammation was found in the score in the group treated with thymol [33].

7 Others activities

L. gracilis essential oils inhibited the growth of *Amoeba polyphaga* trophozoites, at a concentration of 40µg/mL. The major component, carvacrol, was capable of eliminating 100% of trophozoites in the concentration of 75µg/mL [32].

Melo et al. [24] evaluated anti-*Leishmania* of the essential oils from different genotypes of *L. gracilis* and genotypes rich in carvacrol showed leishmanicidal activity suggesting carvacrol as the main active ingredient of oil that already its effect on dysfunction mithocondrial membrane potential of promastigotes of *L. amazonensis* has been confirmed [34].

The molluscicide activity of *L. gracilis* was proven in *Biomphalaria glabrata* snails (LC50 62.2ppm). The major components thymol (24.0%), ρ-cymene (15.9%), methyl thymol (11.7%) and γ-terpinene (10.9%) were found in the essential oil [14].

Table 1: Reported uses of *Lippia gracilis* essential oil leaves.

Activity	Main compound	Concentration	Action mechanism	Reference
Antibacterial	Thymol 41.77% Carvacrol 10.13%	MIC=420µl/L	n.r.	Albuquerque et al. 2006
Antibacterial	Thymol 44.4% Carvacrol 22.2%	n.r.	Synergism	Bitu et al. 2014
Antibacterial	n.r.	MIC=9 µl/Ml	n.r.	Dantas et al. 2010
Antibacterial	Thymol 29.53% Carvacrol 44.45%	MIC=30 µl/Ml	n.r.	Dantas et al. 2015
Antifungical	Thymol 73.5%	MIC=5.0 µl/L	n.r.	Franco et al. 2014
Fungicide	n.r.	C=255, 340 and 425 ppm	n.r.	Fernandes et al., 2015
Fungicide	Thymol 61.84% Carvacrol 54.56%	MIC=46.8µg/mL (LGRA106) MIC=93.7µg/mL (LGRA109)	n.r.	Melo et al. 2013
Amoebicide	Carvacrol 48.92%	MIC=40 µg/Ml	n.r.	Santos et al. 2015
Leishmanicide	Thymol 61.84% Carvacrol 54.56%	IC50=86.3µg/mL (LGRA106) IC50=77.3µg/mL (LGRA110)	n.r.	Melo et al. 2013
Insecticide	1,8-Cineole 56,6%	LC50=282mg/L	n.r.	Dias et al. 2015
Insecticide	Carvacrol 44.4%	LC50=1.99ppm	n.r.	Silva et al. 2008
Acaricide	Carvacrol 40.4% ρ-Cimene 11.4%	LC50= 3.21µl/L	n.r.	Chagas et al. 2016
Acaricide	Thymol (LGRA106= 59.7% and LGRA201= 5.78%) Carvacrol (LGRA106= 0.88% and LGRA201= 35.28%	LGRA106 (LC50=1.02 in susceptible strains and 0.84mg/mL in organophosphate resistant strains) LGRA201 (LC50=1.03 in susceptible strains	n.r.	Costa-Júnior et al. 2016

		and 0.65mg/mL in organophosphate resistant strains Timol (LC50=1.94 in susceptible strains and 1.70mg/mL in organophosphate resistant strains) Carvacrol (LC=2.56 in susceptible strains and 0.80mg/mL in organophosphate resistant strains)		
Acaricide	Thymol Carvacrol	LGRA201 (LC50=1.31mg/mL to larvae and 6.55mg/mL to females) LGRA106 LC50=2.23mg/mL to larvae and 4.66mg/mL to females) LGRA109 (LC50=3.21mg/mL to larvae and 7.15mg/mL to females) LGRA108 (LC50=4.34mg/mL to larvae and 9.21mg/mL to females)	n.r.	Cruz et al. 2013
Cytotoxic	Thymol 55.5% ρ-Cimene 10.8%	IC50=4.33µg/mL to HepG2 cells IC50=22.9µg/mL to K562 cells IC50=7.01µg/mL to B16-F10 cells IC50=16.6µg/mL to PBMC cells	Apoptosis induction	Ferraz et al. 2013
Cytotoxic	Thymol Carvacrol	LGRA106 LC50=31.3µg/mL (HeLa cells, 3T3 and MRC-5 cells), 15.6µg/mL (B16 cells), 62.5 µg/mL (MCF-7 cells) LGRA109 LC50=125µg/mL (HeLa and MRC-5 cells) and 62.5µg/mL (3T3, B16 and MCF-7 cells) LGRA201 LC50=62.5µg/mL (3T3, MCF-7, HeLa and MRC-5) and 31.3µg/mL (B16 cells)	Disruption and increased membrane permeability	Melo et al. 2014
Anti-inflammatory	Carvacrol 44.4%	C=10, 30 and 100mg/kg	Inhibition inflammatory mediators	Guilhon et al. 2011

and antinociceptive				
Antiinflamatory and antinociceptive	Thymol 32.68%	C=50, 100 and 200mg/kg	Inhibition of inflammatory mediators	Mendes et al. 2010
Molluscicidal	Thymol 24% ρ-Cimene 15.9%	LC50=62.2ppm	n.r.	Teles et al. 2010

n.r.: not recorded

Conclusion

The essential oil of *L. gracilis* has been the subject of research due to its potent biological activities. Despite the various pharmacological activities of the essential oil of *Lippia gracilis* its major components, there are few studies that claim the mechanisms of action of these componentes. Despite the occurrence of plant species in the caatinga of the Brazilian Northeast, *L. gracilis* is an easy plant to grow and able to adapt to different environmental conditions, which makes it a promising productivity for the development of products for therapeutic purposes. Thus, studies to the actions of the individual components or synergism between them are necessary to complement research conducted.

REFERENCES

1. LORENZI H, MATOS FJA. Plantas medicinais no Brasil: nativas e exóticas. 2.ed. São Paulo: Nova Odessa: Plantarum, 2008.

2. SANTOS JP, ARAÚJO EL, ALBUQUERQUE UP. Richness and distribution of useful woody plants in the semi-arid region of northeastern Brazil. Journal of Arid Environments. 2008;72:652–663.

3. Siani AC, Sampaio ALF, Sousa MG, Henriques, MGMO, Ramos, MFS. Óleos essenciais - Potencial anti-inflamatório. Revista Biotecnologia Ciência & Desenvolvimento 2000;16:38-43.

4. Bizzo HR, Hovell AMC, Rezende CM. Óleos essenciais no Brasil: Aspectos gerais, desenvolvimento e perspectivas. Química Nova. 2009;32(3):588-594.

5. Albuquerque CC, Camara TR, Mariano RLR, Willadino L, Júnior, CM, Ulisses C. Antimicrobial Action of the Essential Oil of *Lippia gracilis* Schauer. Brazilian Archives of Biology and Technology. 2006;49(4):527-535.

6. Silva WJ, Dória GAA, Maria RT, Nunes RS, Carvalho GA, Blank AF, Alves PB, Marçal RM, Cavalcanti SCH. Effects of essential oils on *Aedes aegypti* larvae: Alternatives to environmentally safe insecticides. Bioresource Technology; 2008;99:3251-3255.

7. Neto RM, Matos FJA, Andrade VSA, Melo MCN, Carvalho CBM, Guimarães SB, Pessoa ODL, Silva SL, Silva SFR, Vasconcelos PRL. The essential oil from *Lippia gracilis* Schauer, Verbenaceae, in diabetic rats. Revista Brasileira de Farmacognosia. 2010;20(2):261-266.

8. Mendes SS, Bomfim RR, Jesus HCR, Alves PB, Blank AF, Estevam CS, Antoniolli AR, Thomazzi SM. Evaluation of the analgesic and anti-inflammatory effects of the essential oil of *Lippia gracilis* leaves. Journal of Ethnopharmacology. 2010;129:391-397.

9. Guilhon CC, Raymundo LJRP, Alviano DS, Blank AF, Arrigoni-Blank M F, Matheus ME, Cavalcanti SCH, Alviano CS, Fernandes PD. Characterization of the anti-inflammatory and antinociceptive activities and the mechanism of the action of *Lippia gracilis* essential oil. Journal of Ethnopharmacology. 2011;135(2):406-413

10. Cruz EM, Pinto JA, Fontes SS, Arrigoni-Blank MF, Bacci L, Jesus HC, Santos DA, Alves PB, Blank AF. Water deficit and seasonality study on essential oil constituents of *Lippia gracilis* Schauer germplasm. Scientific World Journal. 2014;2014.

11. Cavalcanti SCH, Niculau ES, Blank AF, Câmara CAG, Araújo IN, Alves PB. Composition and acaricidal activity of *Lippia sidoides* essential oil against two-spotted spider mite (*Tetranychus urticae* Koch). Bioresource Technology. 2010;101(2):829–832.

12. Lemos ELG, Monte FJQ, Matos FJA, Alencar JW, Craveiro AA, Barbosa RCSB, Lima EO. Chemical composition and antimicrobial activity of essential oils from Brazilian plants. Fitoterapia. 1992;63:266-268.

13. Matos FJA, Machado MI, Craveiro AA, Alencar JW, Silva MG. Medicinal plants northeast Brazil containing thymol and carvacrol - *Lippia sidoides* Cham. and *L. gracillis* H. B. K (Verbenaceae). Journal of Essential Oil Research, v.11, p.666-8,1999.

14. Teles TV, Bomfim RR, Alves PB, Blank AF, Jesus HCR, Quintans-Jr LJ, Serafini MR, Bonjardim LR, Araújo AAS. Composition and evaluation of the lethality of *Lippia gracilis* essential oil to adults of *Biomphalaria glabrata* and larvae of *Artemia salina*. African Journal of Biotechnology. 2010;9:22.

15. Franco CS, Ribeiro AF, Carvalho NCC, Monteiro OS, Silva JKR, Andrade EH, Maia JGS. Composition and antioxidante and antifungical activities of essential oil from *Lippia gracilis* Schauer. African Journal of Biotecnology. 2014;13(30):3107-3113.

16. Cruz EM, Costa-Júnior LM, Pinto JA, Santos DA, Araujo SA, Arrigoni-Blank MF, Bacci L, Alves PB, Cavalcanti SC, Blank AF. Acaricidal activity of *Lippia gracilis* essential oil and its major constituents on the tick *Rhipicephalus* (*Boophilus*) *microplus*. Veterinary Parasitology. 2013;195(1-2):198-202.

17. Dantas LIS, Rocha FAG, Medeiros FGM, Santos JAB. Atividade antibacteriana do óleo essencial de *Lippia gracilis* Schauer sobre patógenos de importância na indústria de alimentos. Holos. 2010;5:114-123.

18. Bittu VCN, Fecundo HTF, Costa JGM, Coutinho HDM, Rodrigues FFF, Santana NM, Botelho MA, Menezes IRA. Chemical composition of the essential oil of *Lippia gracilis* Schauer leaves and its potential as modulator of bacterial resistance. Natural Product Research. 2014;28(6):399-402.

19. Dantas LICS, Araújo GM, Ribeiro NM, Andrade VS, Oliveira FFM, Albuquerque CC, Rocha FAG. Antibacterial potential of *Lippia gracilis* Schauer essential oil against a *Salmonella* sp. strain isolated in tilápia fillet-fish (*Oreochromis niloticus*). International Journal of Scientific Research. 2015;4(6):500-503.

20. Burt SA, Van Der Zee R, Koets P, Graaff Am, Van Kanpen F, Gasstra W, Haagsman Hp, Veldhuizen Eja. Carvacrol induces heat shock protein 60 and inhibits synthesis of flagellin in *Escherichia coli* O157:H7. Applied and environmental microbiology. 2007;73:14.

21. Inamuco J, Veenendaal Akj, Burt Sa, Post Ja, Bokhoven Jlmt, Haagsman Hp, Veldhuizen Eja. Sub-lethal levels of carvacrol reduce *Salmonella Typhimurium* motility and invasion of porcine epithelial cells. Veterinary Microbiology. 2012;157:200–207.

22. Van Alphen LB, Burt SA, Veenedaal AKJ, Bleumink-Pluym NM, Van Putten JPM. The Natural antimicrobial carvacrol inhibits *Campylobacter jejuni* motility and infection of epithelial cells. Plos One. 2012;7:9.

23. Ahmad A, Khan A, Akhtar F, Yousuf S, Xess I, Khan LA, Manzoor M. Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against Candida. European Journal of Clinical Microbiology and Infectious Disease. 2011;30:41–50.

24. Melo JO, Bitencourt TA, Fachin AL, Cruz EM, Jesus HC, Alves PB, Arrigoni-Blank MF, Franca SC, Beleboni RO, Fernandes RP, Blank AF, SCHER R. Antidermatophytic and antileishmanial activities of essential oils from Lippia gracilis Schauer genotypes. Acta Tropica. 2013;128(1):110-115

25. Fernandes LCB, Aluquerque CC, Sales-Júnior R, Oliveira FFM, Gurgel EP, Mesquita MV, Silva MDS. Fungitoxicidade dos extratos vegetais e do óleo essencial de *Lippia gracilis* Schauer sobre o fungo *Monosporascus cannonballus* Pollack e Uecker. Summa Phytopathologica. 2015;41(2):153-155.

26. Dias CN, Alves LPL, Rodrigues KAF, Brito MCA, Rosa CS, Amaral FMM, Monteiro OS, Andrade EHA, Maia JGS, Moraes DFC. Chemical Composition and Larvicidal Activity of Essential Oils Extracted from Brazilian Legal Amazon Plants against *Aedes aegypti* L. (Diptera: Culicidae). Evidence-Based Complementary and Alternative Medicine. 2015:2015.

27. Seo S, Jung CS, Kang J, Lee HR, Kim SW, Hyun J, Park II-K. Larvicidal and acetylcholine esterase inhibitory activity of Apiaceae plant essential oils and their constituents against *Aedes Albopictus*, and formulation development. Journal of Agricultral and Food Chemestry. 2015;63(45):9977-9986.

28. Costa-Júnior LM, Miller RJ, Alves PB, Blank AF, Li AY, Pérez De Léon AA. Acaricidal efficacies of *Lippia gracilis* essential oil and its phytochemicals against organophosphate-resistant and susceptible strains of *Rhipicephalus* (*Boophilus*) *microplus*. Veterinary Parasitology. 2016;228:60-64.

29. Chagas ACS, Oliveira MCS, Giglioti R, Santana RCM, Bizzo HR, Gama PE, Chaves FCM. Efficacy of 11 Brazilian essential oils on lethality of the cattle tick *Rhipicephalus* (*Boophilus*) *microplus*. Ticks and Tick-borne Diseases. 2016;7(3):427–432.

30. Ferraz RP, Bomfim DS, Carvalho NC, Soares MB, Silva TB, Machado WJ, Prata AP, Costa EV, Moraes VR, Nogueira PC, Bezerra DP. Cytotoxic effect of leaf essential oil of *Lippia gracilis* Schauer (Verbenaceae). Phytomedicine. 2013;20(7):615-21.

31. Melo JO, Fachin AL, Rizo WF, Jesus HCR, Arrigoni-Blank MF, Alves PB, Martins MA, França SC, Blank AF. Cytotoxic effects of essential oils from three *Lippia gracilis* Schauer genotypes on HeLa, B16, and MCF-7 cells and normal human fibroblastos. Genetic and Molecular Research. 2014;13(2):2691-2697.

32. Santos IGA, Scher R, Rott MB, Menezes LR, Costa EV, Cavalcanti SCH, Blank AF, Aguiar JS, Silva TG, Dolabella SS. Amebicidal activity of the essential oils of *Lippia* spp. (Verbenaceae) against *Acanthamoeba polyphaga* trophozoites. Parasitology Research. 2015;115(2):535-40.

33. Riella KR, Marinho RR, Santos JS, Pereira-Filho RN, Cardoso JC, Albuquerque-Júnior RLC, Thomazzi SM. Anti-inflammatory and cicatrizing activities of thymol, a monoterpene of the essential oil from *Lippia gracilis*, in rodents. Journal of Ethnopharmacology. 2012;43:656–663.

34. Manzote L, García M, Pastor J, Gil L, Scull R, Maes L, Cos P, Gille L. Essential oil from *Chenopodium ambrosioides* and main components: Activity against *Leishmania*, their mitochondria and other microorganisms. Experimental Parasitology.2014;136:20–26.

3.2 Capítulo 2

Assessment of different *Lippia sidoides* genotypes regarding their acaricidal activity against *Rhipicephalus* (*Boophilus*) *microplus*

Avaliação da atividade carrapaticida do óleo essencial de diferentes genótipos de *Lippia sidoides* sobre *Rhipicephalus* (*Boophilus*) *microplus*

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Abstract

The aim of this study was to select different genotypes of *Lippia sidoides* with the highest activity against larvae and engorged females of the tick *Rhipicephalus* (*Boophilus*) *microplus*. The germplasms studied were LSID006, LSID102, LSID103 and LSID104. The LSID104 genotype, that presented carvacrol as a major constituent, was the germplasm with the worst larvicide effect (LC_{50} 2.99 mg/mL). The LSID006 genotype was the least effective compound against engorged females (LC_{50} 12.46 mg/mL), and it was chemically similar to the LSID102 genotype, which presented the highest acaricide activity (LC_{50} 2.81 mg/mL). We conclude that chemical similarity of the germplasms was not correlated with acaricide activity against *R.* (*B.*) *microplus*. The essential oil of *L. sidoides* is a potent natural agent. However, the

findings of this work provide a better understanding for product development based on this essential oil, which must consider synergic effects or the action of minor compounds.

Keywords: Essential oil, acaricidal, chemical diversity, germplasms, carvacrol, thymol.

Resumo

O objetivo deste trabalho foi selecionar genótipos de *Lippia sidoides* que apresentem maiores atividades em larvas e fêmeas ingurgitadas do carrapato *Rhipicephalus* (*Boophilus*) *microplus*. Os genótipos estudados foram LSID006, LSID102, LSID103 e LSID104. O genótipo LSID104, o único a conter o monoterpeno carvacrol como um dos principais constituintes, foi o que apresentou o menor efeito larvicida (CL_{50} 2,99 mg/mL). O genótipo LSID006 apresentou menor efeito sobre fêmeas ingurgitadas (CL_{50} 12,46 mg/mL), entretanto foi quimicamente similar ao genótipo LSID102, que apresentou a maior atividade carrapaticida (CL_{50} 2,81 mg/mL). Concluí-se que a semelhança química dos genótipos não se correlaciona com a atividade carrapaticida contra *R.* (*B.*) *microplus*. O óleo essencial de *L. sidoides* é um agente natural potente e os resultados deste trabalho proporcionam um melhor entendimento para o desenvolvimento de produtos com base neste óleo essencial, devendo ser considerado os efeitos sinérgicos ou a ação de compostos presentes em menores concentrações.

Palavras-chave: Óleo essencial, acaricida, diversidade química, genótipos, carvacrol, timol.

Introduction

The *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) cattle tick is a blood-sucking ectoparasite that occurs in tropical and subtropical regions (PEREIRA et al. 2008). This species has caused economic losses to the world livestock, inducing intense irritation of the animals, leather depreciation, decreased weight gain, decreased production of meat and milk, and transmitting *Babesia* spp. and *Anaplasma* spp. (GOMES 1998; FURLONG et al., 2005; RECK et al., 2014). In Brazil, these losses exceed 3 billion dollars annually (GRISI et al., 2014).

The most widely used control method is the use of synthetic chemical agents. However, chemical control can lead to selected populations of resistant ticks (GUERRERO et al., 2012) and are costly and contaminate the environment with residues harmful to the hosts and also humans (FREITAS et al., 2005). Therefore, new approaches are needed, and

natural products are potential candidates for acaricidal drugs. These compounds generally have low environmental impact and can lead to slower tick resistance (BORGES et al., 2003; Hu & Coats, 2008).

Lippia sidoides is a Verbenaceae plant known in Brazil as "alecrim-pimenta". Despite the occurrence in northeastern of Brazil, it is now cultivated in several Brazilian states due to its herbal characteristics (MATOS & OLIVEIRA, 1998). *L. sidoides* leaves are approximately constituted with 4% essential oil with carvacrol and thymol as major constituents, depending on the evaluated germplasm (LORENZI & MATOS, 2008; SANTOS et al., 2015). The essential oils of *L. sidoides* have bactericidal, fungicidal and molluscicidal properties which are generally attributed to the major components (MATOS, 2000; MATOS, 2002; CARVALHO et al., 2003; BOTELHO et al., 2007).

In addition to the activities mentioned above, the acaricide activity of the essential oil of *L. sidoides* with high concentration of thymol and carvacrol has been used against *Tetranychus urticae* (CAVALCANTI et al., 2010), *R.* (*B.*) *microplus, R. sanguineus, Amblyomma cajennense* and *Dermacentor nitens* (GOMES et al., 2012; GOMES et al., 2014). However, different genotypes of plants show differences in their chemical composition and thus different bioactivities (CRUZ et al., 2013; COSTA-JÚNIOR et al., 2016). Therefore, the aim of this study was to evaluate the activity of the essential oil of different germplasms of *L. sidoides* plants against larvae and females of *R.* (*B.*) *microplus*.

Materials and methods

Essential oil

Leaves of *L. sidoides* were collected from the active Germplasm Bank of Medicinal Plants that was established with *L. sidoides* plants from different geographical locations (Table 1) at the research farm of the Federal University of Sergipe, Brazil (SANTOS et al., 2015). The harvests of all genotype were performed at the same time. After manual defoliation, the leaves were dried in a forced air circulation oven for five days at 40°C.

The essential oils were extracted by hydrodistillation in a Clevenger apparatus for 140 minutes. Each sample consisted of 75 g of dried leaves from four plants. The essential oils extraction, as well the determination and analysis of their chemical composition, were conducted according Santos et al. (2015).

Briefly, the chemical composition of the essential oils was determined using a gas chromatograph coupled to a mass spectrometer equipped with an AOC-20i auto injector and a fused-silica capillary column. Quantitative analyses were performed by flame ionization gas chromatography (FID). The essential oil components were identified by comparing their mass spectra with the available spectra in the equipment database (NIST05 and WILEY8). Finally,

the measured retention indices were compared with those in the literature (ADAMS, 2007), and the retention times (RT) were determined using the Van Den Dool and Kratz (1963) equation and a homologous series of *n*-alkanes (C_8-C_{18}).

Obtaining the larvae and the engorged females

The larvae and engorged females of *R.* (*B.*) *microplus* ticks used in this work were obtained from colonies maintained at the Biological and Health Science Center of the Federal University of Maranhão (UFMA), Brazil. This study was approved by the Ethics Committee on Animal Use of UFMA under protocol 23115018061/2011-01. Larvae between 14 and 21 days after hatching were used in the Larval Packet Test. Adult engorged female ticks (≥ 4.5 mm in length) were collected from the bodies of artificially infested cattle.

Larval Packet Test

The larval packet test was performed according to Stone and Haydock (1962) and modified by FAO (1984) and Leite (1988), as described below. Two sheets of filter paper (4 cm^2) (Whatman 80 g) were treated with 400 µL of solution containing 3% dimethyl sulfoxide (DMSO) and essential oil. Twelve concentrations, ranging from 0.0612 to 15.00 mg/mL of essential oil isolated from each of the four *L. sidoides* genotypes, were used for the test. Ten concentrations ranging from 0.0612 to 25.00 mg/mL of thymol (Merck) and carvacrol (Sigma–Aldrich) were performed, tested as published before (CRUZ et al., 2013).

Approximately 100 tick larvae were placed in filter papers folded to form a packet and sealed with a plastic clothespin. The packet was placed in an incubator (27ºC and relative humidity ≥ 80%) for 24 hours. After this time, alive and dead larvae were counted. Ticks that did not move were considered dead. The experiment was performed with four replicates for each treatment. Furthermore, a solution of 3% DMSO was used as the negative control.

Adult immersion test

The adult immersion test was performed as described by Drummond et al. (1973). The adult immersion test shows the activity on mortality as well as the interference in reproduction, by evaluation of oviposition and eggs hatching. Engorged female cattle ticks were collected from artificially infested calves. Groups of ten engorged female ticks were weighed to obtain groups with weights ranging from 2.24 to 2.32 g.

Each tick group was dipped in one of twelve concentrations ranging from 0.0612 to 25.00 mg/mL of essential oil isolated from one of the four *L. sidoides* genotypes, using 3% DMSO as solvent for five minutes. Ten concentrations ranging from 1.00 to 25.00 mg/mL of thymol (Merck) and carvacrol (Sigma–Aldrich) were performed, tested and published before

(CRUZ et al., 2013). DMSO (3%) was used for the negative control group. The engorged females were subsequently dried on a paper towel, placed in Petri dishes and maintained in a biochemical oxygen demand (BOD) incubator at 27 ± 1ºC and relative humidity ≥ 80% for 35 days to allow oviposition and hatching of the larvae. The eggs mass were weighed and the hatching was evaluated. The efficacy was calculated according Drummond et al. (1973).

Statistical analysis

Lethal concentrations were calculated using GraphPad Prism 6.0. Significant differences between the average efficiency of each pair of essential oil and/or monoterpene were considered when there was no overlap between the 95% confidence limits of the LC_{50} values (RODITAKIS et al., 2005). The data of the acaricidal activity (on larvae and engorged female) of the essential oils from each genotype were submitted to cluster analysis using DataLab 3.5 software. The dissimilarity matrix was simplified with dendrograms using Ward's clustering method. The dendrograms were drawn using the PHY FY website (FREDSLUND, 2006).

Results

Twenty six components of the *L. sidoides* essential oil were identified (Table 2). The LSID006, LSID102, LSID103, and the LSID104 genotypes presented, respectively, 21, 14, 19 and 16 compounds. The most abundant chemical compound in LSID006, LSID102, and LSID103 was thymol (54.4%; 38.7%; 64.8%, respectively), and the most abundant compound in LSID104 was the thymol isomer, carvacrol (43.7%).

Lippia sidoides oils showed efficacy against larvae and engorged female ticks (Table 3). The LSID104 genotype, which uniquely presented carvacrol as its major constituent, had one of the worst larvicide effects. LSID006 had the highest larvicide effect. LSID103 had the highest amounts of thymol and presented a lower larvicide activity than LSID006. The LSID103 and LSID006 are in different clusters based on acaricidal activity (Figure 1).

LSID102 presented higher efficacy against engorged females (LC50 = 2.81 mg/mL) (Table 3). Similar to the results with larvae, no direct relationship between clustering analysis was found based on the chemical constituents and the acaricidal effect because LSID006 was the least effective compound against engorged females and is chemically similar to the LSID102 genotype (Figure 1). Thymol acetate is present only in LSID102 and LSID103 (Table 2), the two genotypes with the largest acaricidal efficacy against engorged females (Table 3), which could indicate the possibility of a synergistic effect of this compound.

Clustering analysis of *L. sidoides* genotypes based on acaricidal activity showed that LSID102 and LSID103 are closely related (Figure 1). These genotypes were the most

effective against engorged females (Table 3). Both genotypes are more closely related to thymol than carvacrol, although all of them represent the same cluster (Figure 1).

Discussion

The difficulties in preparing proper formulations, differences in the chemical composition of plants of the same species due to extrinsic and intrinsic factors and differences on the activity of the formulations from the same vegetal species are hindrances that need to be addressed in order to enable progress to transposing the efficacy obtained from the laboratory to the field (BORGES et al., 2011). This study selected *L. sidoides* genotypes with highest efficacies on *R.* (*B.*) *microplus* advancing knowledge for the standardization of a compound.

The susceptibility to acaricidal compounds is related to the life stages of the tick, as well as the physiological process involving blood feeding. In general, the immature stages of ticks seem to be more susceptible to synthetic acaricide effects than others stages (SOUZA & GONZALES, 1980; PINHEIRO, 1987). The essential oil of *L. sidoides* was most efficient against larvae than nymphs of *R. sanguineus* and *A. cajennense* (GOMES et al., 2014). However the animals are always infested by ticks at different life stages and the best compound should be effective against all of them. In the present study a cluster analysis was performed to select *L. sidoides* genotypes with the highest efficacies against *R. (B.) microplus* larvae and engorged female.

Several phytochemical studies demonstrated the presence of thymol as the major compound of *L. sidoides* (CAVALCANTI et al., 2010; VERAS et al., 2012; GOMES et al., 2014). The exception observed in LSID104 (CAVALCANTI et al., 2010) could be a result of the chemical distance explained by the clustering analysis of *L. sidoides* genotypes, where LSID104 was grouped alone and the other genotypes showed considerable chemical similarity (SANTOS et al., 2015).

Carvacrol was the most efficient compound against larvae mainly organophosphate resistant strain larvae (CRUZ et al., 2013; COSTA-JUNIOR et al., 2016), the essential oil that presented as its major constituent, had one of the worst larvicide effects. This result suggests that despite carvacrol alone having elevated larvicide action, it is not the main bioactive compound, and the different oil constitutions play an important role in the acaricidal action. We hypothesize that the high levels of thymol (54.4%) in LSID006 are responsible for this action, but LSID103 has higher amounts of thymol and presented a lower larvicide activity because LSID103 and LSID006 are in the same cluster based on chemical constituents (Santos et al. 2015) but are in different clusters based on acaricidal activity (Figure 1). β-bisabolene is present only in LSID103 and could have antagonistic activity. Additionally,

some compounds exist only in LSID006 (β-pinene; 1,8 cineole; ipsdienol; aromadrendene; β-selinene) and could contribute to the elevated larvicide action of this genotype. The synergistic effects of β-pinene, 1,8 cineole and aromadendrene have recently been reported (MULYANINGSIH et al., 2010; RODENAK et al., 2014; ZHANG et al., 2015), but to the best of our knowledge, the minor compounds of *L. sidoides* essential oil have not been studied to evaluate their synergistic capabilities in *R.* (*B.*) *microplus*.

The synergy studies conducted in *Lippia* spp. corroborate our results. The activity of the essential oil of *L. sidoides* and *L. gracilis* and chemical components against the fungus *Thielaviopsis paradoxa* recently have been reported. The compounds p-cymene, 1.8 cineole, α-terpinene and β-caryophyllene had no efficiency when tested alone, and the authors proposed that they act in synergy with other compounds because the thymol concentration required to control the fungus was higher than the concentration of the *L. sidoides* essential oil (CARVALHO et al., 2013).

Different plant genotypes can present distinct essential oil profiles (GIL et al., 2002; DRAGLAND et al., 2005; PEIXOTO et al., 2015). In this context, it is important to study the relation between these composition variations and the interference in the bioactivity. For example, LSID102 and LSID104 presented similar leishmanicidal activity (Concentration that inhibits 50% - IC50 = 74.1 and 54.8 µg/mL, respectively) (FARIAS-JUNIOR et al., 2012), although both genotypes are in different clusters based on their chemical constitution (SANTOS et al., 2015). In addition, no significant differences were observed in the acaricidal activity of the essential oils of different *L. sidoides* genotypes against *T. urticae,* and after acaricidal analysis with selected compounds it was suggested that the evaluated components act synergistically to achieve the acaricidal effect (CAVALCANTI et al., 2010).

The bioactivity of thymol has been reported against ticks and insects (NOVELINO et al., 2007; WALIWITIYA et al., 2010; CRUZ et al., 2013), and the larvicide activity of *L. sidoides* essential oil against *Aedes aegypti* was attributed to this monoterpene (CARVALHO et al., 2003). The LSID102 and LSID103 genotypes were the most effective against engorged females (Table 3). Both genotypes are more closely related to thymol than carvacrol, although all of them represent the same cluster (Figure 1). The presence of thymol in these genotypes is associated with the activity against engorged females.

Although the genotypes with higher efficiency against larvae (LSID006) and engorged females (LSID102) have thymol as their major constituent, which could be an indicative that this monoterpene is involved in the acaricidal effect, the general chemical balance among the essential oil compounds leads to different acaricidal effects. Both genotypes are in different clusters based on acaricidal activity (Figure 1), which suggests that there could be different action modes of these essential oils during different life stages of the *R.* (*B.*) *microplus*.

Although the activity of *L. sidoides* essential oil has been described on *R.* (*B.*) *microplus*, to our knowledge, this is the first evaluation of the relationship among the activity of different genotypes of *L. sidoides* and the acaricide effect.

Conclusions

The results indicated that the chemical differences in the *L. sidoides* genotypes influence the acaricidal activity against *R.* (*B.*) *microplus*. In addition, the clustering analysis of *L. sidoides* genotypes based on acaricidal activity suggests that the essential oils have different modes of action in larvae and in engorged females. We conclude that the different constitutions of the essential oils, as well as the relationships among the compounds, play important roles in the acaricidal action. However, further studies are needed to verify the global acaricidal effects of the minor compounds of *L. sidoides* essential oil. The findings of this work facilitate the understanding and the development of innovative strategies aimed to control the cattle tick *R.* (*B.*) *microplus*.

Acknowledgements

The authors wish to thank CNPq (The Brazilian National Council for Scientific and Technological Development) for awarding a fellowship to L.M. Costa-Júnior, and A.F. Blank, CAPES (Brazilian Federal Agency for support and evaluation of graduate education) for the scholarship to T.A. Penha and E.M.O. Cruz and FAPEMA (Maranhão State Research Foundation) for the scholarship to S.A. Araújo. We also thank CNPq and FAPEMA for financial support.

References

Adams RP. *Identification of essential oil components by gas chromatography/mass spectroscopy*. 4th. Illinois: Allured Pub Corporation; 2007.

Borges LMF, Ferri PH, Silva WJ, Silva WC, Silva JG. *In vitro* efficacy of extracts of *Melia azedarach* against the tick *Boophilus microplus*. *Med Vet Entomol* 2003; 17(2): 28-31.

Borges LMF, Sousa LAD, Barbosa CS. Perspectives for the use of plant extracts to control the cattle tick *Rhipicephalus* (*Boophilus*) *microplus*. *Rev Bras Parasitol Vet* 2011; 20(2): 89-96.

Botelho MA, Nogueira NAP, Bastos GM, Fonseca SC, Lemos TL, Matos FJ, et al. Antimicrobial activity of the essential oil from *Lippia sidoides*, carvacrol and thymol against oral pathogens. *Braz J Med Biol Res* 2007; 40(3): 349-356.

Carvalho AFU, Melo VMM, Craveiro AA, Machado MIL, Bantim MB, Rabelo EF. Larvicidal activity of the essential oil from *Lippia sidoides* Cham. against *Aedes aegypti* Linn. *Mem Inst Oswaldo Cruz* 2003; 98(4): 569-571.

Carvalho RRC, Laranjeira D, Carvalho-Filho JLS, Souza PE, Blank AF, Alves PB, et al. *In vitro* Activity of essential oils of *Lippia sidoides* and *Lippia gracilis* and their major chemical components against *Thielaviopsis paradoxa*, causal agent of stem bleeding in coconut palms. *Quim Nova* 2013; 36(2): 241-244.

Cavalcanti SCH, Niculau ES, Blank AF, Câmara CAG, Araújo IN, Alves PB. Composition and acaricidal activity of *Lippia sidoides* essential oil against two-spotted spider mite (*Tetranychus urticae* Koch). *Bioresour Technol* 2010; 101(2): 829-832.

Costa-Júnior LM, Miller RJ, Alves PB, Blank AF, Li AY, Pérez de León AA. Acaricidal efficacies of *Lippia gracilis* essential oil and its phytochemicals against organophosphate-resistant and susceptible strains of *Rhipicephalus* (*Boophilus*) *microplus*. *Vet Parasitol* 2016; 228: 60-64.

Cruz EMO, Costa-Júnior LM, Pinto JAO, Santos DA, Araujo SA, Arrigoni-Blank MF, et al. Acaricidal activity of *Lippia gracilis* essential oil and its major constituents on the tick *Rhipicephalus (Boophilus) microplus*. *Vet Parasitol* 2013; 195(1-2): 198-202.

Dragland S, Rohloff J, Mordal R, Iversen TH. Harvest regimen optimization and essential oil production in five tansy (*Tanacetum vulgare* L.) genotypes under a northern climate. *J Agric Food Chem* 2005; 53(12): 4946-4953.

Drummond RO, Ernst SE, Trevino JL, Gladney WJ, Graham OH. *Boophilus annulatus* and *Boophilus microplus*: Laboratory test of insecticides. *J Econ Entomol* 1973; 66(1): 130-133.

FAO. *Ticks and tick borne disease control: a practical field manual*. Roma: FAO; 1984.

Farias-Júnior PA, Rios MC, Moura TA, Almeida RP, Alves PB, Blank AF, et al. Leishmanicidal activity of carvacrol-rich essencial oil from *Lippia sidoides* Cham. *Biol Res* 2012; 45(4): 399-402.

Fredslund J. PHY FI: fast and easy online creation and manipulation of phylogeny color figures. *BMC Bioinformatics* 2006; 7: 315.

Freitas DRJ, Pohl PC, Vaz IS. Characterization of acaricide resistance in *Boophilus microplus*. *Acta Sci Vet* 2005; 33(2): 109-117.

Furlong J. *Carrapato: problemas e soluções*. Embrapa Gado de Leite, Juiz de Fora; 2005.

Gil A, De La Fuente EB, Lenardis AE, López Pereira M, Suárez SA, Bandoni A, et al. Coriander essential oil composition from two genotypes grown in different environmental conditions. *J Agric Food Chem* 2002; 50(10): 2870-2877.

Gomes A. *Controle do carrapato do boi: um problema para quem cria raças européias*. Embrapa, Campo Grande, 1998.

Gomes GA, Oliveira-Monteiro CM, Senra TOS, Zeringota V, Calmon F, Matos RS, et al. 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). *Parasitol Res* 2012; 111(6): 2423-2430.

Gomes GA, Oliveira-Monteiro CM, Julião LS, Maturano R, Senra TOS, Zeringota V, et al. Acaricidal activity of essential oil from *Lippia sidoides* on unengorged larvae and nymphs of *Rhipicephalus sanguineus* (Acari: Ixodidae) and *Amblyomma cajennense* (Acari: Ixodidae). *Exp Parasitol* 2014; 137: 41-45.

Grisi L, Leite RC, Martins JRS, Barros ATM, Andreotti R, Cançado PHD, et al Reassessment of the potential economic impact of cattle parasites in Brazil. *Rev Bras Parasitol Vet* 2014; 23(2): 150-156.

Guerrero FD, Lovis L, Martins JR. Acaricide resistance mechanisms in *Rhipicephalus* (*Boophilus*) *microplus*. *Rev Bras Parasitol Vet* 2012; 21(1): 1-6.

Hu D, Coats J. Evaluation of the environmental fate of thymol and phenethyl propionate in the Laboratory. *Pest Manag Sci* 2008; 64(7): 775-779.

Leite RC. *Boophilus microplus (Canestrini, 1887): susceptibilidade, uso atual e retrospectivo de carrapaticidas em propriedades das regiões fisiográficas da baixada do Grande-Rio e Rio*

de Janeiro, uma abordagem epidemiológica. 122p. [Tese] . Rio de Janeiro: Universidade Federal Rural do Rio de Janeiro, 1988.

Lorenzi H, Matos FJA. *Plantas medicinais no Brasil: nativas e exóticas*. Plantarum, Nova Odessa. 2008.

Matos FJA, Oliveira F. *Lippia sidoides* Cham. – Farmacognosia, química e farmacologia. *Rev Bras Farm* 1998; 79(3-4): 84-87.

Matos FJA. *Plantas medicinais – guia de seleção e emprego de plantas usadas em fitoterapia no nordeste do Brasil*. Imp. Universitária, Fortaleza; 2000.

Matos FJA. *Farmácias vivas: sistema de utilização de plantas medicinais projetado para pequenas comunidades.* UFC, Fortaleza; 2002.

Mulyaningsih S, Sporer F, Zimmermann S, Reichling J, Wink M. Synergistic properties of the terpenoids aromadendrene and 1,8-cineole from the essential oil of *Eucalyptus globulus* against antibiotic-susceptible and antibiotic-resistant pathogens. *Phytomedicine* 2010; 17(13): 1061-1006.

Novelino AMS, Daemon E, Soares GLG. Avaliação da atividade repelente do timol, mentol, salicilato de metila e ácido salicilico sobre larvas de *Boophilus microplus* (Canestrini, 1887) (Acari: Ixodidae). *Arq Bras Med Vet Zootec* 2007; 59(3): 700-704.

Peixoto MG, Costa-Júnior LM, Blank AF, Lima AS, Meneses TS, Santos DA, et al. Acaricidal activity of essential oils from *Lippia alba* genotypes and its major components carvone, limonene, and citral against *Rhipicephalus microplus*. *Vet Parasitol* 2015; 210(1-2): 118-122.

Pereira MC, Labruna MB, Szabó MPJ, Klafke GM. *Rhipicephalus (Boophilus) microplus: Biologia, Controle e Resistência.* São Paulo, 192 pp. 2008.

Pinheiro VRE. Avaliação do efeito carrapaticida de alguns piretróides sintéticos sobre o *Amblyomma cajennense* (Fabricius, 1787). *A Hora Vet* 1987; 7: 20-26.

Reck J, Marks FS, Rodrigues RO, Souza UA, Webster A, Leite RC, et al. Does *Rhipicephalus microplus* tick infestation increase the risk for myiasis caused by *Cochliomyia hominivorax* in cattle? *Prev Vet Med* 2014; 113(1):59-62.

Rodenak KB, Polo M, Montero Villegas S, Galle M, Crespo R, García de Bravo M. Synergistic antiproliferative and anticholesterogenic effects of linalool, 1,8-cineole, and simvastatin on human cell lines. *Chem Biol Interact* 2014; 214: 57-68.

Santos PL, Araújo AA, Quintans JS, Oliveira MG, Brito RG, Serafini MR, et al. Preparation, Characterization, and Pharmacological Activity of *Cymbopogon winterianus* Jowitt ex Bor (Poaceae) Leaf Essential Oil of β-Cyclodextrin Inclusion Complexes. *Evid Based Complement Alternat Med* 2015; 502454.

Souza AP, Gonzales JC. Suscetibilidade dos ínstares parasitários do *Boophilus microplus*, ao ethion, amitraz e arsenito de sódio. *Arq Fac Vet (UFRGS)* 1980; 8: 117-123.
Stone BF, Haydock KP. A method for measuring the acaricide-susceptibility of the cattle tick *Boophilus microplus* (Can.). *Bull Entomol Res* 1962; 53(3): 563-578.

Van Den Dool H, Kratz PD. A generalization of retention index system including linear temperature programmed gas–liquid partition chromatography. *J Chromatography A* 1963; 11: 463-471.

Veras HNH, Rodrigues FFG, Colares AV, Menezes IRA, Coutinho HD, Botelho MA, Costa JG. Synergistic antibiotic activity of volatile compounds from the essential oil of *Lippia sidoides* and thymol. *Fitoterapia* 2012; 83(3): 508-512.

Waliwitiya R, Belton P, Nicholson RA, Lowenberger CA. Effects of the essential oil constituent thymol and other neuroactive chemicals on flight motor activity and wing beat frequency in the blowfly *Phaenicia sericata*. *Pest Manag Sci* 2010; 66(3): 277-289.

Zhang Z, Guo S, Liu X, Gao X. Synergistic antitumor effect of α-pinene and β-pinene with paclitaxel against non-small-cell lung carcinoma (NSCLC). *Drug Res* 2015; 65(4): 214-218.

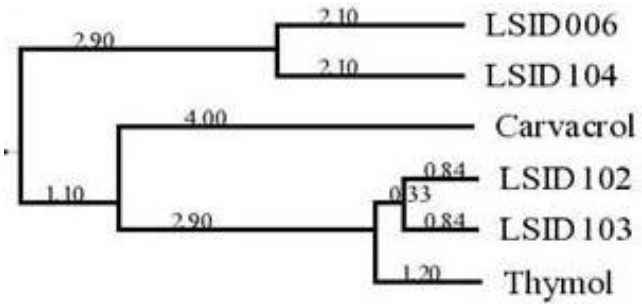

Figure 1. Clustering of *Lippia sidoides* genotypes based on acaricidal activity with the Euclidean distances.

Table 1. Identification and geographical origin of the *Lippia sidoides* genotypes used in the present study.

Code	Origin (State/Country)	Geographical data	Voucher nº
LSID006	Ceará/Brazil	5°14'05.4"S;38°11'35.0"W	8223
LSID102	Sergipe/Brazil	9°58'07.6"S;37°51'49.2"W	8224
LSID103	Sergipe/Brazil	9°58'08.6"S;37°51'50.3"W	8225
LSID104	Sergipe/Brazil	9°58'09.2"S;37°51'50.3"W	8226

Table 2. Essential oil composition (%) from *Lippia sidoides* genotypes characterized by gas chromatography associated with a mass spectrophotometer.

Compound	TR (min)	LSIDI 006	LSIDI 102	LSIDI 103	LSIDI 104
α-Thujene	6.567	1.55	1.09	1.01	1.66
α-Pinene	6.783	0.69	0.34	0.47	0.49
β-Pinene	8.183	0.32	-	-	-
Myrcene	8.592	3.16	3.35	5.32	3.52
α-Phelandrene	9.167	-	-	-	0.20
δ-3-Carene	9.233	0.23	0.12	0.18	0.10
α-Terpinene	9.542	2.04	1.91	1.53	3.12
p-Cimene	9.817	19.18	34.11	13.89	17.83
Limonene	9.975	0.94	0.49	0.71	0.44
1,8 Cineole	10.083	0.29	-	-	-
β-(Z)-Ocimene	10.233	-	-	-	0.29
γ-Terpinene	11.017	5.10	6.84	4.41	16.56
Linalol	12.583	0.27	-	0.10	-
Ipsdienol	14.133	1.23	-	-	-
NI	15.142	0.20	-	0.40	0.78
Terpinen-4-ol	15.542	1.18	0.66	0.96	0.91
Methyl thymol	17.233	2.16	9.42	1.87	4.13
Thymol	19.625	54.40	38.68	64.82	6.05
Carvacrol	19.858	-	0.60	-	43.69
Thymol Acetate	21.450	-	1.76	2.06	-
E-Methyl Cinnamate	22.808	-	-	0.96	-
β-Cariofilene	24.000	5.04	0.63	0.67	-
Aromadendrene	24.617	0.26	-	-	-
α-Humulene	25.192	0.24	-	0.23	-
β-Selinene	26.350	0.20	-	-	-
β-Bisabolene	26.900	-	-	0.24	0.23
Oxide of Cariophyllene	29.242	1.32	-	0.18	-
Total	**-**	**99.9**	**100.0**	**100.0**	**100.0**

Table 3. Acaricidal activity (LC_{50}) of the essential oil from *Lippia sidoides* genotypes.

Access	LC_{50} (mg/mL)	IC 95%	R^2
Larvae			
LSID006	0.93[b]	0.65 - 1.31	0.97
LSID102	3.36[c]	3.15 - 3.58	0.96
LSID103	3.90[d]	3.65 - 4.17	0.94
LSID104	2.99[cd]	1.62 - 5.50	0.86
Carvacrol*	0.22[a]	0.08 – 0.60	0.78
Thymol*	3.86[cd]	3.26 - 4.58	0.82
Engorged females			
LSID006	12.46[d]	11.28 - 13.77	0.95
LSID102	2.81[a]	2.62 - 3.01	0.99
LSID103	4.31[b]	3.92 - 4.74	0.99
LSID104	11.48[d]	11.08 - 11.91	0.99
Carvacrol*	4.46[b]	4.33 - 4.60	0.99
Thymol*	5.50[c]	5.41 - 5.58	0.99

*Tested by our group and published in Cruz et al. (2013).

3.3 Capítulo 3

Histopathology of ovary of *Riphicephalus microplus* female treated with *Lippia sidoides* and *Lippia gracilis* essential oils and its main components.

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Abstract

Rhipicephalus microplus is responsible to pathogen transmission, reducing weight gain, production of calves and milk, and leather quality. The use of plants for therapeutic purposes has been the target of research worldwide. The combined effect of different chemotypes of essentials oils, enhance biological activity, allows reducing the chance of selection for the target organism resistance. Little is known about the effects of essential oils of *Lippia sidoides* and *Lippia gracilis* and its majority compounds thymol and carvacrol in morphophysiology of ovaries of ticks. Therefore, the aim of this study was to identify the morphological changes in the ovaries of *R. microplus* females treated with essentials oils of *L. sidoides* (LSID102 and LSID103), *L. gracilis* (LGRA106 and LGRA201), thymol and carvacrol through histological techniques. Groups of 10 engorged females were submitted to immersion test in different concentrations of essentials oils. A negative control (DMSO 3% solution) was performed. Only the group treated with LGRA106 in concentration 4.66 mg/mL had no change compared to control. The other groups showed the following changes in oocytes I to V: vacuolation, chorion deformation, disorganization of yolk granules and irregularities in cell limit. Thus, the essential oils tested in this study may be a potent

product for the control of cattle tick for causing losses in vitelogenesis this parasite and thereby preventing further cyclic life.

Keywords: *Rhipicephalus microplus*, Ovary, essential oil, thymol, carvacrol.

1. INTRODUCTION

Rhipicephalus microplus is responsible to pathogen transmission, reducing weight gain, production of calves and milk, and leather quality (Jonsson, 2006; Grisi et al., 2014). Chemical acaricidal compounds quickly led to the use of synthetic products as main method of ticks control around the world (Guerrero et al. 2012). The use of plants for therapeutic purposes has been the target of research worldwide. Chemical compounds derived from plants generally don't cause environmental impact and can lead to a slower tick resistance (Borges et al., 2003).

The Verbenaceae family, present in northeastern Brazil, is distributed in tropical and subtropical regions, consists of approximately 175 genera and 2300 species, among them are: *Lippia sidoides* and *L. gracilis*. The *Lippia* genus consists of shrubs, herbs and small trees, usually aromatic and present in South and Central America as well as Central Africa (Lorenzi and Matos, 2008).

Essential oils of *L. sidoides* and *L. gracilis* have shown activity against ticks and female larvae of several species of ticks (Cruz et al., 2013; Gomes et al., 2012; Gomes et al., 2014; Matos et al., 2014; Costa-Júnior et al., 2016). The activities of the oils are mainly due to its major components, thymol and carvacrol, which already showed efficacy when tested alone (Cruz et al., 2013; Matos et al., 2014; Araújo et al., 2015; Costa-Júnior et al., 2016) and when evaluated in combination (Novato et al., 2015; Araújo et al., 2016). Some studies have tested the acaricide action of different genotypes of the species *L. sidoides* and *L. gracilis* and observed their effectiveness, especially considering that there is a synergistic effect between the main thymol and carvacrol compounds even when the concentration of one significantly above the another (Cruz et al., 2013; Costa-Júnior et al., 2016).

The essential oils consist of the mixture of several chemical components. The combined effect of different chemotypes, enhance biological activity, allows reducing the chance of selection for the target organism resistance (Araújo et al., 2016). Despite this importance, little is known about the effects of essential oils of *L. sidoides* and *L. gracilis* in morphophysiology of ovaries of ticks. Histopathological studies help to understanding mechanisms of action of substances in organisms. The action of substances in the

reproductive organs of the cattle tick females may be related to impairment of vitellogenesis inhibiting the continuation of its life cycle, which is important to control this ectoparasite.

2. MATERIAL AND METHODS

2.1 Essential oils

Two genotypes of *L. gracilis* (106 and 201) and two genotypes of *L. sidoides* (102 and 103) from different origins (Table 1) were cultivated under same soil, irrigation and fertilization conditions and agricultural practices as part of the Germplasm Bank of Medicinal Plants at the Experimental Farm of the Federal University of Sergipe (UFS). Leaves were collected and dried the same day in a convention dryer at 40°C for five days.

The essential oil was extracted by hydrodistillation in a Clevenger apparatus. The analysis of the essential oil chemical composition was performed using a gas chromatograph coupled to a mass spectrometer (GC-MS) (Shimadzu, Corporation, Kyoto, Japan) equipped with an AOC-20i auto injector (Shimadzu) and a fused-silica capillary column (5%-phenyl-95%-dimethylpolysiloxane, 30 m x 0.25 mm id., 0.25 µm film, J&W Scientific). Helium was used as the carrier gas at a flow rate of 1.2 mL min^{-1}. Quantitative analysis of the chemical constituents was performed by flame ionization gas chromatography (FID), using a Shimadzu GC-17A (Shimadzu). Quantification of each constituent was estimated by area normalization (%). Compound concentrations were calculated from the GC peak areas and they were arranged in order of GC elution.

The essential oil components were identified by comparing their mass spectra with the available spectra in the equipment database (NIST05 and WILEY8). Additionally, the measured retention index was compared with those in the literature (Adams, 2007). The relative retention index (RRI) was determined using the Van Den Dool and Kratz (1963) equation and a homologous series of *n*-alkanes (C_8-C_{18}) injected under the chromatography conditions described above. The means of the chemical constituents and essential content were subjected to the analysis of variance F test and were compared using the Scott-Knott test at 5% probability.

2.2 Ticks collection

Engorged *R. microplus* females (≥4.5 mm) resistant to synthetic pyrethroids and amitraz were collected from artificially infested calves. The resistance of the strain was previously evaluated according Drummond et al. (1973).

This procedure is registered in the Ethics Committee for Animal Use - CEUA / UFMA under protocol nº 23115 018061/2011 – 01.

2.3 Adult immersion test

Adult immersion test was performed according to Drummond et al. (1973). *R. microplus* engorged females were washed, dried and weighed in 5 specimens of groups seeking the weights of the most homogeneous groups possible. Each tick group was submerged for five minutes at different concentrations (table 2) of essential oils of *L. sidoides*, *L. gracilis*, thymol and carvacrol. A negative control (DMSO 3% solution) was performed. All tests were performed in duplicates. After tests, ticks were stored in incubator (27ºC, UR≥80%) for 7 days. A total of 180 ovaries were analyzed.

2.4 Histology

Individual ticks were anesthetized in refrigerator at 4° for thermal shock for 5 minutes. Ticks were dissected and their ovaries were removed under a stereomicroscope (Leica®), using a saline solution (NaCl 7.5 g/L, Na2HPO4 2.38 g/L and KH2PO4 2.72 g/L). The ovaries were stored into tubes with 2,5% glutaraldehyde for 24h. The material was then dehydrated in increasing ethanol concentrations (70% to 100%); embedded in Historesin® (Leica, USA) during 24h at 4°C and transferred to plastic moulds containing resin with a catalyst. After polymerization, sections of 5µm were performed using an ultra-microtome (Leica Ultracut UCT, USA) and stained with hematoxylin and eosin, following routine histological procedures. Ovaries were analyzed according to Saito et al. (2005).

3. RESULTS

The results allowed the visualization and characterization of the ovary and the different stages of development of oocytes. The ovary of *R. microplus* was defined as panoistic, characterized by the absence of nurse cells. In the control group, treated with DMSO 3%, no changes were observed in tissues and it was possible to characterize 5 oocyte development stages. In all treated groups with essential oils, it was possible observe decreased of number of mature oocytes in relation to the control group. All essential oils, except LGRA-106 with 4.66 mg/mL concentration, caused damages in oocytes. The histopathological changes in *R. microplus* caused by *L. gracilis*, *L. sidoides*, thymol and carvacrol are presented in table 1.

3.1 Treatment with 4.66 mg/mL of LGRA-106

No changes were observed when compared with control group (Figure 1E, F, G and H)

3.2 Treatment with 8.15 mg/mL of LGRA-106

In oocytes I, no changes were observed (Figure 1I)

Oocytes II presented smaller than in the control group, with vacuoles in peripheral region (Figure 1I).

Oocytes III and IV showed smaller size than in the control group. Vacuoles in peripheral region and thick chorion were observed too (Figure 1J, K).

Oocytes V showed irregular cell limit and vacuoles in peripheral region (Figure 1L).

3.3 Treatment with 6.55 mg/mL of LGRA-201

No changes were observed in oocytes I.

Oocytes II showed smaller size than in the control group with irregular cell limit and vacuolation in peripheral region of cell (Figure 1M)

Oocytes III, like oocytes II, presented a smaller size than control and peripheric vacuoles. Thick chorion was observed too (Figure 1N)

Oocytes IV and V presented vacuoles mainly in peripheral region of cell (Figure 1O, P).

3.4 Treatment with 7.65 mg/mL of LGRA-201

Oocytes I did not exhibit changes when compared with control group.

Oocytes II presented vacuolation in peripheral region (Figure 1Q).

Oocytes III exhibit peripheric vacuolation with loss in its granulation and thick chorion was evident (Figure 1R)

In oocytes IV it was possible observed vacuoles in peripheral region and around yolk granules, a discrete fusion of some granules and thick chorion were observed too (Figure 1S).

In oocytes V, vacuoles in cytoplasm, fusion of yolk granules and thick chorion were very evident causing loss of oocyte shape (Figure 1T).

3.5 Treatment with 2.80 mg/mL of LSDI-102

No changes were founded in oocyte I.

In oocytes II, vacuolation can be observed in peripheral region and around the germ vesicle. The cell limit showed irregular (Figure 2E).

The oocytes III presented thick chorion, in addition to peripheral vacuolation and around the germ vesicle (Figure 2F).

The oocytes IV showed smaller than in the control group. Vacuoles in periphery and thick chorion were evident too (Figure 2G).

In oocytes V there was cytoplasmic vacuolation with discrete granule fusion. In addition, it was possible to observe highly thick chorion. As in stage IV, the oocytes V were smaller than in the control group (Figure 2H).

3.6 Treatment with 4.66 mg/mL of LSDI-102

In oocytes I, there were no changes when compared to the control group.

In oocytes II and III vacuolation in periphery were evident (Figure 2I, J).

The oocytes IV presented vacuoles in peripheral region with fusion of yolk granules (Figure 2K).

The oocytes V presented changes more evident than the others stages with vacuoles around and inside the yolk granules and intense fusion of granules throughout the cytoplasm (Figure 2L).

3.7 Treatment with 4.31 mg/mL of LSDI-103

The oocytes I did not present morphological alterations with this treatment.

The oocytes II and III presented vacuoles in peripheral region (Figure 2M, N).

The oocytes IV showed vacuolation in periphery and around yolk granules (Figure 2O).

The oocytes V were characterized by smaller size than control group, peripheric vacuolation and around the yolk granules, with fusion of granules (Figure 2P).

3.8 Treatment with 7.21 mg/mL of LSDI-103

There were no changes in oocytes I.

The oocytes II showed vacuoles mainly in peripheral region (Figure 2Q).

The oocytes III presented smaller size than control, with vacuolation around yolk granules and in periphery. Thick chorion was evident in these cells (Figure 2R).

The oocytes IV presented intense vacuolation in periphery when compared with previous treatment (4.31 mg/mL) (Figure 2S).

The oocytes V were characterized by present fusion of yolk granules and vacuoles around them (Figure 2T).

3.9 Treatment with 5.49 mg/mL of Thymol

The oocytes I showed irregularities in limit cells (Figure 3F).

The oocytes II presented vacuoles in periphery and irregular limit cell (Figure 3G).

The oocytes III showed vacuolation in periphery with chorion thinner than in the control group (Figure 3H).

The oocytes IV exhibit vacuoles in peripheral region of cells and irregular limit (Figure 3I).

The oocytes V were characterized by vacuolation around yolk granules and periphery, with thick chorion and discrete fusion of yolk granules (Figure 3J).

3.10 Treatment with 5.77 mg/mL of Thymol

No changes were observed in oocytes I (Figure 3K).

In oocytes II, vacuolation can be observed around germ vesicle and periphery (Figure 3L).

In oocytes III, vacuolation in peripheral region were observed and chorion showed thinner than control group (Figure 3M).

The oocytes IV presented the same changes that previous treatment but the alterations were more intense (Figure 3N)

The oocytes V showed the same changes that previous treatment, however, the fusion of yolk granules was more intense with loss of the cellular form (Figure 3O).

3.11 Treatment with 4.46 mg/mL of Carvacrol

The oocytes I have no changes after treatment (Figure 3P).

The oocytes II presented vacuolation mainly in peripheral region and around germ vesicle, with irregularities in limit of cell (Figure 3Q).

The oocytes III showed vacuoles mainly in periphery of cells (Figure 3R).

Oocytes IV appear extensively vacuolated in periphery and around yolk granules (Figure 3S).

Oocytes V concentrate vacuoles in periphery region and around yolk granules which were in the process of merging, causing loss of oocytes shape. The chorion, at this stage, was thick (Figure 3T).

3.12 Treatment with 5.04 mg/mL of Carvacrol

The oocytes I presented intact after this treatment (Figure 3U).

The oocytes II showed irregular cells limit, with vacuoles in periphery region and around germ vesicle (Figure 3V).

Oocytes III exhibit extensive vacuolation in all cytoplasm, leading to loss of its granulation. (Figure 3W).

In oocytes IV, vacuolation concentrated in peripheric region were evident (Figure 3X).

Oocytes V presented smaller size than control. Vacuoles around yolk granules and intense fusion of granules were founded leading to loss of their original form. Chorion showed highly thick in this treatment (Figure 3Y).

4. DISCUSSION

The results of this study show that the effects of *L. gracilis* and *L. sidoides* essential oils and thymol and carvacrol in reproductive efficiency is due changes in morphology of the reproductive organs, decreasing the ovarian viability. The action of these essential oils and monoterpenes showed that, even at low concentrations, these components were able to cause changes in the germinative cells.

All changes caused by tested essential oils and their majority component were dose-dependent and can be characterized mainly by decrease in size, vacuoles in cytoplasm, chorion deformation, loss of granulation and fusion of yolk granules. These alterations had previously been described in tick-reproducing organs tested with fipronil (Oliveira et al., 2008), permethrin (Roma et al., 2010), *Ricinus communis* oil (Arnosti et al., 2011), *Melia azedarach* extract (Sousa et al., 2013) and thymol (Matos et al., 2014). Previously studies showed potent action of essential oils of *L. sidoides* (Gomes et al., 2012;

Monteiro et al., 2014; Soares et al. 2016), *L. gracilis,* thymol and carvacrol (Cruz et al., 2013; Soares et al., 2016) in cattle tick reproduction, reducing oviposition of *R. microplus* females. Our results confirm the action of *L. sidoides* and *L. gracilis* essential oils in oviposition of *R. microplus* females.

Oocytes in the early stage of development, mainly oocytes II presented some vacuoles in the cytoplasm, which moved the germ vesicle to the cell pole near the pedicel. At higher concentrations, the cytoplasmic vacuolation process becomes more intense. It is suggested that these vacuoles are autophagic and are responsible for the degradation of organelles that have been damaged by the action of the essential oils and monoterpenes tested. Similar results were observed in studies with fipronil (Oliveira et al., 2008), permethrin (Roma et al., 2010), *Ricinus communis* (Arnosti et al., 2011) and thymol (Matos et al.,2014) for *Rhipicephalus sanguineus* females. The presence of vacuoles and cell disorganization in oocytes indicated cellular toxicity caused by chemical compounds of *L. sidoides* and *L. gracilis* essential oils. Vacuolation is due changes in cell physiology as detoxification mechanism, so that the cells continue their metabolic processes. Moreover, organelles no ability to perform its functions may be present (Arnosti et al. 2011).

In oocytes III and IV, in all treated groups, more significant changes were observed, such as reduction of size of cells, vacuoles since periphery to the cell center, around yolk granules and germ vesicle and loss of yolk granules. These changes were more intense in higher concentrations of tested substances. In oocytes III of *R. microplus* it is suggested the production of endogenous elements and exogenous incorporation of vitellogenic elements leading to development of these oocytes (Saito et al., 2005). In our results, the smaller size of oocytes III exposed to treatments with LGRA-106, LGRA-201 and LSDI-103 essential oils can be due reduction of synthesis and incorporation of vitellin elements by cells, preventing the development of these oocytes.

Vitellogenin is a protein that is 11% of the concentration of total protein in the hemolymph (Soneshine et al. 2013). Its concentration increases with female engorgement and this regulation occurs through the uptake of vitellogenin by oocytes (Seixas et al., 2010). Its suggest that the compounds used in this study interferes in hormonal regulation leading to decrease in production of vitellogenic elements and reduces the release of vitellogenin though inhibition of ecdisteroids hormones, which can be related with loss of yolk granules founded mainly in oocytes III treated with LGRA-201 (7.65mg/mL) e carvacrol (5.04mg/mL), however, other studies are necessary to confirm this hypothesis.

Thick chorion was observed in oocytes III and IV treated with LGRA-106 (8.15mg/mL) and LGRA-201 (7.65mg/mL) and oocytes V treated with LGRA-201 (7.65mg/mL), LSDI-102 (2.80mg/mL), LSDI-103 (4.31mg/mL and 7.21mg/mL), thymol

(5.49mg/mL and 5.77mg/mL) and carvacrol (5.04mg/mL). The chorion is a membrane that protects the oocytes from adverse conditions (Saito et al., 2005; Oliveira et al., 2005). In our studies, increased chorion thickness in oocytes III, IV and V may be a mechanism of cellular defense against the toxicity of the substances used in the treatments.

In addition to the presence of vacuoles throughout the cytoplasm, the fusion of yolk granules in oocytes V were evident in all group tested. It was also observed in oocytes V treated with LGRA-201, thymol and carvacrol in the highest concentrations, disruption of yolk granules with releasing of your contents into cell cytoplasm, changing its original shape. These results corroborate with studies with *R. sanguineus* female exposed with fipronil (Oliveira et al., 2008) and permethrin (Roma et al., 2010).

Thymol was the only one compound that caused changes in oocytes I. However, in the other oocytes, the severity of injuries caused by carvacrol was greater. Studies about the reproductive efficiency of *R. microplus* female showed greater efficacy of carvacrol when compared with thymol but there was no statistical difference between them (Cruz et al., 2013).

Monoterpenes are composed of lipophilic molecules are able to penetrate the cell membrane, increasing their fluidity and permeability. Under physiological conditions phenolic compounds can dissociate leading to accumulation of their polar portion near the membrane (Herrmann and Wink, 2011; Matos et al., 2014). The chorionic layer has protective function against mechanical stimuli and begins its formation in the stage of oocytes III, getting denser eggs, preventing their predation and drying and allowing gas exchange (Oliveira et al., 2005). Our studies showed irregularities of plasmic membrane and chorion deformation in mature oocytes because the action of chemical components thymol and carvacrol, which are phenolic compounds that that are changing membrane permeability and layer, allowing the action of essential oils.

The changes caused by action of *L. gracilis* and *L. sidoides* essential oils in tested ovaries were more severe than those caused by thymol and carvacrol used alone suggesting synergic effect among chemical compounds present in essential oils and an important role of minority compounds in this action. These results confirm those found in previous studies by our research group, in which these oils had a greater effect on the reproductive efficiency of *R. microplus* females than their main components (Cruz et al., 2013; Soares et al., 2016).

In conclusion, the exposure of *R. microplus* engorged females to *L. gracilis* and *L. sidoides* essential oils and its main components affects the embryonic development of this parasite for interfering in its vitellogenesis. Therefore, the tested components may be potent products for the cattle tick control.

REFERENCES

ADAMS, R.P. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. **Allured Publishing Corporation**, Illinois. pp 804, 2007.

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. Acaricidal activity of thymol against larvae of *Rhipicephalus microplus* (Acari: Ixodidae) under semi-natural conditions. **Parasitology Research**, v.114, n.9, p.3271-6, 2015.

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

ARNOSTI, A.; BRIENZA, P.D.; FURQUIM, K.C.; CHIERICE, G.O.; BECHARA, G.H.; CAL-LIGARIS, I.B.; CAMARGO-MATHIAS, M.I. Effects of ricinoleic acid esters from castor oil of *Ricinus communis* on the vitellogenesis of *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) ticks. **Experimental Parasitology**, v.127, p.575–580, 2011.

BORGES, L.M.; FERRI, P.H.; SILVA, W.J.; SILVA, W.C.; SILVA, J.C. In vitro efficacy of extracts of *Melia azedarach* against the tick *Boophilus microplus*. **Medical and Veterinary Entomology**, v.172, p.28–31, 2003.

CRUZ, E.M.; COSTA-JÚNIOR, L.M.; PINTO, J.A.; SANTOS, D.A.; ARAUJO, S.A.; ARRIGONI-BLANK, M.F.; BACCI, L.; ALVES, P.B.; CAVALCANTI, S.C.; BLANK, A.F. Acaricidal activity of *Lippia gracilis* essential oil and its major constituents on the tick *Rhipicephalus* (*Boophilus*) *microplus*. **Veterinary Parasitolology**, v.195, n.1-2, p.198-202, 2013.

COSTA-JÚNIOR, L.M.; MILLER, R.J.; ALVES, P.B.; BLANK, A.F.; LI, A.Y.; PÉREZ DE LÉON, A.A.; 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.

DRUMMOND, R. O., ERNST, S. E., TREVINO, J. L., GLADNEY, W. J., GRAHAM, O. H. *Boophilus annulatus* and *Boophilus microplus*: Laboratory test of insecticides. **Journal Economical Entomology**, v.66, n.1, p.130-133, 1973.

GOMES, G.A.; OLIVEIRA-MONTEIRO, C.M.; JULIÃO, L.S.; MATURANO, R.; SENRA, T.O.S.; ZERINGOTA, V.; CALMON, F.; MATOS, R.S.; DAEMON, E.; CARVALHO, G.C.. Acaricidal activity of essential oil from *Lippia sidoides* on unengorged larvae and nymphs of *Rhipicephalus sanguineus* (Acari: Ixodidae) and *Amblyomma cajennense* (Acari: Ixodidae). **Experimental Parasitoogy**, v.137, p.41-45, 2014.

GOMES, G.A., OLIVEIRA-MONTEIRO, C.M.; SENRA, T.O.S.; ZERINGOTA, V.; CALMON, F.; MATOS, R.S.; DAEMON, E.; GOIS, R.W.S.; SANTIAGO, G.M.P.; CARAVALHO, G.C. 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**, v.111, p.2423-2430, 2012.

GRISI, L.; LEITE, R.C.; MARTINS, J.R.S.; BARROS, A.T.M.; ANDREOTTI, R.; CANÇADO, P.H.D.; PÉREZ DE LEÓN, A.A.; PEREIRA, J.B.; VILLELA, H. S. Reassessment of the potential economic impact of cattle parasites in Brazil. **Brazilian Journal of Veterinary Parasitology**, v.23, n.2, p.150-156, 2014.

GUERRERO, F.D.; LOVIS, L.; MARTINS, J. R. Acaricide resistance mechanisms in *Rhipicephalus (Boophilus) microplus*. **Brasilian Journal of Veterinary Parasitology**, v.21, n.1, p.1-6, 2012.

HERRMANN, F.; WINK, M. Synergistic interactions of saponins and monoterpenes in HeLa cells, Cos7 cells and in erythrocytes. **Phytomedicine**, v.18, p.1191-1196, 2011.

JONSSON, N.N. The productivity effects of cattle tick (Boophilus microplus) infestation on cattle, with particular reference to Bos indicus cattle and their crosses. **Veterinary Parasitology**, v.137, n.1-2, p.1-10, 2006.

LORENZI, H.; MATOS, F.J.A. Plantas medicinais no Brasil: nativas e exóticas. 2.ed. Nova Odessa: **Plantarum**, 544p, 2008.

MATOS, R.S.; DAEMON E.; CAMARGO-MATHIAS, M.I.; FURQUIM, K.C.S.; SAMPIERI, B.R.; REMEDIO, R.N.; ARAÚJO, L.X.; NOVATO, T.P.L. Histopathological study of ovaries of *Rhipicephalus sanguineus* (Acari: Ixodidae) exposed to different thymol concentrations. **Parasitology Research**, v.113, p.4555–4565, 2014.

MONTEIRO, C.M.A.; ARAÚJO, L.X.; GOMES, G.A.; SENRA, T.O.S.; CALMON, F.; DAEMON, E.; CARVALHO, M.G.; BITTENCOURT, V.R.E.P.; FURLONG, J.; PRATA, M.C.A. Entomopathogenic nematodes associated with essential oil of Lippia sidoides for control of Rhipicephalus microplus (Acari: Ixodidae). **Parasitolology Research**, v.113, p.189–195, 2014.

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

OLIVEIRA, P.R.; BECHARA, G.H.; DENARDI, S.E.; NUNES, E.T.; CAMARGO-MATHIAS, M.I. Morphological characterization of the ovary and oocytes vitellogenesis of the tick *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae). **Experimental Parasitology**, v.110, n.2, p. 146-156, 2005.

OLIVEIRA, P.R.; BECHARA, G.H.B.; CAMARGO-MATHIAS, M.I. Evaluation of cytotoxic effects of fipronil on ovaries of semi-engorged *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) tick female. **Food and Chemical Toxicology**, v.46, p.2459–2465, 2008.

ROMA, G.C.; FURQUIM, K.C.; BECHARA, G.H.; CAMARGO-MATHIAS, M.I. Permethrin-induced morphological changes in oocytes of *Rhipicephalus sanguineus* (Acari: Ixodidae) semi-engorged females. **Food and Chemical Toxicology**, v.48, p.825–830, 2010.

SAITO, K.C., BECHARA, G.H., OLIVEIRA, P.R., NUNES, E.T., DENARDI, S.E., CAMARGO-MATHIAS, M.I. Morphological, histological, and ultrastructural studies of the ovary of the tick *Boophilus microplus* (Canestrini, 1887) (Acari: Ixodidae). **Veterinary Parasitology**, v.129, 299–311, 2005.

SEIXAS, A.; OLDIGES, D.P.; VAZ, I.S.J.R.; TERMIGNONI, C. Endocrinologia e controle da vitelogênese em carrapatos. **Acta Science Veterinary**, v.38, n.2, p.95-111, 2010.

SOARES, A.M.; PENHA, T.A.; ARAÚJO, S.A.; CRUZ, E.M.; BLANK, A.F.; COSTA-JUNIOR, L.M. Assessment of different Lippia sidoides genotypes regarding their acaricidal activity against *Rhipicephalus* (*Boophilus*) *microplus*. **Brazilian Journal of Veterinary Parasitology**, v.25, n.4, p.401-405, 2016.

SOUSA, L.A.; ROCHA, T.L.; SABÓIA-MORAIS, S.M.; BORGES, L.M. Ovary histology and quantification of hemolymph proteins of *Rhipicephalus* (*Boophilus*) *microplus* treated with *Melia azedarach.* **Revista Brasileira de Parasitologia Veterinária**, v.22, n.3, p.339-345, 2013.

SONENSHINE, D.E. Biology of ticks, v. 1. 2Ed. Oxford University, New York, pp. 280–303, 2013.

VAN DEN DOOL, H., KRATZ, P.D. A generalization of retention index system including linear temperature programmed gas–liquid partition chromatography. **Journal of Chromatography**, v.11, p.463–471, 1963.

Table 1: *Lippia gracilis* and *Lippia sidoides* genotypes maintained at the Active Germplasm Bank of Medicinal Plants (UFS) and used in the present study.

Code	Origin (State/Country)	Geographical data	Voucher N
LGRA106	Sergipe/Brazil	11 19’ 16.7” S; 37 55’ 09.2” W	14733
LGRA201	Bahia/Brazil	11 19’ 22.4” S; 37 55’ 12.6” W	14734
LSID102	Sergipe/Brazil	9 58' 07 6'' S; 37 51' 49.2'' W	8224
LSID103	Sergipe/Brazil	9 58' 08.6'' S; 37 51' 50.3'' W	8225

Table 2: Concentrations of *Lippia gracilis* and *Lippia sidoides* essential oils and thymol and carvacrol used in adult immersion test of *Rhipicephalus microplus*.

	Concentrations (mg/mL)	
	LC_{50}	LC_{75}
LGRA106	4,66	8,15
LGRA201	6,55	7,65
LSID102	2,80	4,66
LSID103	4,31	7,21
THYMOL	5,49	5,77
CARVACROL	4,46	5,04
DMSO 3%	Negative control	

Table 3: Histological changes caused by *Lippia sidoides*, *Lippia gracilis*, thymol and carvacrol essential oils.

	Lippia gracillis		*Lippia sidoides*		Thymol	Carvacrol
	106	201	102	103		
Oocyte I	-	-	-	-	Irregular cell limit	-
Oocyte II	Vacuolation	Vacuolation; Irregular cell limit	Vacuolation; Irregular cell limit	Vacuolation	Vacuolation; Irregular cell limit;	Irregular cell limit; Vacuolation;
Oocyte III	Vacuolation; Thick chorion; Small size	Vacuolation; Thick chorion; Small size	Vacuolation; Thick chorion;	Vacuolation; Thick chorion; Small size	Vacuolation; Fine chorion	Cytoplasm disorganization; Vacuolation
Oocyte IV	Vacuolation; Thick chorion	Vacuolation; Fusion of yolk granules; Thick chorion	Vacuolation; Fusion of yolk granules; Thick chorion	Vacuolation	Vacuolation; Irregular cell limit	Vacuolation
Oocyte V	Vacuolation	Vacuolation; Fusion of yolk granules; Cytoplasm disorganization	Vacuolation; Fusion of yolk granules; Thick chorion	Vacuolation; Fusion of yolk granules; Thick chorion	Vacuolation; Fusion of yolk granules; Cytoplasm disorganization; Thick chorion	Vacuolation; Fusion of yolk granules; Cytoplasm disorganization; Thick chorion

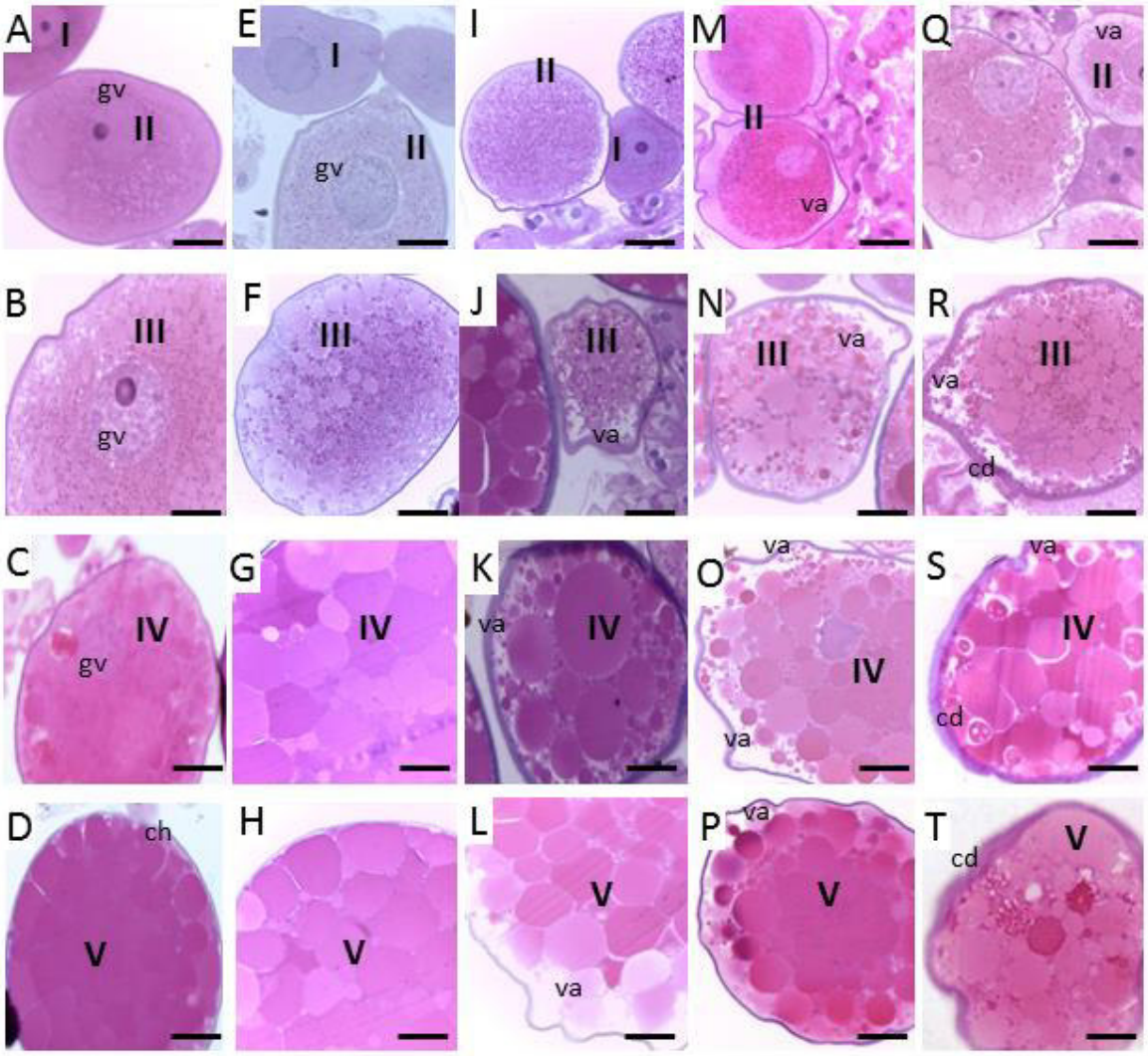

Figure 1: Histological sections of *Rhipicephalus microplus* oocytes stained with hematoxylin and eosin (HE). (A-D) Oocytes of control group (DMSO 3%). (E-H) Detail of oocytes treated with 4.66mg/mL of *Lippia gracilis* genotype 106 essential oil. (I-L) Detail of oocytes treated with 8.15mg/mL of *L. gracilis* genotype 106 essential oil. (M-P) Detail of oocytes treated with 6.65mg/mL of *L. gracilis* genotype 201 essential oil. (Q-T) Detail of oocytes treated with 7.65mg/mL of *L. gracilis* genotype 201 essential oil. I- oocyte I; II- oocyte II; III- oocyte III; IV- oocyte IV; V- oocyte V; gv- germ vesicle; ch- chorion; va- vacuole; cd- chorion deformation. Bars: 20µm.

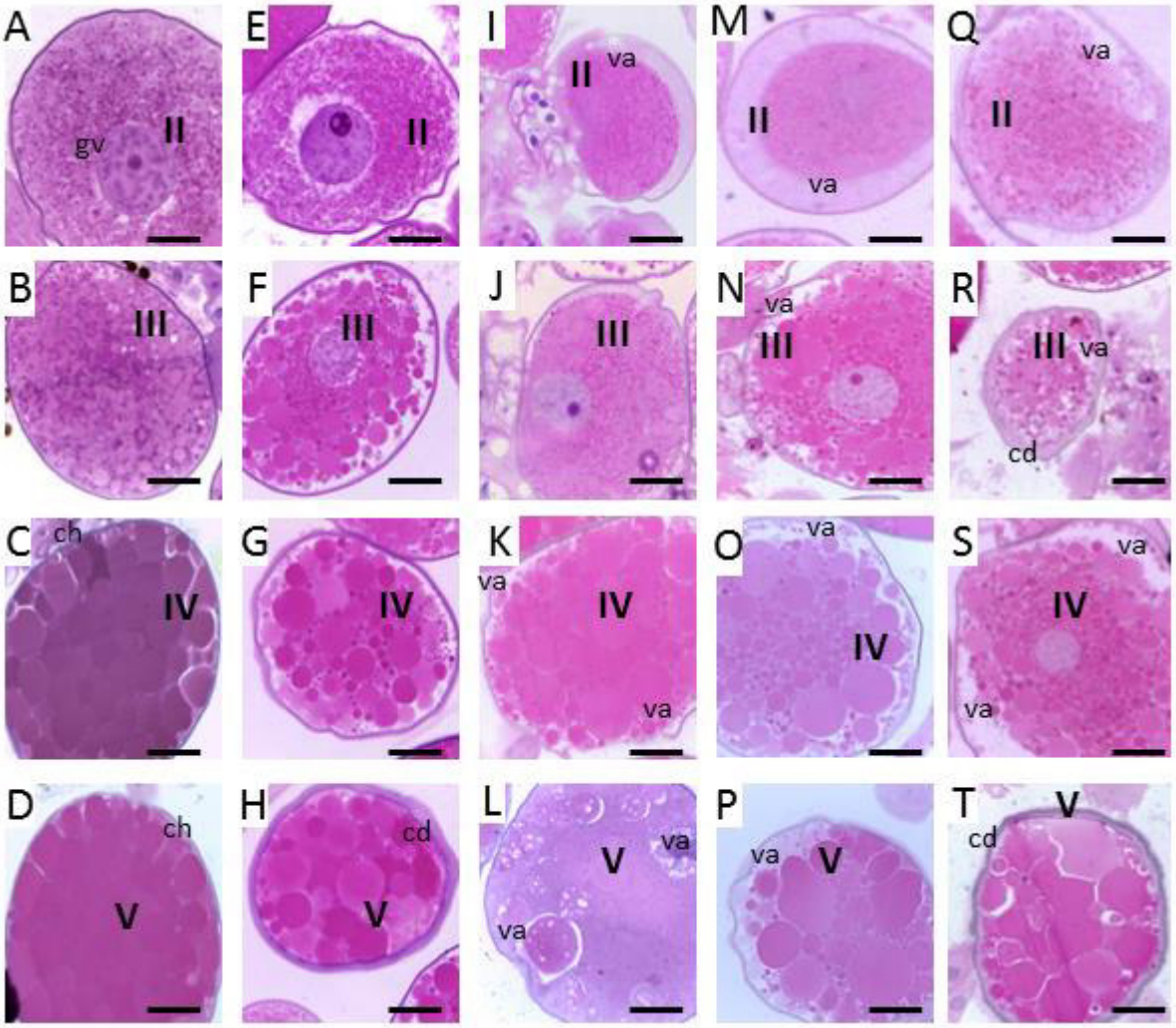

Figure 2: Histological sections of *Rhipicephalus microplus* oocytes stained with hematoxylin and eosin (HE). (A-D) Oocytes of control group (DMSO 3%). (E-H) Detail of oocytes treated with 2.80mg/mL of *Lippia sidoides* genotype 102 essential oil. (I-L) Detail of oocytes treated with 4.66mg/mL of *L. sidoides* genotype 102 essential oil. (M-P) Detail of oocytes treated with 4.31mg/mL of *L. sidoides* genotype 103 essential oil. (Q-T) Detail of oocytes treated with 7.21mg/mL of *L. sidoides* genotype 103 essential oil. II- oocyte II; III- oocyte III; IV- oocyte IV; V- oocyte V; gv- germ vesicle; ch- chorion; va- vacuole; cd- chorion deformation. Bars: 20µm.

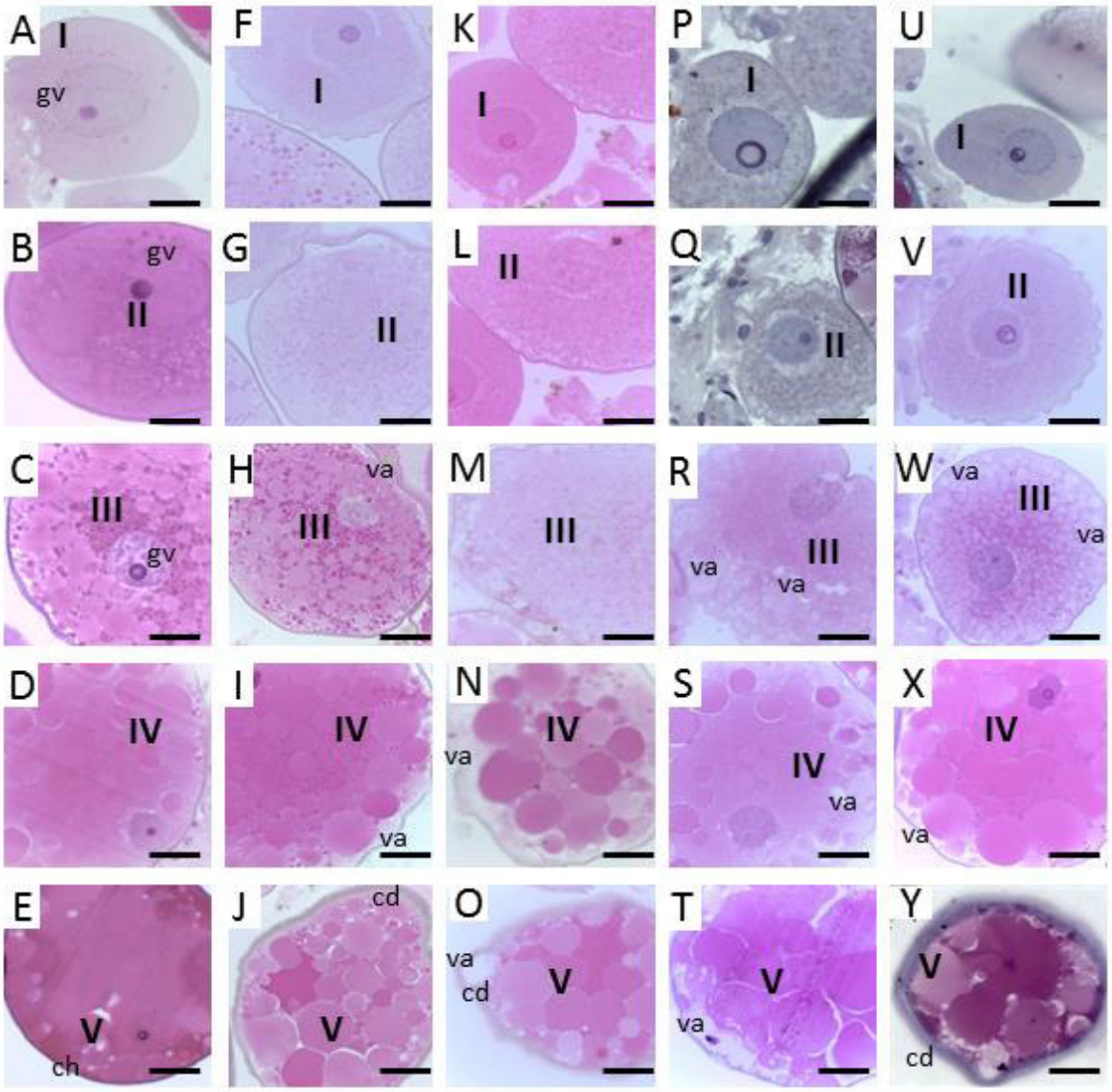

Figure 3: Histological sections of *Rhipicephalus microplus* oocytes stained with hematoxylin and eosin (HE). (A-E) Oocytes of control group (DMSO 3%). (F-J) Detail of oocytes treated with 5.49mg/mL of thymol. (K-O) Detail of oocytes treated with 5.77mg/mL of thymol. (P-T) Detail of oocytes treated with 4.46mg/mL of carvacrol. (T-X) Detail of oocytes treated with 5.04mg/mL of carvacrol. I- oocyte I; II- oocyte II; III- oocyte III; IV- oocyte IV; V- oocyte V; gv- germ vesicle; ch- chorion; va- vacuole; cd- chorion deformation. Bars: 20µm.

4 CONSIDERAÇÕES FINAIS

- ✓ Os óleos essenciais de *Lippia gracilis* e *L. sidoides* são potentes compostos bioativos, portanto, a produção dessas espécies de plantas é promissora para o mercado de óleos essenciais com fins terapêuticos;
- ✓ Os óleos essenciais de *L. sidoides* e *L. gracilis* e seus componentes químicos apresentam potencial para produção de produtos carrapaticidas para diferentes fases de desenvolvimento de *R. microplus*;
- ✓ As alterações morfológicas rm ovários de *R. microplus* provocadas pelos óleos estudados e seus componentes majoritários permitiu elucidar o mecanismo de ação dessas substâncias;
- ✓ Apesar da ação dos óleos essenciais serem atribuídas aos compostos majoritários, deve ser dada atenção aos componentes encontrados em concentrações menores já que os mesmos podem estar interferindo na ação dos compostos de maiores concentrações.