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ATIVIDADE INSETICIDA DE COMPOSTOS DE ORIGEM VEGETAL EM *Mahanarva spectabilis* (Distant, 1909) (HEMIPTERA: CERCOPIDAE)

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2020

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas – Comportamento e Biologia Animal, Área de Concentração: Comportamento e Biologia Animal da Faculdade de Ciências Biológicas da Universidade Federal de Juiz de Fora como requisito parcial para obtenção do grau de doutora.

Orientador: Prof. Dr. Alexander Machado Auad

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“A História está repleta de pessoas que, como resultado do medo, ou por ignorância, ou por cobiça de poder, destruíram conhecimentos de imensurável valor que em verdade pertenciam a todos nós. Nós não devemos deixar isso acontecer de novo. ”

(Carl Sagan)

RESUMO GERAL

A cigarrinha das pastagens *Mahanarva spectabilis* (Distant, 1909) (Hemiptera: Cercopidae) pode limitar a produção das forrageiras. O uso de gramíneas resistentes e aplicação de inseticidas sintéticos nos adultos são alguns dos métodos recomendados para o controle desse inseto praga. Inseticidas botânicos são apontados como alternativas aos inseticidas químicos sintéticos no manejo de pragas por serem de baixa toxicidade para o ambiente e para a saúde humana. Neste contexto, a presente tese está estruturada de forma a desenvolver e avaliar um método alternativo, menos laborioso e confiável, para investigar a atividade inseticida com uso de constituintes puros de origem vegetal e, a partir desses resultados, avaliar as atividades inseticidas do timol, carvacrol, eugenol, cinamaldeído e trans-anetol sobre os ovos, ninfas e adultos de *M. spectabilis* em condições de laboratório. O método alternativo proposto na pesquisa para teste com bioinseticidas não foi o ideal, devido a alta mortalidade dos adultos no tratamento controle. Após estabelecida a metodologia eficiente, verificou-se que as concentrações mais eficazes de timol foram 1,2; 2,5 e 5,0% ocasionando, respectivamente, 97, 86 e 98% de inviabilidade de ovos e mortalidade de ninfas e adultos. Eugenol, carvacrol e timol apresentaram eficiências superiores a 85% na avaliação realizada 48 horas após a aplicação das soluções em ovos de *M. spectabilis*. Nos testes com as ninfas do inseto praga, timol e carvacrol a 2,5% e eugenol a 2,0 e 2,5% apresentaram eficiências intermediárias, com valores acima de 61%. A maior eficiência (95%) foi constatada com trans-anetol a 2,5%. Já nos testes com adultos, apenas o tratamento com trans-anetol a 2,5% obteve eficácia de até 90%, enquanto nos demais tratamentos as taxas de eficiência não ultrapassaram 51%. Assim, neste estudo conseguimos definir uma metodologia para testes com produtos bioinsetidas sobre *M. spectabilis*, utilizando o timol como modelo e evidenciando seu potencial no controle deste inseto-praga. E, levando-se em consideração que o controle desse inseto deve se concentrar em diminuir o número de ninfas e adultos e que somente são considerados eficientes os inseticidas que atingirem um valor médio de 90% (± 10%) de mortalidade, recomendamos o trans-anetol como potencial inseticida natural para o controle de ninfas e adultos de *M. spectabilis* devido à alta taxa de atividade inseticida demonstrada. Ademais, estes compostos podem ser uma alternativa aos inseticidas sintéticos e levar ao desenvolvimento de novas classes de bioinseticidas.

Palavras-chave: Cigarrinha-das-pastagens. MIP. Bioinseticidas.

GENERAL ABSTRACT

Spittlebug *Mahanarva spectabilis* (Distant, 1909) (Hemiptera: Cercopidae) can limit forage production. The use of resistant grasses and application of synthetic insecticides in adults are some of the recommended methods for the control of this insect pest. Botanical insecticides are considered as alternatives to synthetic chemical insecticides in pest management because they are of low toxicity to the environment and human health. In this context, the present thesis is structured to develop and evaluate an alternative method, less laborious and reliable, to investigate the insecticidal activity using pure constituents of plant origin and, from these results, to evaluate the insecticidal activities of thymol, carvacrol, eugenol, cinnamaldehyde and trans-anethole on *M. spectabilis* eggs, nymphs and adults under laboratory conditions. The alterative method proposed in the research for bioinsecticide testing was not ideal due to the high mortality of adults in the control treatment. After establishing the efficient methodology, it was found that the most effective thymol concentrations were 1.2; 2.5 and 5.0% leading, respectively, to 97, 86 and 98% of egg unviability and mortality of nymphs and adults. Eugenol, carvacrol and thymol showed efficiencies higher than 85% in the evaluation performed 48 hours after the application of solutions in *M. spectabilis* eggs. In the tests with insect pests, 2.5% thymol and carvacrol and 2.0 and 2.5% eugenol showed intermediate efficiencies, with values above 61%. The highest efficiency (95%) was found with 2.5% trans-anethole. In adult tests, only 2.5% trans-anethole treatment was effective up to 90%, while in other treatments the efficiency rates did not exceed 51%. Thus, in this study we were able to define a methodology for testing bioinserted products on *M. spectabilis*, using thymol as a model and highlighting its potential to control this insect pest. And considering that the control of this insect should focus on reducing the number of nymphs and adults and that only insecticides that achieve an average mortality of 90% (± 10%) are considered efficient, we recommend trans-anethole as a natural insecticide potential for the control of *M. spectabilis* nymphs and adults due to the high rate of insecticide activity demonstrated. Moreover, these compounds may be an alternative to synthetic insecticides and lead to the development of new classes of bioinsecticides.

Keywords: Spittlebugs. IPM. Biopesticides

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PRIMEIRA PARTE

INTRODUÇÃO GERAL

As cigarrinhas-das-pastagens são consideradas as principais pragas associadas às forrageiras, causando grandes prejuízos à pecuária leiteira e de corte no Brasil (SOUZA, SILVA, *et al.*, 2008). Estes hemípteros podem promover a redução do crescimento, queda da produção de matéria seca, bem como da qualidade da forrageira, constituindo um problema relevante dentro da bovinocultura em toda América Tropical (VALÉRIO e NAKANO, 1988; SOUZA, SILVA, *et al.*, 2008). Os danos provocados pelas cigarrinhas variam para cada espécie de gramínea, sendo que os prejuízos econômicos podem atingir cifras alarmantes dependendo da região, condições climáticas e manejo (BERNARDO, ROCHA, *et al.*, 2003). Estima-se que as perdas que estes insetos têm causado variam de US$ 840 a US$ 2,1 milhões de dólares por ano em todo mundo (THOMPSON, 2004).

As espécies de cigarrinhas de maior ocorrência no Brasil são *Notozulia entreriana* (Berg, 1879), *Deois flavopicta* (Stall, 1854), *Deois schach* (Fabricius, 1787), *Deois incompleta* (Walker, 1851), *Aeneolamia selecta* (Walker, 1858) (VALÉRIO e KOLLER, 1992), *Mahanarva fimbriolata* (Stål, 1854) e *Mahanarva spectabilis* (Distant, 1909) (AUAD, SIMÕES, *et al.*, 2007). Entre as principais espécies, *M. spectabilis* é considerada uma praga limitante na produção de gramíneas forrageiras, como o capim-elefante (*Pennisetum purpureum* Schum.) (AUAD, SIMÕES, *et al.*, 2007). O ataque das ninfas de cigarrinha causam prejuízos consideráveis podendo levar a planta a um desequilíbrio hídrico (OLIVEIRA e ALVES, 1988). No entanto, as principais injúrias são causadas pelos adultos que, ao sugarem a seiva das folhas, inoculam toxinas causando fitotoxemia na planta perceptível através de pontos amarelos que se tornam avermelhados e posteriormente evoluem para estrias cloróticas longitudinais reduzindo a capacidade fotossintética da planta e resultando na queima das pastagens (NAVES, 1980; NILAKHE, 1982; COSENZA, ANDRADE, *et al.*, 1983; REIS, BOTELHO e MENDES, 1983; VALÉRIO, WIENDL e NAKANO, 1988; VALÉRIO, 2009). Ademais, essas toxinas resultantes da alimentação conferem impalatabilidade às pastagens, dificultando a alimentação do gado (PACHECO, 1982). Assim, o ataque severo e por tempo prolongado das cigarrinhas-das-pastagens afetam significativamente a produção e qualidade da forragem (AGUIRRE, CARDONA, *et al.*, 2013).

Os principais métodos de Manejo Integrado de Pragas realizados com cigarrinha, segundo Valério e Koller (1992), são: inclusão de gramíneas resistentes às cigarrinhas-das-pastagens; diminuição da altura da gramínea e da quantidade de palha acumulada ao nível do solo, resultando em condições desfavoráveis ao desenvolvimento e sobrevivência de ovos e ninfas; aplicação do fungo *Metarhizium anisopliae* e aplicação de inseticidas químicos nos adultos. Entretanto, o controle químico dessa praga é considerado economicamente e ecologicamente impraticável, além disso, o uso desses produtos levam ao acúmulo de resíduos (FERRUFINO e LAPOINT, 1989; VALÉRIO, 2005). Ademais, esses inseticidas podem dizimar populações inteiras de organismos não-alvo (SARWAR, 2015).

Diversas estratégias de controle para *M. spectabilis* vêm sendo pesquisadas nos últimos anos nos campos de resistência de plantas, controle biológico, controle cultural, assim como o comportamento, tendo como exemplo os trabalhos de RESENDE, AUAD, *et al.*, 2012; CAMPAGNANI, CAMPOS, *et al.*, 2017; SILVA, AUAD, *et al.*, 2017; VERÍSSIMO, AUAD, *et al.*, 2018; ALVARENGA, AUAD, *et al.*, 2019a; ALVARENGA, AUAD, *et al.*, 2019b e SILVA, AUAD, *et al.*, 2019, no entanto, ainda não há nenhum produto inseticida registrado para ser agregado ao controle dessa espécie. Em vista disso, um recurso alternativo promissor é o uso de inseticidas botânicos que têm sido apontados como uma alternativa atraente no manejo de pragas quando comparados aos inseticidas químicos sintéticos em razão de representarem pouca ameaça para o ambiente e para a saúde humana. (ISMAN, 2006). De acordo com Mazzonetto e Vendramim

(2003), o uso de métodos alternativos podem favorecer, principalmente, o pequeno agricultor já que são de fácil utilização, não exigem pessoal qualificado e são mais baratos.

Inseticidas botânicos possuem vantagens ecotoxicológicas em comparação com os tradicionais inseticidas sintéticos devido a presença de particularidades pertinentes (baixa toxicidade humana, rápida degradação e impacto ambiental reduzido), que os tornam inseticidas adequados para agricultura orgânica (BOURGUET, GENISSEL e RAYMOND, 2000; CHERMENSKAYA, STEPANYCHEVA, *et al.*, 2010). Esses inseticidas possuem em sua composição metabólitos secundários, tais como alcalóides, amidas, chalconas, flavonas, fenóis, lignanas, neolignanas ou kavapironas que são importantes em interações planta-inseto e podem ser utilizadas em programas de manejo integrado de pragas (PARMAR, JAIN, *et al.*, 1997; ISMAN, 2006; MARTÍNEZ, PLATA-RUEDA, *et al.*, 2015). Eles agem como repelentes com odores desagradáveis ou irritantes, reguladores de crescimento, ação deterrente na oviposição e alimentação e atividade biocida (BOURGUET, GENISSEL e RAYMOND, 2000; CHERMENSKAYA, STEPANYCHEVA, *et al.*, 2010; MARTÍNEZ, PLATA-RUEDA, *et al.*, 2015).

A composição do óleo vegetal é bastante complexa, podendo conter de 30 a 65 constituintes, aproximadamente, em concentrações variadas (BAKKALI, AVERBECK, *et al.*, 2008; SENTHIL-NATHAN, 2013; PAVELA, 2015). Os constituintes principais são apresentados em 25% a 60% da concentração em comparação com outros componentes presentes em quantidades mínimas (BAKKALI, AVERBECK, *et al.*, 2008). Os compostos utilizados neste estudo são tidos como componentes majoritários e podem ser encontrados nos óleos essenciais de plantas de diversas famílias. Como por exemplo, o óleo essencial de tomilho (*Thymus vulgaris*) que possui o timol (40.04%) e o carvacrol (2.45%) como primeiro e segundo componentes presentes em maior quantidade, o óleo de cravo da Índia (*Syzygium aromaticum*) que apresenta o eugenol (91.3%) como componente principal , o óleo de canela (*Cinnamomum verum*) que contém o cinamaldeído

(90.17%) como componente majoritário e, por fim, o óleo essencial de anis estrelado (*Illicium verum*) que possuí o trans-anetol (90.1%) como componente em maior quantidade (MÈTOMÈ, ADJOU, *et al.*, 2017; PARK, JEON, *et al.*, 2017; CHANSANG, CHAMPAKAEW, *et al.*, 2018; DA ROCHA VORIS, DOS SANTOS DIAS, *et al.*, 2018).

Óleos essenciais de plantas e seus componentes principais possuem atividade inseticida significativa contra diversas espécies de insetos, havendo diversas pesquisas que os intitulam como potenciais pesticidas ecológicos (BAKKALI, AVERBECK, *et al.*, 2008; KIM, YANG e LEE, 2013; SENTHIL-NATHAN, 2015). Como por exemplo, a pesquisa realizada por Tak, Jovel e Isman (2016), que avaliaram a atividade inseticida do timol em *Trichoplusia ni* (Hübner, 1803) (Lepidoptera: Noctuidae) exibindo atividade significativa sobre essa praga (LD50= 32.6), da mesma forma o timol e também carvacrol foram testados quanto suas propriedades inseticidas em *Pochazia shantungensis* (Chou; Lu, 1977) (Hemiptera: Ricaniidae) (LD50= 28.52, 56.74 mg/L) considerados promissores como inseticidas sobre adultos e ninfas desta espécie (PARK, JEON, *et al.*, 2017), igualmente, o óleo de *Syzygium* aromaticum, rico em eugenol, foi testado sobre *Callosobruchus maculatus* (Fabr., 1775) (Coleoptera: Chrysomelidae) e apresentou mortalidade de 20% sobre o inseto (MÈTOMÈ, ADJOU, *et al.*, 2017), já a eficácia do cinamaldeído foi testada contra *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) (CHANSANG, CHAMPAKAEW, *et al.*, 2018) (LD50= 3.49 µg/mg) mostrando seu potencial como inseticida naturai alternativo sobre o mosquito e, por fim, a atividade biológica do extrato de *Illicium verum*, rico em trans-anetol, foi avaliada sobre *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (LC50= 0.266 mg/L) sendo considerado promissor no desenvolvimento de pesticidas para o manejo desta praga (LI, ZHOU, *et al.*, 2017).

Assim, considerando às propriedades destas classes de substâncias naturais e estudos existentes que comprovam a atividade dos monoterpenos timol, carvacrol e os fenilpropanóides eugenol, cinamaldeído e trans-anetol como antioxidantes, antimicrobianos, acaricidas, repelentes, e / ou inseticidas (LETIZIA, COCCHIARA, *et al.*, 2003; ALI, KHAN, *et al.*, 2005; YANG, LEE, *et al.*, 2005; LIMA, MORAES, *et al.*, 2008; DOLAN, JORDAN, *et al.*, 2009; CETIN, CILEK, *et al.*, 2010; NA, KIM, *et al.*, 2011; SOUTO, HARADA e DE SOUZA MAIA, 2011; ZENG, ZHU, *et al.*, 2011), essas substâncias foram escolhidas com a finalidade de testar sua atividade inseticida sobre *M. spectabillis*. Até o momento, nenhum estudo foi feito para avaliar o desempenho dessa espécie de cigarrinha na presença de nenhuma destas substâncias provenientes de plantas. Portanto, este estudo tem como objetivos determinar a metodologia adequada para aplicação tópica das soluções e avaliar a toxicidade dos constituintes puros timol e carvacrol, eugenol , cinamaldeído e trans-anetol em ovos, ninfas e adultos de *M. spectabilis* em condições de laboratório.

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SEGUNDA PARTE - ARTIGOS

ARTIGO 1: INSECTICIDAL EFFECTS OF THYMOL ON *Mahanarva spectabilis* (HEMIPTERA: CERCOPIDAE) IN TWO EVALUATION METHODOLOGIES

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Insecticidal effects of thymol on *Mahanarva spectabilis* (Hemiptera: Cercopidae) in two evaluation methodologies

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Abstract

The objective of this study was to develop and evaluate an alternative, less laborious and reliable method for investigating the insecticidal effect of thymol on *Mahanarva spectabilis* (Hemiptera: Cercopidae). Two methods for evaluating the effects of thymol on *M. spectabilis* (conventional and alternative) were tested for optimum survival rates. The conventional method proved to be the most suitable for investigating the insecticidal effect of thymol on *M. spectabilis*. Thymol concentrations of 1.2, 2.5, and 5.0% resulted in the highest mortality rates of eggs, nymphs and adults, respectively. Thus, the study determined a methodology to evaluate the effects of insecticide products on spittlebugs, which indicated that thymol may be used effectively for the control of *M. spectabilis.*

Keywords: Spittlebugs, integrated pest management (IPM), bioinsecticides.

INTRODUCTION

Among the pest species that occur in Brazil, the spittlebug *Mahanarva spectabilis* (Distant) is a limiting pest in the production of forage grasses (Auad et al., 2007). The main current control strategies for this insect pest have some limitations, for example, the long time between the discovery of resistant forages and the release of a cultivar (Auad and Resende, 2018), and the fact that chemical control is economically and ecologically impracticable (Ferrufino and Lapointe, 1989; Valério 2005). Therefore, alternative measures to control *M. spectabilis* are required.

Naturally occurring plant products have been used to protect agricultural crops against pest insects for many years in various parts of the world (Kpatinvoh et al., 2017). These products are attractive alternatives to the synthetic chemical insecticides used in pest management, due to the fact that they supposedly pose little threat to the environment or to human health (Isman, 2006).

Studies have shown the potential of pure thymol in the control of several insect pests of agricultural importance (Tak and Isman, 2017; Oliveira et al., 2018). The method used to evaluate the effects of pesticides on any insect can have an effect on the final results obtained in the study (Studebaker and Kring 2003). The only study on the potential control of spittlebugs with bioinsecticides was performed by Garcia et al. (2006), who used the spray application method in plants. After spraying, individual *Mahanarva fimbriolata* (Stål) spittlebugs were released onto the plants. However, this method is very laborious, and due to the lack of indication of bioinsecticides for the control of spittlebugs, the study is aimed to develop and evaluate a reliable and less laborious method for investigating the insecticidal effect of thymol on *M. spectabilis*.

METHODOLOGY

Mahanarva spectabilis were collected in the experimental field of Embrapa Cattle Dairy in Coronel Pacheco, MG, Brazil, and transported to the Laboratory of Entomology in Juiz de Fora, MG, Brazil. Elephant grass plants (*Pennisetum purpureum*) were propagated in plastic pots (500 mL), containing soil and manure in a 1:1 ratio. The plants were kept in a greenhouse and irrigated daily, until they were used in the experiments (after 60 days). Thymol crystals and dimethyl sulfoxide (DMSO) were purchased in standard chemical form from Sigma-Aldrich®. The concentrations used in this study were based on previous studies by Novato et al. (2015), Senra et al. (2013), and preliminary tests.

The conventional method consisted of packing the insects in groups of 10 into plastic pots (500 ml) containing an elephant grass plant. The plastic pots containing the plants were wrapped in a cage made of voile fabric to prevent the escape of *M. spectabilis* nymphs and adults (Figure 1). The alternative method consisted of placing *M. spectabilis* nymphs and adults in groups of 10 in Petri dishes (9 cm), lined with filter paper moistened with distilled water, containing sections of elephant grass leaves as a food source for adults and root sections as a food source for nymphs (Figure 1). In order to evaluate the reliability of the two evaluation methods used, the mortality rates of *M. spectabilis* submitted to each methodology were compared. The experimental design used in the bioassays was completely randomized, with 10 replicates for each methodology. The method that resulted in higher survival rates of the insects up to 48 h after treatment application was considered the better of the two methods. The data were analyzed using analysis of variance (ANOVA). Results were considered significant at $p < 0.05$.

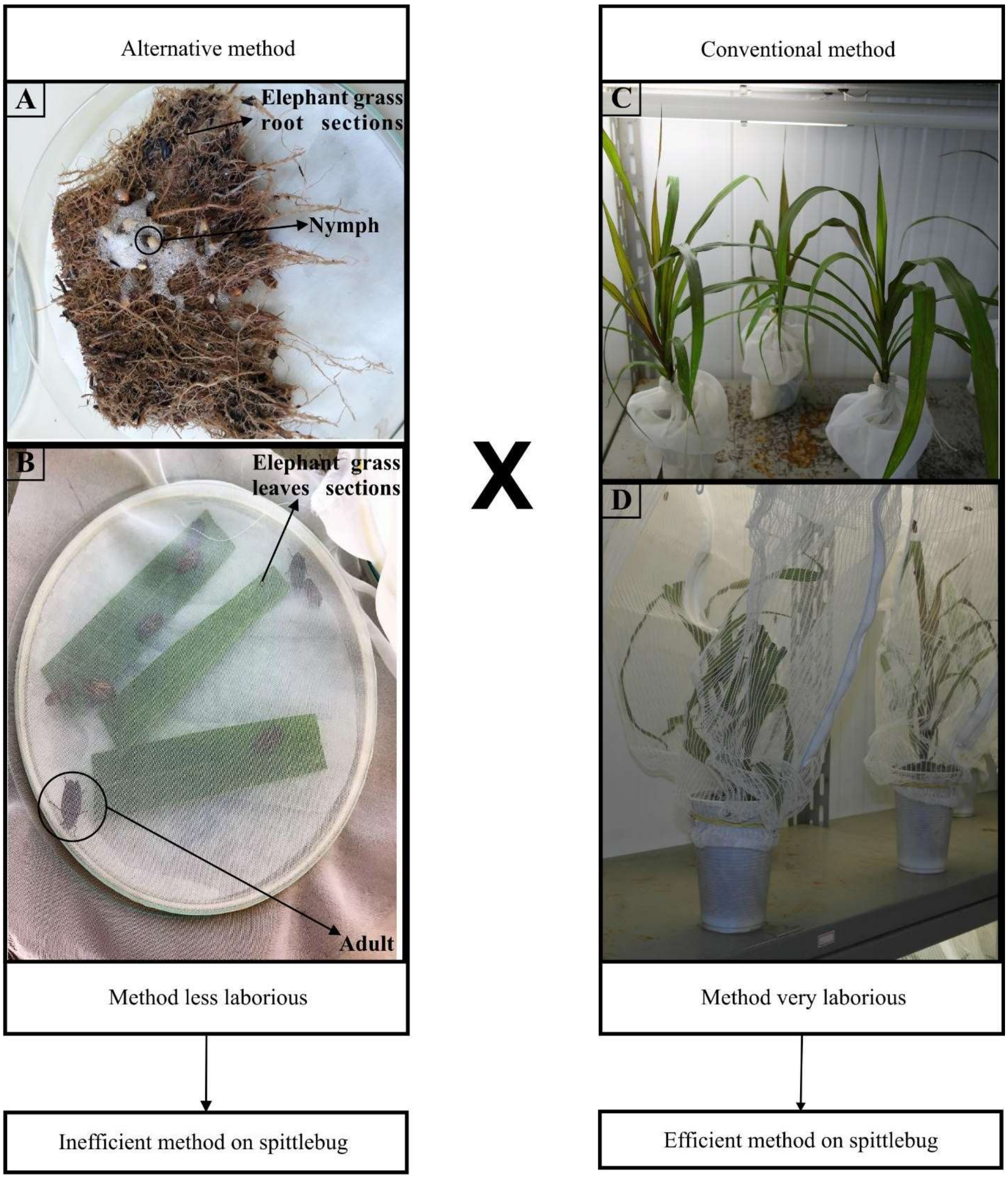

Figure 1. Methodologies for evaluating the effects of thymol on *M. spectabilis* used in this study. Alternative method: Plates lined with filter paper, containing sections of elephant grass roots and *M. spectabilis* nymphs (A); plates containing elephant grass leaf sections and *M. spectabilis* (B). Conventional method: Vases containing *M. spectabilis* nymphs (C) and adults (D).

After the selection of the preferred evaluation method, 10 µL of thymol solutions at concentrations of 2.5, 3.75, 5.0, 7.5, and 10.0% was used. A 1.0% DMSO solution was used as control. Solutions were applied to the dorsal region of *M. spectabilis* nymphs and adults (using a V3-Plus micropipette, 5-10 µL). After application, nymphs and adults were kept room at 25 ± 2 ºC with a 12:12 h light:dark cycle, and at a relative humidity of 70 ± 10%.

Mahanarva spectabilis eggs were obtained according to Auad and Resende (2018). In the egg test, 340 µL (20 µL/egg) of the thymol solutions were applied in concentrations of 0.3, 0.6, 0.9, 1.2, and 1.5%, and 1.0% DMSO was used as the control. Solutions were applied to groups of 17 eggs at an advanced embryonic stage (S4), arranged in Petri dishes that contained filter paper that was moistened daily. The concentrations were selected based on preliminary tests, in which the lowest concentration used in the tests using nymphs and adults resulted in 100% egg unviability. The Petri dishes containing the eggs were also kept in an air-conditioned room as described above.

The insecticidal effect of thymol on eggs, nymphs, and adults was evaluated at 24 and 48 h after application. The experimental design used in the bioassays was completely randomized, with 10 replicates for each concentration. Control efficiency was calculated using Abbott's formula (1925). The data were transformed by the square root of $(x + 0.5)$ for ANOVA, and the means were compared by the Tukey test (with significance set at $p < 0.05$). The analyses were conducted using SAS software version 9.2 (SAS Institute, Cary, North Carolina, 2008).

RESULTS

No significant difference in nymph survival rates was found when comparing the two methodologies used ($F = 0.217$; $df = 1$; $p = 0.6470$). However, adult survival was significantly higher in the tests using the conventional method (70%) as compared to the alternative method

(0%) (F = 39.44; df = 1; p = 0,0001). Thus, the tests to evaluate the effects of thymol on *M. spectabilis* nymphs and adults were performed using the conventional method.

In the tests to evaluate the effect of thymol on *M. spectabilis*, using the conventional method, nymph survival rates were significantly lower for all thymol concentrations used as compared to the control at 24 (F = 38.65, df = 5, p = 0.0001) and 48 h (F = 50.50, df = 5, p = 0.0001) after treatment application. All thymol concentrations resulted in efficiency above 70% and 80% after 24 h and 48 h, respectively (Figure 2A, B).

The adult survival of *M. spectabilis* was significantly lower at thymol concentrations of 3.75, 5.0, 7.5, and 10.0%, as compared to the control at 24 (F = 41.45, df = 5, p = 0.0001) and 48 h (F = 30.55, df = 5, p = 0.0001) after treatment application. When using thymol at concentrations above 5.0%, the efficacy rates were 94% and 97% after 24 and 48 h, respectively. At a concentration of 2.5%, thymol was not efficient for adult control (Figure 2C, D). When using a 5.0% thymol solution, a state of agitation followed by paralysis and death in *M. spectabilis* nymphs and adults was observed.

The viability of *M. spectabilis* eggs was significantly lower at thymol concentrations of 0.9, 1.2, and 1.5%, as compared to the control at 24 h (F = 29.14, df = 5, p = 0.0001) after treatment application. The viability of *M. spectabilis* eggs was significantly lower at all thymol concentrations used when compared to the control at 48 h (F = 26.84; df = 5; p = 0.0001) after treatment application. When using the 1.5% thymol solution, 81% of eggs were not viable at 24 h after treatment application, and this increased to 88% at 48 h after treatment application (Figure 2E, F).

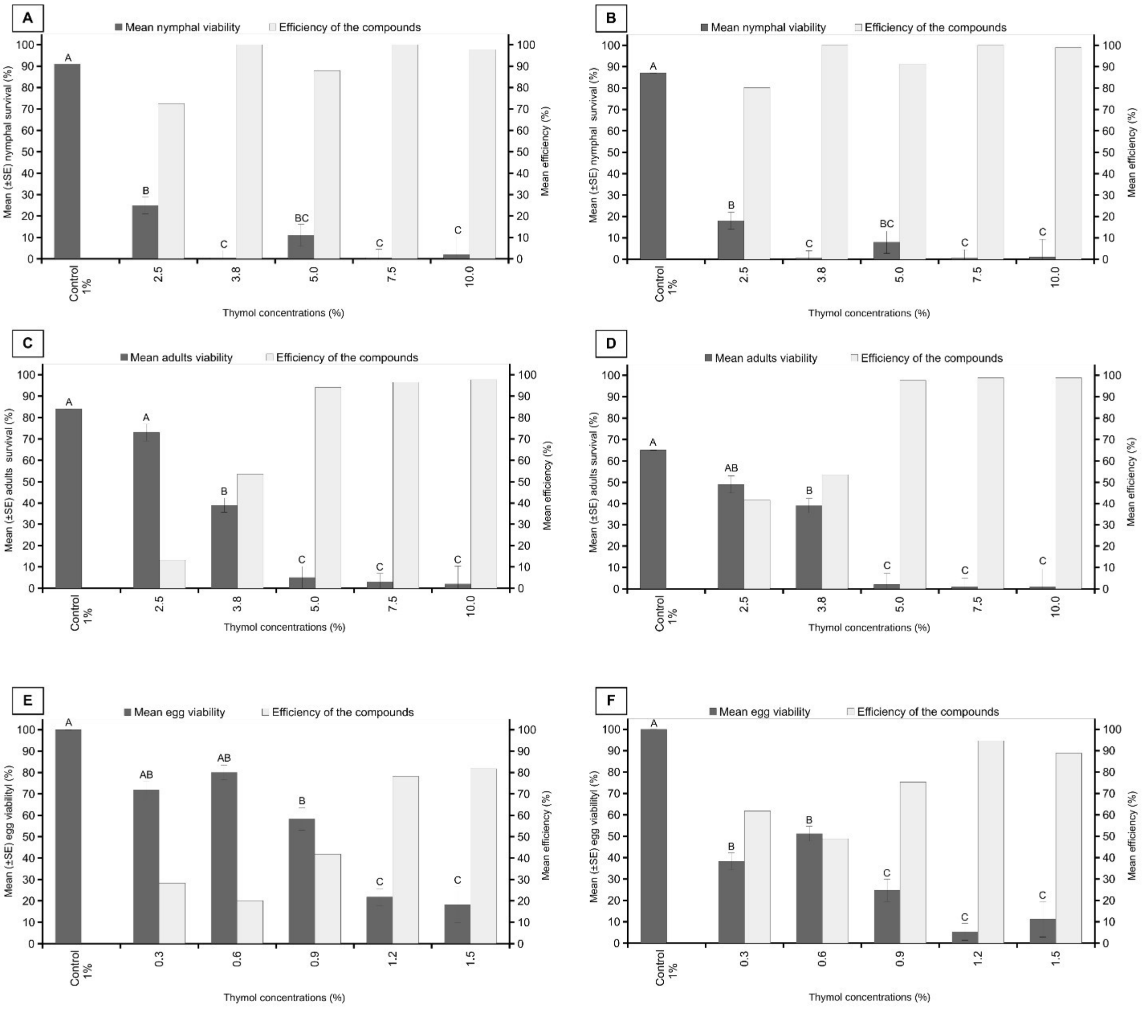

Figure 2. Insecticidal activity of thymol against *M. spectabilis* nymphs 24h (A) and 48 h (B); adults at 24h (C) and 48 h (D), and eggs at 24 (E) and 48 h (F) after application. Different letters in the columns represent significant differences between the treatments by the Tukey test ($p < 0.05$). Control efficiency was calculated using Abbott's formula (1925).

DISCUSSION

The conventional method, although more laborious, resulted in low adult mortality of *M. spectabilis*, when compared to the alternative method used in this study. This indicates the efficiency and reliability of the conventional method in tests evaluating the effects of thymol and/or other chemical compounds on spittlebugs. Garcia et al. (2006) successfully employed a similar method using plant pots to verify the insecticidal activity of Neem-based formulations on *M.*

fimbriolata. The inefficiency of the alternative method using Petri dishes, evidenced by the significant mortality of adults, suggests that the jumping behavior of adults may also have affected this result. For the nymphs, the alternative method was effective, showing that the spittlebugs were able to use the elephant grass root sections as a sufficient source of nutrients. The alternative method was efficient for studying the effect of insecticides on nymphs of *Orius insidiosus* (Say) (Studebaker and Kring, 2003) and the effects of thymol on larvae of *Diaphania hyalinata* (Linnaeus) (Melo et al., 2018). It should be noted that the behavior of the insects is related to the efficiency of the method used, and the inefficiency of the alternative method described here in studies on adult insects limits its indication for tests with bioinsecticides, which seek to control both nymphs and adults.

The relationship between thymol concentrations and insect mortality obtained in this study was also observed by Ismail (2018) in tests used to evaluate the toxic effects of thymol on *Megaselia scalaris* (Loew). In addition, the agitation behavior exhibited by *M. spectabilis* has also been reported by Hummelbrunner and Isman (2001) in thymol toxicity tests on *Spodoptera litura* (Fabricius). These researchers attributed this effect to the action of thymol in the octopaminergic system, which is exclusive to insects, and of considerable interest as a target location for control agents.

Thymol has already been shown to have an ovicidal effect, as reported in studies on *Rhodnius prolixus* (Stal) (Figueiredo et al., 2017) and *Nezara viridula* (Linnaeus) (Werdin González et al., 2011). The egg shell is described as a barrier against insecticides; however, it contains some areas that could allow the penetration of insecticides, aeropiles, and micropiles (Campbell et al., 2016). Caperucci and Camargo-Mathias (2006) described the presence of numerous small pores in the exochorion of eggs of *M. fimbriolata*, which probably facilitate the oxygenation of the internal structures of the eggs. These structures may explain the high rate of unviability observed in thymol treatments of *M. spectabilis* eggs, requiring 10 times lower concentrations to promote embryo mortality than those applied to nymphs and adults. This result was also observed by Kovaříková et al. (2017), when evaluating the effects of botanical insecticides on *Aleyrodes proletella* (Linnaeus) eggs and nymphs. These researchers attributed this difference to the serosa layer that protects the nymphs. This study suggest that, in addition to the presence of pores in eggshell, this difference may occur because of the foam secreted by the nymphs of spittlebugs, which may function as a partial elimination mechanism for the irritant.

CONCLUSION

The conventional method, although more laborious, is the most efficient way to evaluate the insecticidal activity of thymol and/or other chemical compounds on adults and nymphs of *M. spectabilis* and other species of spittlebugs. Furthermore, thymol is recommended as an active substance with a potential use in combination with other control methods in integrated pest management (IPM) programs.

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ARTIGO 2: INSECTICIDAL ACTIVITY OF COMPOUNDS OF PLANT ORIGIN ON *Mahanarva spectabilis* (HEMIPTERA: CERCOPIDAE)

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Insecticidal Activity of Compounds of Plant Origin on *Mahanarva spectabilis* (Hemiptera: Cercopidae)

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Abstract

The damage caused by spittlebugs varies according to the species of grass, and the losses can reach alarming levels. Measures for population control are currently restricted to the use of resistant grasses and the diversification of pastures. Therefore, alternative control measures are necessary, such as the use of botanical insecticides. The aim of this study was to evaluate the insecticidal activities of thymol, carvacrol, eugenol, cinnamaldehyde, and trans-anethole on *Mahanarva spectabilis* eggs, nymphs, and adults under laboratory conditions. In the egg tests, treatments with eugenol, carvacrol, and thymol showed the highest mortalities, presenting efficiencies higher than 85% after 48 h of application. In the nymph tests, the treatments with thymol and carvacrol at 2.5% and eugenol at 2.0% and 2.5% showed intermediate efficiencies, with values above 61%. The highest mortality was observed in the treatment with trans-anethole at 2.5%, with an efficiency of 95%. In the tests with adults, only treatment with trans-anethole at 2.5% obtained an efficiency reaching 90%; in the other treatments, the efficiency did not exceed 51%. These results showed that, at these concentrations, trans-anethole presents a high rate of insecticidal activity on *M. spectabilis* nymphs and adults and, therefore, is recommended as a potential natural insecticide for the control of this pest.

Keywords: spittlebug; IPM; botanical insecticide; biopesticides; main compounds

INTRODUCTION

Spittlebugs are the main pests associated with forage grasses in the tropical Americas [1]. The damage caused by spittlebugs varies for each species of grass, and economic losses can reach alarming figures depending on the region, climatic conditions, and management [2]. The global losses caused by these insects are estimated to range from US$840 million to US$2.1 billion per year [3]. *Mahanarva spectabilis* (Distant, 1909) (Hemiptera: Cercopidae) is considered a limiting pest in the production of forage grasses such as elephant grass (*Pennisetum purpureum*, Schumacher) [4]. Although the nymphs of this species cause considerable damage leading to a water imbalance in the plants, the adults are responsible for greater plant losses because they suck the sap and inject a toxin that initiates the yellowing and drying of the forage [5,6]. In addition, this toxin makes pastures unpalatable and hinders the feeding of cattle [7].

The best method to control spittlebugs would be the diversification of pastures and the use of resistant grasses [8]. Although the inclusion of resistant grasses is a promising technique for reducing the damage caused by spittlebugs, there is a long time between the discovery of resistant forage and the release of a cultivar [9]. The chemical control of this pest is considered economically unviable because pasture is considered a low-value crop per unit area and, in addition, the use of insecticides leads to the accumulation of residues [10,11]. Furthermore, these insecticides can decimate entire populations of nontarget organisms [12]. Therefore, alternative control measures are necessary, such as the use of botanical insecticides, which are attractive alternatives for pest management compared with synthetic chemical insecticides because they present little threat to the environment and human health [13–16]. Natural products are generally effcient, low cost, and less harmful than synthetic products to nontarget organisms, and because of their biodegradability, they are ecologically appropriate [17,18]. Both commercially available formulations and rudimentary essential oils have shown promise in pest control for crops [19]. Recent research has shown a growing interest in the bioactive e effects of essential oils and their derivatives on insects [20–23].

Pure compounds and/or their essential oils thymol, carvacrol, eugenol, trans-anethole, and cinnamaldehyde have already been tested against *Trichoplusia ni* (Hübner, 1803) [24], *Pochazia shantungensis* (Chou and Lu) [25], *Callosobruchus maculatus* (Fabricius, 1775) [26], *Myzus persicae* (Sulzer, 1776), and *Aedes aegypti* (Linnaeus, 1762) [27,28]. Neem-based formulations were also tested against *Mahanarva fimbriolata* (Stal, 1854) [29]. Among the various mechanisms of action of these compounds are the inhibition of acetylcholinesterase and neurotoxic effects involving actions on the receptors of gamma-aminobutyric acid (GABA) and octopamine [30].

Thus, the objective of the present study was to evaluate the insecticidal activities of the monoterpenes thymol and carvacrol, the phenylpropanoid eugenol, the flavonoid trans-anethole, and the ether cinnamaldehyde against the eggs, nymphs, and adults of *M. spectabilis.*

MATERIALS AND METHODS

Acquisition and Maintenance of Insects and Plants

To obtain eggs, *M. spectabilis* adults were collected from the experimental field of Embrapa Dairy Cattle in Coronel Pacheco, MG, Brazil (21º 33’22” S latitude, 43º 6’15” W longitude, at a height above sea level of 414 m). These insects were taken to the Entomology Laboratory of Embrapa Dairy Cattle in Juiz de Fora, MG, Brazil, where they were kept in acrylic cages (30 × 30 × 60 cm) that contained elephant grass plants (*P. purpureu*m cv. Roxo de Botucatu) for feeding at a temperature of 25 ± 2 ºC. The base of each cage was wrapped with gauze moistened with distilled water, which served as a substrate for oviposition; the wrapped eggs were then placed under a set of sieves and subjected to running water, and the eggs were retained in the finest sieves (mesh opening 400). Then, 300 eggs were grouped in Petri dishes (9 cm in diameter) lined with filter paper and were placed in an air-conditioned chamber, maintained at 25 ± 2 ºC with 70% ± 10%

relative humidity (RH) and a photoperiod of 12:12 h (L:D) until they reached the S4 development stage, which is characterized by two red spots on each side of the operculum; the operculum corresponds to the eyes, and the red spots represent the nymph's abdominal pigments [31]. The filter papers were moistened daily, and the development of the eggs was observed. Eggs were used at the S4 stage of development to ensure that the lack of hatching during the tests was caused by the insecticidal substance and not the diapause period of the eggs, which were retained at embryonic stage S2 for approximately 200 days.

To obtain nymphs and adults for bioassays, fourth- and fifth-instar nymphs were collected from the experimental field and sent to the laboratory, where they were placed on elephant grass plants with roots exposed for feeding. Adults were also collected from the field and taken to the laboratory, where they were kept in elephant grass pots covered with voile to prevent the insects from escaping until they were used in the experiment. Prior to bioassays, nymphs and adults collected from the field were conditioned in the laboratory and kept under controlled conditions for 24 h to adapt to the laboratory environment.

Elephant grass (*P. purpureum*) plants were used in 10 cm (single-node) stakes, propagated in plastic pots (500 mL) containing substrate (soil/fertilizer in the proportion 1:1). Seedlings were collected from the experimental field of Embrapa Dairy Cattle in Coronel Pacheco. The seedlings were kept in a greenhouse and irrigated daily until they were used in the experiments (60 days).

Reagents

All compounds were purchased in standard chemical form from Sigma-Aldrich® (Saint Louis, MO, USA). Thymol crystals, eugenol, trans-anethole, and dimethyl sulfoxide (DMSO) were obtained at analytical purity of >99%, and carvacrol and cinnamaldehyde were obtained at purities of 98% and ≥95%, respectively. All solutions were placed in an ultrasonic bath, model Elma E 60

H (Elma Ultrasonic System®, Singen, Germany), for approximately 5–7 min, and the thymol solutions were heated to 40 ºC for complete emulsion of the crystals.

Evaluation of Insecticidal Activity

In the bioassay of the susceptibility of eggs to botanical compounds, 15 eggs were grouped in Petri dishes (5 cm) lined with filter paper, and each egg received an application of 10 µL (Micropipette V3-PLUS 0.5–10 µL) of solution at concentrations of 0.5%, 1.0%, 1.5%, 2.0%, and 2.5%; these solutions were prepared using 1% DMSO as a solvent or a DMSO control. Each concentration of each compound was replicated 10 times, and the plates of each replicate were maintained in a phytotron-type controlled environment (2.5 × 2.20 × 2.80 m) at 25 ± 2 ºC with a photoperiod of 12:12 h (L:D) and RH of 70% ± 10%. The ovicidal activity of each compound was evaluated in a stereomicroscope after 24 and 48 h of application, and the eggs that darkened in color, indicating embryonic death, were considered unviable.

In the tests with nymphs and adults, 10 µL of solution was applied to the dorsal region of each insect at the same concentrations mentioned above, and the insects were then assembled into groups of 10 in plastic pots (500 mL) that contained elephant grass plants. Each concentration of each compound was replicated 10 times, and the pots of each replicate were maintained in a phytotron-type controlled environment. The potted plants containing the nymphs had the roots exposed to jets of running water to facilitate feeding. The pots were wrapped in voile secured by elastic at the base of the leaves to prevent the nymphs from escaping. For the test with adults, a cage accommodating the leaves was adapted to avoid insect escape. The pots were then transferred to an air-conditioned chambre maintained at 25 ± 2 ºC with a 12 h photophase and RH of 70% ± 10%. Then, the number of survivors was counted 24 and 48 h after the treatments. The insects were considered dead when they presented evidence of paralysis, tipping, and immobility when touched by the bristles of a fillet-type brush after 60 s under these conditions.

Statistical Analysis

According to the National Health Surveillance Agency (ANVISA) [32], tests with insecticides and related products are considered satisfactory when the mortality obtained in the positive control reaches an average value of 90% (±10%) in relation to the control. In the present research, we considered treatments to be satisfactory if they were efficient according to ANVISA. The experimental design consisted of randomized blocks composed of 25 treatments and a control with 10 replicates. In each repetition, 15 eggs or 10 nymphs or adults were used. The control efficiency of the treatments was calculated using the Abbott formula [33]. The data were transformed by the square root of $(x + 0.5)$ for the analysis of variance (ANOVA) of repeated measurements, and the means were compared by the Scott Knott test ($p < 0.05$). The analyses were performed using the free software RStudio with R version 3.5.1 (packages: ScottKnott [v1.2-7]) [34,35].

RESULTS

In the evaluation performed 24 h after the application of the treatments, the number of viable eggs was significantly lower at concentrations of 1.0%, 1.5%, 2.0%, and 2.5% in the thymol treatments and at all concentrations in the treatments with carvacrol and eugenol when compared with the control ($F = 62.37$; *df* = 25; $p < 0.0001$); the same results were not obtained for the other treatments. In the present research, the treatments with thymol at a concentration of 1.5%; carvacrol at concentrations of 1.0% and 2.5%; and eugenol at concentrations of 1.0%, 1.5%, 2.0%, and 2.5% showed the best results, presenting control efficiencies higher than 85%. In addition, thymol treatments at concentrations of 1.0% and 2.0% and carvacrol at 1.5% showed intermediate control efficiencies above 69%. Efficiencies below 60% were found for thymol at 0.5% and 2.5%, carvacrol at 0.5% and 2.0%, and eugenol at 0.5%, whereas zero efficiency was observed for all concentrations of cinnamaldehyde, and efficiencies below 5% were observed for all concentrations of trans-anethole (Figure 1A).

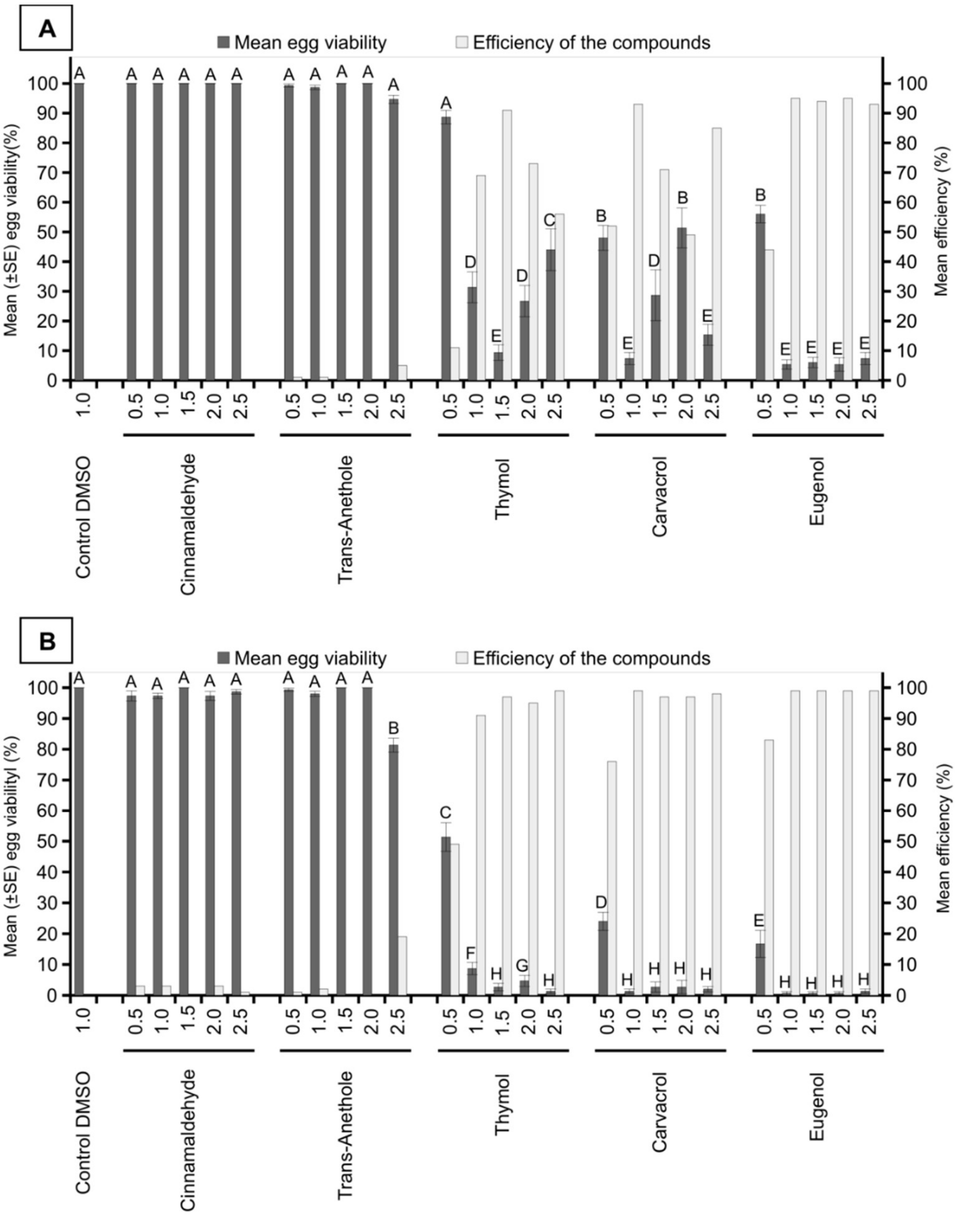

Figure 1. Insecticidal activity of compounds of plant origin (concentrations of 0.5%, 1.0%, 1.5%, 2.0%, and 2.5%) against *Mahanarva spectabilis* eggs after 24 (**A**) and 48 h (**B**) of application. The control efficiency of the treatments was calculated using the Abbott formula. Different letters in the columns represent significant differences between the treatments by the Scott Knott test ($p < 0.05$).

At 48 h after application, the number of viable eggs was significantly lower in the treatments with thymol, carvacrol, and eugenol at all concentrations and in that with trans-anethole at 2.5%

when compared with the control treatment (F = 266.90; *df* = 25; $p < 0.0001$). The treatments with thymol, carvacrol, and eugenol at concentrations of 1.0%, 1.5%, 2.0%, and 2.5% presented the best results in relation to the other treatments, showing control efficiency values greater than 91% and reaching 99%. In addition, the carvacrol and eugenol treatments at the lowest dose (0.5%) presented control efficiencies of 76% and 83%, respectively. The treatments with cinnamaldehyde and trans-anethole at different concentrations showed control efficiencies below 5% and 20%, respectively (Figure 1B).

In the first evaluation, performed 24 h after the treatments, the number of surviving nymphs was significantly lower in the thymol treatments at 1.5% and 2.5%; carvacrol at 2.5%; eugenol at 1.5%, 2.0%, and 2.5%; and trans-anethole at 2.5% when compared with that in the control treatment (F = 20.95; *df* = 25; $p < 0.0001$). The best results were observed in the treatments with carvacrol and trans-anethole at a concentration of 2.5%, with control efficiency values of 60% and 87%, respectively. In the other treatments, the control efficiency was lower than 60% (Figure 2A).

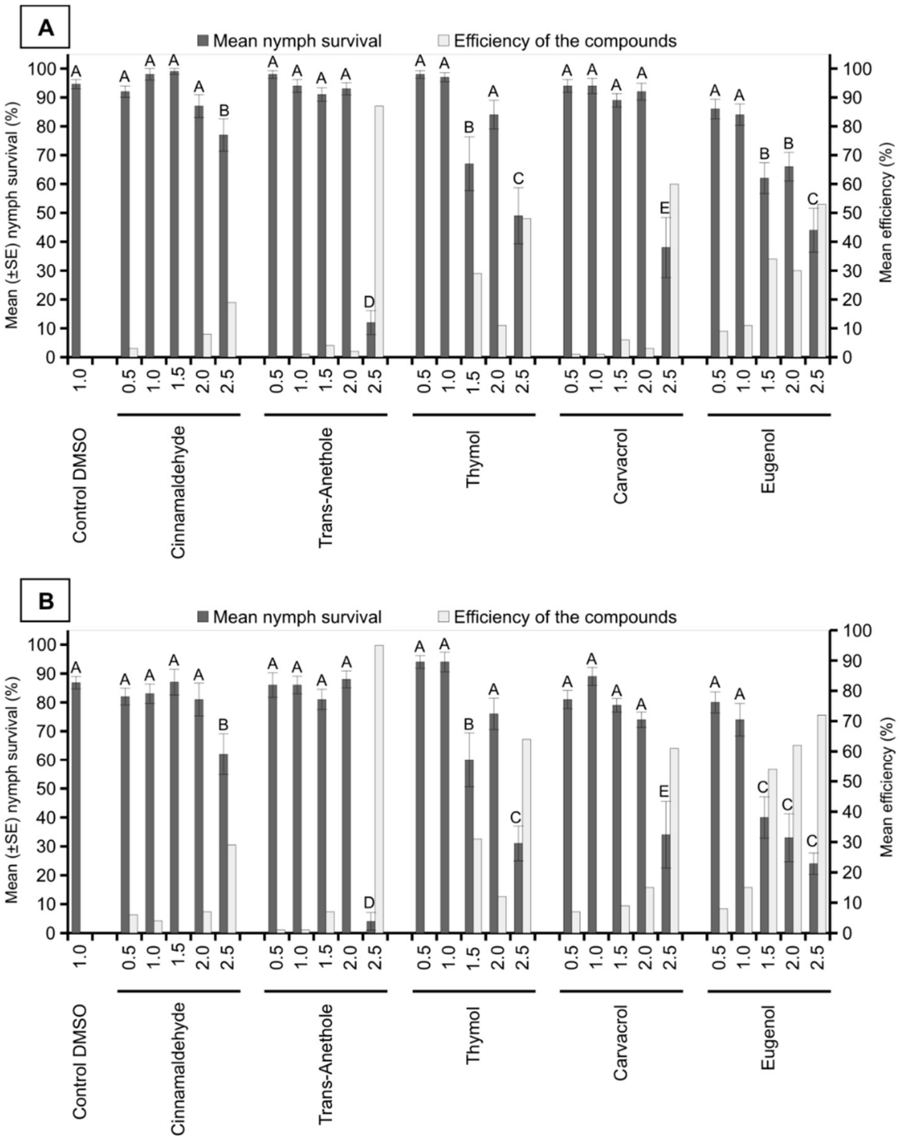

Figure 2. Insecticidal activity of compounds of plant origin (concentrations of 0.5%, 1.0%, 1.5%, 2.0%, and 2.5%) against *M. spectabilis* nymphs after 24 (**A**) and 48 h (**B**) of application. The control efficiency of the treatments was calculated using the Abbott formula. Different letters in the columns represent significant differences between the treatments by the Scott Knott test ($p < 0.05$).

In the evaluation after 48 h of application, treatments with thymol at 1.5% and 2.5%; carvacrol at 2.5%; eugenol at 1.5%, 2.0%, and 2.5%; and cinnamaldehyde and trans-anethole at 2.5% presented significantly fewer surviving nymphs when compared with the control treatment ($F = 24.14$; *df* = 25; $p < 0.0001$). In the treatments with thymol and carvacrol at 2.5% and eugenol at 2.0% and 2.5%, intermediate control efficiency was observed, with values above 61%. The best result was observed in the treatment with trans-anethole at 2.5%, with a control efficiency of 95%. In the other treatments, the control efficiency was below 60% (Figure 2B).

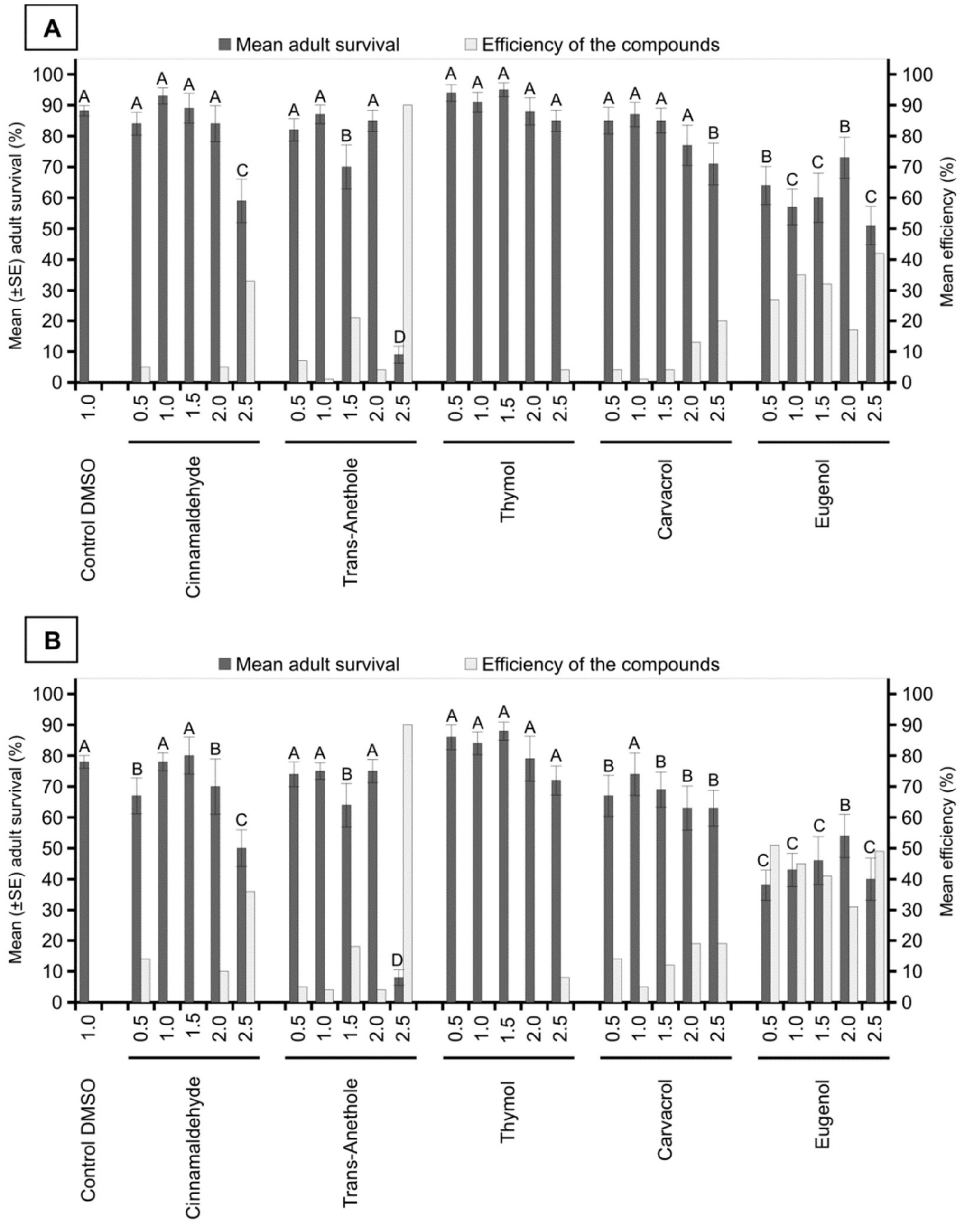

Figure 3. Insecticidal activity of compounds of plant origin (concentrations of 0.5%, 1.0%, 1.5%, 2.0%, and 2.5%) against *M. spectabilis* adults after 24 (**A**) and 48 h (**B**) of application. The control efficiency of the treatments was calculated using the Abbott formula. Different letters in the columns represent significant differences between the treatments by the Scott Knott test ($p < 0.05$).

In the evaluation performed 24 h after application, the number of surviving adults was significantly lower in the treatments with carvacrol at 2.5%, eugenol at all concentrations, cinnamaldehyde at 2.5%, and trans-anethole at 1.5% and 2.5% when compared with that in the control treatment (F = 18.39; *df* = 25; $p < 0.0001$). Only the treatment with trans-anethole at 2.5% showed a control efficiency of 90%, whereas in the other treatments, the efficiency ratio did not exceed 42% (Figure 3A).

In the evaluation performed 48 h after application, the number of surviving adults was significantly lower in the carvacrol treatments at 0.5%, 1.5%, 2.0%, and 2.5%; eugenol at all concentrations; cinnamaldehyde at 0.5%, 2.0%, and 2.5%; and trans-anethole at 1.5% and 2.5% when compared with that in the control treatment (F = 12.73; *df* = 25; $p < 0.0001$). Once again, only in the treatment with trans-anethole at a concentration of 2.5% did the control efficiency reach 90%, whereas in the other treatments, the efficiency ratio did not exceed 51% (Figure 3B).

DISCUSSION

Compounds from essential oils are attractive alternatives for the management of this pest because they are safer than synthetic insecticides both for the environment and human health [36]. The results of the experiments showed that an evaluation performed 24 h after application was not able to determine the unviability rate of eggs, and even with increased concentrations, there was no corresponding increase in mortality. This may have occurred due to the necessity of a longer time for the product to act on the eggs, which suggests that evaluations of bioinsecticide tests should be performed 48 h after their application. At 48 h after application, treatments with thymol, carvacrol, and eugenol showed rates varying between 91% and 99% for *M. spectabilis* egg unviability. This result can be explained by the presence of structures described as numerous small pores present in the exochorion of eggs of the species *M. fimbriolata* [37]. These pores are probably responsible for the oxygenation of the inner structures of the eggs and can also act as facilitators for the introduction of toxic materials into the egg membrane [38].

The ovicidal effects of these monoterpenes against other entomological pests, such as the effect of thymol and carvacrol on the eggs of *Rhodnius prolixus* (Stal, 1859) (Hemiptera: Reduviidae) and the effect of eugenol on eggs of *Bradysia procera* (Winnertz, 1868) (Diptera: Sciaridae), have already been mentioned in the literature [39,40]. The modes of action of thymol and its isomer carvacrol, such as thymol's ability to block GABA and/or octopaminergic insect systems [41–43] and the inhibitory effects of acetylcholinesterase exhibited by carvacrol [44] on domestic flies, ticks, and cockroaches, have also been reported in the literature. Although the neurotoxic action of these monoterpenes is widely described in the literature, the toxicity of these compounds becomes more visible when the nervous system of the insect embryo is developing [39].

The efficiency rates of eugenol on eggs varied between 83% and 99% egg unviability in both evaluations. The ovicidal effect of this compound was evaluated in tests in which eugenol was applied to the eggs of *Sitophilus granarius* (Linnaeus, 1758) and *Sitophilus zeamais* (Motschulsky, 1855) (Coleoptera: Curculionidae); these studies concluded that eugenol completely inhibits egg hatching in these insects [45]. Eugenol was also reported as a neuroinsecticide against the ant *Camponotus pennsylvanicus* (De Geer, 1773) (Hymenoptera: Formicidae), and the octopaminergic system acts as a mediator of its insecticidal activity [46].

The compounds used in this study were more effective against eggs than against the nymphs of *M. spectabilis*. Similar results were found in tests comparing the effectiveness of some insecticides, including *Quassia amara* (Linnaeus) (Simaroubaceae), NeemAzal, and other botanical insecticides, against *Aleyrodes proletella* (Linnaeus, 1758) (Hemiptera: Aleyrodidae); this difference was attributed to the serous layer that protects the nymphs [47]. In our research, we suggest that, in addition to the presence of pores in the egg shells, this difference may have occurred due to the froth secreted by the nymphs of spittlebugs. It is proposed that the main functions of this foam are to confer protection against predation and to form a microhabitat that avoids high-temperature dryness and helps the thermoregulation of nymphs [48–50]. However, there may be a similarity between the foam produced by the spittlebugs and the foam that snails secrete during a physical attack or exposure to chemicals [51]. Thus, we suggest that the production of froth by the nymphs soon after the application of the treatments functions to partially eliminate the irritant.

At least one concentration of thymol, carvacrol, and eugenol exhibited control efficiency rates above 60% on the nymphs at 48 h. These compounds have already been proved effective on other insects, for example, the potential of eugenol, thymol, and carvacrol on nymphs of *R. prolixus* (Stal, 1859) and *Triatoma infestans* (Klug, 1834) (Hemiptera: Reduviidae), which are vectors of Chagas' disease [52]. Likewise, the insecticidal properties of thymol and carvacrol, derived from the essential oil of *Thymus vulgaris* (Linnaeus) (Lamiaceae), were proved on *P. shantungensis* nymphs [25]. In the nymph tests, trans-anethole obtained efficiency rates between 87% and 95%, superior to those observed in nymphs of *Trialeurodes vaporariorum* (Westwood, 1856) (Hemiptera: Aleyrodidae), in which the compound showed 50% interference in nymphal growth [53]. Studies have shown the toxic effects of phenylpropanoid trans-anethole on other pest insects, such as *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) [54] and *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) [55]. Among the effects of trans-anethole, there are reports of changes in the biological parameters of insects, such as the inhibition of acetylcholinesterase [56], which may explain its efficacy in the nymphs of this study.

The least efficient compound against *M. spectabilis* nymphs was cinnamaldehyde, which was in contrast to the results found in tests of the insecticidal activity of cinnamon essential oils, their constituents, and the analogues of (E)-cinnamaldehyde on *Metcalfa pruinosa* nymphs and adults (Say, 1830) (Hemiptera: Flatidae) [57]. This difference in toxicity may have occurred due to the particularities of the target species of each study, such as physiology and resistance mechanisms.

In the tests with adults, the efficiency rates of the treatments were much lower than those obtained in the tests with nymphs. We can infer that this difference may have occurred due to the adult integument being more chitinized, acting as a physical barrier to the absorption of the applied compounds, a fact already mentioned in the literature to explain the absence of adult infection of *M. spectabilis* by entomopathogenic nematodes [58]. However, trans-anethole presented an efficiency rate of 90% when applied at the highest concentration to the adults of *M. spectabilis*. This efficiency can be explained by the double bond of the propenyl group present in trans-anethole, suggested in toxicity tests on *Callosobruchus chinensis* (Linnaeus, 1758) (Coleoptera: Bruchidae) to be responsible for the high insecticidal activity of this compound [59]. Eugenol showed an efficiency rate below 60% in adults, unlike the results found in adults of *M. pruinosa*, against which eugenol was mentioned as the most toxic compound [57]. In our evaluations, thymol presented a significantly lower activity than that of carvacrol in adults, as found in tests of the activity of these compounds on *Culex quinquefasciatus* larvae and pupae (Say, 1823) (Diptera: Culicidae) [60]. It is worth noting that the only structural difference between these two compounds is the position of the hydroxyl group on the benzene ring relative to the larger aliphatic chain and may be related to this difference in activity [60]. However, it is important to note that the efficacy of thymol is well reported for several insect species, such as *Anopheles stephensi* (Liston, 1901) (Diptera: Culicidae) [61], *S. zeamais* [62], and *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) [19].

CONCLUSION

This research shows, for the first time, the activity of some major compounds of vegetal origin in the different phases of life of *M. spectabilis*. Eugenol, carvacrol, and thymol were the most eficient compounds in reducing the number of spittlebug eggs. However, control of this insect should focus on decreasing the number of nymphs and adults. For these phases, the trans-anethole activity had control efficiency rates higher than 85%, a value within the recommended insecticide registration range. Thus, taking into account the concentrations tested, this compound is recommended as a potential natural insecticide for the control of *M. spectabilis*.

Author Contributions: The study was conceived and designed by M.L.D., A.M.A., and M.L.D. A.M.A., M.C.M., T.T.R., and S.E.B.S. performed the experiments. M.G.F., M.L.D., and A.M.A. performed the statistical analysis, and M.L.D. and A.M.A. wrote the manuscript. All the authors have approved the manuscript.

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

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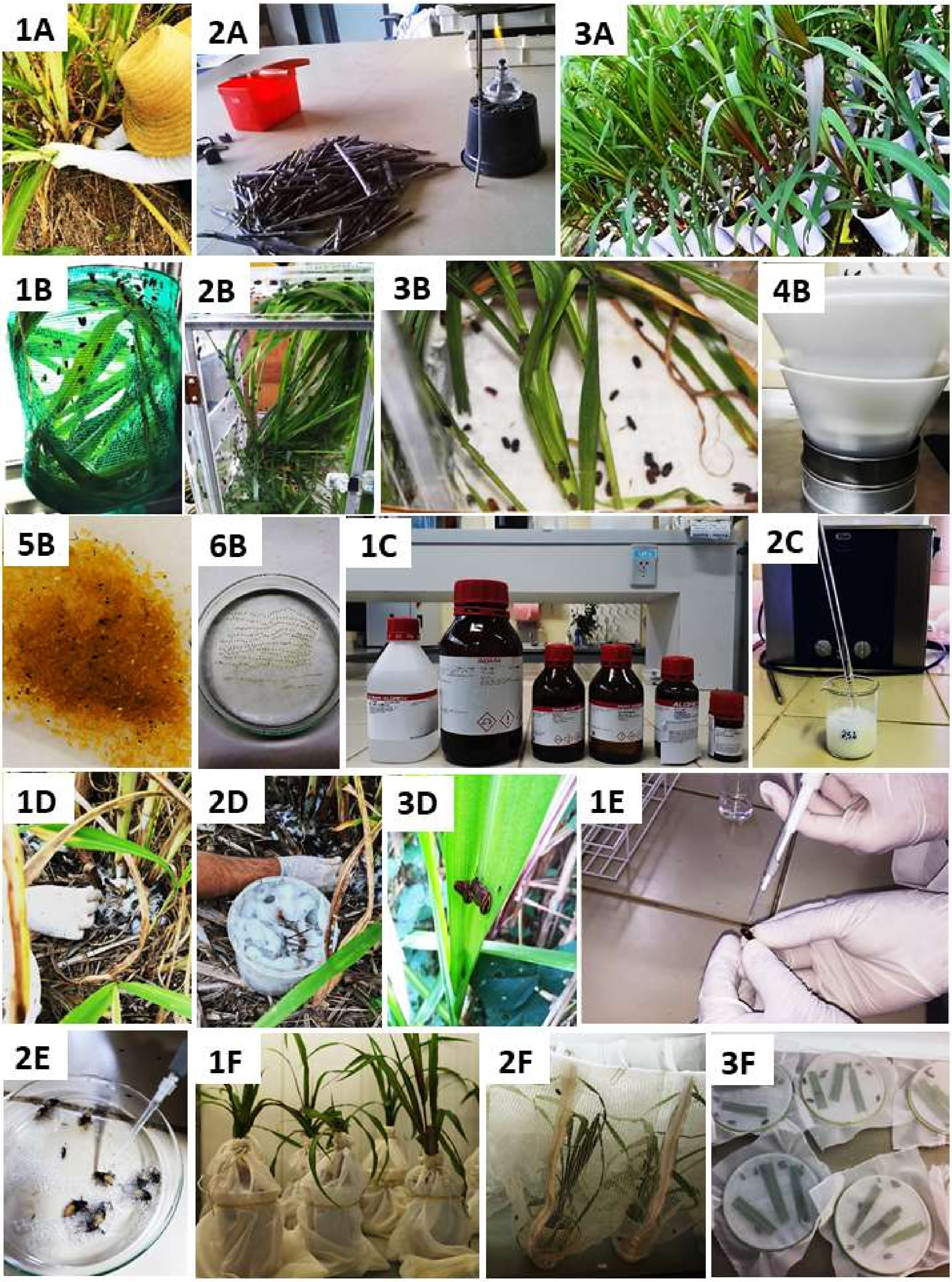
CONSIDERAÇÕES FINAIS

No decorrer desta tese destacamos a relevância da escolha de uma metodologia adequada que forneça todas as necessidades ao inseto alvo e que seja confiável, de fácil replicação e que exija o menor esforço possível ao pesquisador. Nesse contexto, estabelecemos a melhor metodologia de manutenção da cigarrinha *M. spectabilis* e, assim, determinamos o grau de atividade inseticida do do timol, um composto de origem vegetal. Os resultados obtidos da aplicação desta metodologia demonstraram-na eficaz, validando sua utilização em outros testes de atividade inseticida, com esta e outras espécies de cigarrinhas-das-pastagens, de modo confiável e preciso. Para alcançar este resultado, realizamos um extenso levantamento das metodologias empregadas nesses testes com diversas espécies de insetos praga por meio de artigos científicos. Desse processo foram adotados o método em placas de Petri, mais citado na literatura, e o único método descrito utilizando produto comercial de origem vegetal sobre a cigarrinha *M. fimbriolata*. Ambos os métodos foram adaptados para esta pesquisa levando em conta estudos de campo e o comportamento da espécie alvo *M. spectabilis*. Num segundo momento, após a escolha da metodologia convencional como sendo a mais adequada, verificamos o grau de atividade inseticida do composto timol sobre ovos, ninfas e adultos de *M. spectabilis* com a realização de experimentos que exigiram grande esforço da equipe envolvida. Como conclusão dessa etapa, verificamos o potencial do uso do timol como substância ativa no controle da cigarrinha, podendo seu uso ser associado a outras técnicas de manejo integrado de pragas.

O segundo produto desta tese foi um artigo no qual testamos 5 produtos de origem vegetal sobre a cigarrinha *M. spectabilis*, empregando a metodologia descrita no artigo anterior. Num esforço amostral bem maior do que o aplicado nos testes de metodologia, foram triados 4.500 ovos e coletados 3.000 ninfas e 3.000 adultos de cigarrinha no total. Como resultado deste grande esforço, podemos recomendar o composto trans-anetol como potencial inseticida natural no controle de *M. spectabilis*. Sendo assim, a totalidade dos resultados alcançados com esta pesquisa possibilitou a determinação da metodologia mais adequada de manutenção de *M. spectabilis* para realização de testes de toxicidade de produtos em condições de laboratório, bem como determinar o trans-anetol como potencial inseticida natural no manejo dessa espécie. Mas, mais que estes relevantes resultados, o maior impacto desta tese advém da inovação ao testar estes produtos sobre esta espécie de cigarrinha, que ainda não possui inseticida registrado para o seu controle, ainda mais sendo este um produto de origem natural de baixo impacto humano e ambiental e de baixo custo, podendo ser adquirido por pequenos produtores.

ANEXOS



ANEXO 1: Descrição das etapas de obtenção de plantas e insetos, da avaliação da atividade inseticida dos compostos sobre ovos, ninfas e adultos de *M. spectabilis* em condições de laboratório e apresentação das metodologias utilizadas. 1-3A. Propagação das plantas: 1. Coleta das plântulas; 2. Estacas de 10 cm; 3. Propagação em vasos plásticos (500 mL); 1-6B. Obtenção dos ovos: 1. Transporte dos adultos coletados no campo ao laboratório; 2. Gaiola acrílico contendo os adultos; 3. Detalhe da gaze de oviposição; 3. Conjunto de peneiras para lavagem das gazes; 6. Massa de ovos triados das gazes; 7. Disposição dos ovos em placas de Petri (9 cm); 1-2C. Preparação das soluções: 1. Compostos e o solvente utilizados nos testes; 2. Solubilização das soluções em ultrassom; 1-3D. Coleta de ninfas e adultos: 1. Coleta de ninfas com pincel filete; 2. Disposição das ninfas em Beckers para transporte; 3. Coleta manual dos adultos em campo; 1-2E. Aplicação das soluções: 1. Aplicação dos tratamentos no abdômem dos adultos; 2. Aplicação dos tratamentos no dorso das ninfas; 1-3F. Método em vasos de plantas e em placas de Petri: 1. Ninfas em vasos de plantas após aplicação dos tratamentos; 2. Adultos em vasos de plantas após aplicação dos tratamentos; 3. Adultos em placas de Petri após aaplicação dos tratamentos.