FEDERAL UNIVERSITY OF JUIZ DE FORA
INSTITUTE OF BIOLOGICAL SCIENCES
MASTERS IN BIODIVERSITY AND NATURE CONSERVATION

Vinícius Ferraz Nascimento

EVALUATION OF LETHAL AND REPELLENT ACTION OF AQUEOUS VEGETABLE EXTRACTS FOR THE CONTROL OF SPITTLEBUGS (HEMIPTERA: CERCOPIDAE)

Juiz de Fora
2021

Vinícius Ferraz Nascimento

EVALUATION OF LETHAL AND REPELLENT ACTION OF AQUEOUS VEGETABLE EXTRACTS FOR THE CONTROL OF SPITTLEBUGS (HEMIPTERA: CERCOPIDAE)

Dissertation presented to the Postgraduate Program in Biodiversity and Nature Conservation, from the Federal University of Juiz de Fora as a partial requirement to obtain the title of Master in Biodiversity and Nature Conservation. Concentration area: Animal Behavior

Advisor: Ph.D. Alexander Machado Auad

Juiz de Fora

2021

Ficha catalográfica elaborada através do programa de geração
automática da Biblioteca Universitária da UFJF,
com os dados fornecidos pelo(a) autor(a)

Ferraz Nascimento, Vinícius.
Evaluation of lethal and repellent action of aqueous vegetable extracts for the control of spittlebugs (Hemiptera: Cercopidae) / Vinícius Ferraz Nascimento. -- 2021.
57 p. : il.

Orientador: Alexander Machado Auad
Dissertação (mestrado acadêmico) - Universidade Federal de Juiz de Fora, Instituto de Ciências Biológicas. Programa de Pós-Graduação em Biodiversidade e Conservação da Natureza, 2021.

1. Inseticida botânico. 2. Repelência. 3. Compostos orgânicos voláteis. 4. Olfatometria. 5. Agroecologia. I. Machado Auad, Alexander, orient. II. Título.

Vinícius Ferraz Nascimento

EVALUATION OF LETHAL AND REPELLENT ACTION OF AQUEOUS VEGETABLE EXTRACTS FOR THE CONTROL OF SPITTLEBUGS (HEMIPTERA: CERCOPIDAE)

Dissertation presented to the Postgraduate Program in Biodiversity and Nature Conservation, from the Federal University of Juiz de Fora as a partial requirement to obtain the title of Master in Biodiversity and Nature Conservation. Concentration area: Animal Behavior

Approved on March 19, 2021.

Prof. Alexander Machado Auad, Ph.D.
Brazilian Agricultural Research Corporation - Embrapa

Prof. Dra. Marcelle Leandro Dias, Ph.D.
Self-employed

Prof. Fábio Prezoto, Ph.D.
Federal University of Juiz de Fora - UFJF

I dedicate this work to my parents Eliana Ferraz Nascimento and Welinton Moreira Nascimento who believed and encouraged me, and gave me the opportunity to study.

ACKNOWLEDGMENTS

First of all, I thank the God who lit my way during this journey.

To my advisor Dr. Alexander Machado Auad, for his patience in orientation, for his teachings, for believing in my potential and for the encouragement that made it possible to conclude this dissertation.

To my parents Eliana and Welinton, my brother Levy and my family for the immense effort and support provided during the master's degree. I dedicate my work to them.

To friends and colleagues at Embrapa Dairy Cattle's entomology laboratory: Tiago Resende, Marcelle Leandro, Amanda Mendoza, Sandra Elisa and Lenon Reis for their collaboration and support at work, and for their friendship.

To all the staff and professionals at Embrapa Dairy Cattle for their support in carrying out the research.

Embrapa Dairy Cattle for providing structure and providing materials for carrying out the research.

To friends and colleagues in the Postgraduate Program: Raphael Machado, Samuel Lima and Zacarias Rosalina for their conversations, laughter and encouragement.

To my graduate friends and for life Breno Alexandre, Kayro Barra Mansa, João Pedro Gama, Thiago Santos, Ricardo Alves, Dalila Pereira and Lauro Santos, who even from a distance, supported me at all times.

To the professors of the Postgraduate Program in Biodiversity and Nature Conservation for the classes, teachings, advice and support during the master's degree.

The coordination, to the servers and supporters of the Postgraduate Program in Biodiversity and Nature Conservation for their support, commitment and disposition.

The Federal University of Juiz de Fora for its physical structure and support.

CAPES for the financial support to the project.

To IFSULDEMINAS - Campus Machado, and its employees and teachers, for their support and professional qualification.

"A lot of small people in many small places, doing small things will change the face of the Earth" (African Proverb)

ABSTRACT

Spittlebugs (Hemiptera: Cercopidae) are species considered to be the main pests of forage grasses throughout Tropical America. In Brazil, *Mahanarva spectabilis* (Distant) is the main and most limiting pest associated with elephant grass (*Pennisetum purpureum* Schum.). These insects have the potential to generate great economic losses for the milk and meat production chain, harming producers. Among the spittlebugs control tactics, the use of resistant grasses, biological control and the application of synthetic insecticides in adults are the best known methods. The use of synthetic insecticides is economically viable only when combined with other spittlebugs control strategies, but they are environmentally unsafe products, so it is necessary to research tactics that are less aggressive to the environment, socially viable and of low cost for mainly small farmers. In this context, the insecticidal and repellent action that plant compounds have against different insect-pest species stands out. Thus, the objectives of this research were to evaluate whether aqueous extracts of the aromatic plants A. *sativum*, *R. graveolens*, *C. verum*, *C. citratus*, *S. aromaticum*, *I. verum*, *E. globulus*, *N. tabacum* and *T. vulgaris* have an insecticidal effect on spittlebug nymphs and the choice of spittlebugs adults can be altered for elephant grass, by attraction or non-attraction, in olfactometry tests. In the first stage of the research, the insecticidal effect of the extracts on nymphs of *M. spectabilis* was evaluated. The results indicated that the tobacco extract was the most effective among all nine tested. At a concentration of 20%, after 48 hours, it reached an efficiency of 76%. Then, bioassays were carried out comparing 5 concentrations of tobacco extract (5%, 10%, 15%, 20% and, 25%), where the extract in the concentration of 25% reached an efficiency greater than 92%. As for the extraction methods, the infusion and decoction methods were shown to be equivalent to the standard extraction method (UAE). Tobacco extract is recommended as a strategy to control *M. spectabilis*, at a concentration of 25%, with its extraction by infusion and decoction. In the second stage, olfactometry bioassays were performed. The combinations of aqueous extracts applied to the host plant were tested against fresh air and against the host plant without the extract. The aqueous vegetable extracts of tobacco, star anise and eucalyptus were not attractive for the *M. spectabilis*, and can be used as a management tactic in the case of elephant grass.

Keywords: botanical insecticide, repellency, volatile organic compounds, olfactometry, agroecology

RESUMO

As cigarrinhas-das-pastagens (Hemiptera: Cercopidae) são espécies consideradas as principais pragas de gramíneas forrageiras em toda a América Tropical. No Brasil, a *Mahanarva spectabilis* (Distant) é a principal e mais limitante praga associada ao capim-elefante (*Pennisetum purpureum* Schum.). Esses insetos têm potencial para gerar grandes prejuízos econômicos para a cadeia produtiva de leite e carne, prejudicando produtores. Dentre as táticas de controle da cigarrinha, o uso de gramíneas resistentes, o controle biológico e a aplicação de inseticidas sintéticos em adultos são os métodos mais conhecidos. O uso de inseticidas sintéticos é economicamente viável somente quando combinado com outras estratégias de controle da cigarrinha, porém são produtos ambientalmente inseguros, por isso é necessário pesquisar táticas menos agressivas ao meio ambiente, socialmente viáveis e de baixo custo para principalmente os pequenos agricultores. Nesse contexto, destaca-se a ação inseticida e repelente que compostos vegetais possuem contra diferentes espécies insetos-praga. Assim, os objetivos desta pesquisa foram avaliar se extratos aquosos das plantas aromáticas *A. sativum*, *R. graveolens*, *C. verum*, *C. citratus*, *S. aromaticum*, *I. verum*, *E. globulus*, *N. tabacum* e *T. vulgaris* têm efeito inseticida sobre as ninfas da cigarrinha e se podem alterar a escolha dos adultos das cigarrinhas por capim elefante, por atração ou não-atração, em testes de olfatometria. Na primeira etapa da pesquisa foi avaliado o efeito inseticida dos extratos sobre ninfas de *M. spectabilis*. Os resultados indicaram que o extrato de tabaco foi o mais eficaz entre todos os nove testados. Na concentração de 20%, após 48 horas, atingiu uma eficiência de 76%. Em seguida, foram realizados bioensaios comparando 5 concentrações do extrato de tabaco (5%, 10%, 15%, 20% e, 25%), onde o extrato na concentração de 25% atingiu uma eficiência superior a 92%. Quanto aos métodos de extração, os métodos de infusão e decocção mostraram-se equivalentes ao método de extração padrão (UAE). O extrato de tabaco é recomendado como estratégia de controle de *M. spectabilis*, na concentração de 25%, com sua extração por infusão e decocção. Na segunda etapa foram realizados os bioensaios de olfatometria. As combinações dos extratos aquosos aplicados na planta hospedeira foram testadas contra o ar fresco e contra a planta hospedeira sem o extrato. Os extratos vegetais aquosos de tabaco, anis estrelado e eucalipto não foram atrativos para *M. spectabilis*, e podem ser usados como tática de manejo no caso do capim elefante.

Palavras-chave: inseticida botânico, repelência, compostos orgânicos voláteis, olfatometria, agroecologia

LIST OF ILUSTRATIONS

LIST OF TABLES

TABLE OF CONTENTS

CHAPTER 1

1 GENERAL INTRODUCTION

Spittlebugs (Hemiptera: Cercopidae) are a complex of species that can cause serious damage to grasses, compromising the forage supply and leading to economic losses (AUAD *et al*., 2011; CONGIO, 2010). These insects are considered as the main pests of forage grasses in all Tropical America, causing great damage to the dairy and beef cattle in Brazil (SOUZA *et al*., 2008; VALÉRIO *et al*., 1997). The damage caused by the spittlebugs varies for each grass species, location, climatic conditions and, management (VALÉRIO, 2013). In attacked areas, there is a reduction in the volume of dry matter, a reduction in the mass of green grass, a reduction in the growth, and a significant drop in the nutritional quality of the plant's tissues, which considerably limits the support capacity of pastures, implying a decrease in the production of the herd (of milk and meat), and in serious environmental and social problems (AUAD *et al*., 2007; PEREIRA *et al*., 2018; RIBEIRO and CAZAROTTO, 2018; SOUZA *et al*., 2008; TEIXEIRA *et al*., 2017).

The main species of spittlebugs present in Brazil are the *Deois flavopicta* (Stal, 1854), *Zulia entreriana* (Berg, 1879), *Deois incompleta* (Walker, 1851), *Deois schach* (Fabricius, 1787), *Mahanarva fimbriolata* (Stal, 1854) (CASTRO *et al*., 2007), and *Mahanarva spectabilis* (Distant, 1909) (AUAD *et al*., 2007), with the occurrence varying according to the region of the country. In Brazil, the spittlebug *M. spectabilis* (Distant) is the main and more limiting pest associated with the elephant grass (*Pennisetum purpureum* Schum.) (AUAD *et al*., 2007). These insects have the potential to generate economic losses of million-dollar figures, it is estimated that the losses generated vary from the US $ 840 to the US $ 2.1 million per year worldwide (THOMPSON, 2004).

In this sense, research is needed to identify techniques that could minimize the negative impacts involved in the occurrence and dissemination of the spittlebugs, supporting the farmers. The use of tolerant and resistant plants, the diversification of the pastures (ALVARENGA *et al*., 2019), cultural, biological, and chemical control are the existing practices to combat this pest (TEIXEIRA *et al*., 2020), even so, is necessary to research new technologies, mainly transferring the results in the rural environment, as the farmer's understanding of the technologies contributes to the economic, social and environmental development (SABONARO e CARMO, 2020). Since we live in a scenario where about 880 million people live in the world on less than US $ 1.00 a day and depend on livestock for their livelihood

(BESBES and MOLINA FLORES, 2013). Thus, from the social point of view, the creation of alternative forms of pest control, of low cost and easy acquisition, are fundamental measures (ROEL, 2001). The use of insecticides and botanical repellents is a widespread strategy in pest management, which can be integrated with pasture management.

The chemical compounds present in plants, act as attractants, food stimulants, repellents, deterrents, sources of hormones, pheromones, and kairomones, and are involved in several metabolic and behavioral processes in the insects (LOVATTO *et al*., 2012). Humans observed these characteristics in plants and started to use them to their advantage, historical sources suggest that the use of plants in protection against insects dates back more than 3000 years, in Europe (PAVELA, 2016). Botanical insecticides are an excellent alternative to the use of synthetic or chemical insecticides for crop protection, as they are effective in combating pest insects, and are less prone to the occurrence of forms of resistance (BARBOSA *et al*., 2006; HIKAL *et al*., 2017). Against the spittlebug *M. spectabilis*, Dias *et al*. (2019) demonstrated that compounds of plant origin can be efficient control alternatives.

Several plant species stand out among the botanical families that present active ingredients with insecticidal activity, providing efficiency of their extracts, which in direct action can cause changes in the hormonal system, infertility, physiological disturbances, inhibit food, impair oviposition and lead to the death of the insects in various stages of their life cycle (ROEL, 2001). The repellency or attractive action, on the other hand, is closely related to the volatile organic compounds (VOCs) released by the plants. These compounds act in the defense of the plants promoting protection against the abiotic and biotic stresses and also play crucial roles in the ecological interactions of plants with other organisms (DUDAVERA *et al*., 2007; RIFFEL and COSTA, 2015).

Compounds made from the plants used in this study have shown insecticidal and repellent efficiency on the insects in different studies. Garlic (*Allium sativum* L.) is a plant that has significant amounts of bioactive organosulfur compounds, which are mainly concentrated in its bulbs (garlic cloves) (MOREIRA, *et al*., 2006). Prowse *et al*., (2008) found the significant insecticidal activity of garlic juice on *Delia radicum* (L.) (Diptera: Anthomyiidae) and *Musca domestica* L. (Diptera: Muscidae). Mobki *et al*. (2013) reported a strong repellent effect of the garlic extract on *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Star anise has trans-anethole as its main constituent. The insecticidal effect of this substance has already been tested against *M. spectabilis*, and the results were promising (DIAS *et al*., 2019). Addiotionally, Singh, D. and Singh, A. (1991) obtained 100% repellency for *Musca domestica* L. (Diptera: Muscidae).

The Rue plants (*Ruta graveolens* L.) have glycosides (rutin), aromatic lactones, rutalin, rutacridone, and terpenes (MARTINS *et al*., 2005). These compounds are responsible for the insecticidal effects demonstrated on the tomato pests (*Lycopersicon esculentum* Mill.) (BARBOSA, *et al*., 2011). The VOCs present in the rue essential oil promoted repellency of *Cydia pomonella* L. (Lepidoptera: Tortricidae) in the laboratory tests (LANDOLT *et al*., 1999). Cinnamon (*Cinnamomum verum* J. Presl.) is rich in an aromatic compound called (E) - cinamaldehyde (SENRA *et al*., 2013), its insecticidal activity has already been proven on different species of diptera (BENELLI *et al*., 2018; SAMARASEKERA *et al*., 2005). The repellent activity of lemongrass (*Cymbopogon citratus* Stapf.) was evaluated by researchers Lima *et al*. (2008), who proved the effectiveness of the essential oil on *Brevicoryne brassicae* (Linnaeus, 1758) (Hemiptera: Aphididae). The methanolic extract of lemongrass also showed a larvicidal effect on *Anopheles arabiensis* Patton (Diptera: Culicidae) (KARUNAMOORTHI and ILANGO, 2010).

Clove (*Syzygium aromaticum* L.) is a rich source of eugenol, a substance with a recognized insecticidal effect (SANTIN *et al*., 2011). Gorri (2018) performed tests of clove extract on various stages in the life cycle of *Spodoptera frugiperda* (JE Smith, 1797) (Lepidoptera: Noctuidae), and found satisfactory results in action by contact. The essential oil of clove showed 100% repellency against the adults of *T. castaneum* (ABO-EL-SAAD *et al*., 2011). Eucalyptus (*Eucalyptus globulus* Labill) has components whose insecticidal and insect repellent activity is known, such as ρ-cymene, γ-terpinene, and 1.8 cineol (eucalyptol) (LIU *et al*., 2008; LEVYA *et al*., 2020). Sampaio *et al*. (2018) proved that the aqueous extract of eucalyptus leaves showed efficiency on the mortality, oviposition, and the emergence of the brabrid *Zabrotes subfasciatus* (Bohemann, 1833) (Coleoptera: Bruchidae). Its essential oil also has repellent properties, as observed by the González-Guiñez *et al*. (2016), who proved its repellency against the adults of *Sitophilus zeamais* Motschulsky (Coleoptera, Curculionidae).

Among the researches carried out with the plant extracts, tobacco (*Nicotiana tabacum* L.) is always remembered for its insecticidal potential against numerous agricultural pests. Tobacco has alkaloid nicotine, an efficient insecticide with direct effect, which also has a repellent property in its volatile form (SILVA *et al*. 2017; TAYOUB *et al*., 2015). The researchers, Rizvi *et al*. (2016) showed that the smoke extract significantly reduced the infestation levels of *Trichoplusia binotalis* Hiibner (Lepidoptera: Noctuidae) in cabbage plants (*Brassica oleracea* L.), compared to the controls. Sagheer *et al*. (2013), recorded that the acetonic tobacco extract showed repellency against *T. castaneum*. One another species, popularly known as thyme (*Thymus vulgaris* L.), has scientifically active constituents recognized as thymol and carvacrol, which have, among the other properties, insecticidal (PEREIRA, 2006) and repellent activity (CHINTALCHERE *et al*. 2013).

From the aforementioned information, the insecticidal and repellent action of plant compounds against pest insects is emphasized, suggesting, therefore, that they may have the same action against the target pest of the present project, spittlebug *Mahanarva spectabilis* (Distant, 1909) (Hemiptera : Cercopidae). Thus, the objectives of this research were to evaluate whether aqueous extracts from the aromatic plants *A. sativum*, *R. graveolens*, *C. verum*, *C. citratus*, *S. aromaticum*, *I. verum*, *E. globulus*, *N. tabacum* and *T. vulgaris* have an insecticidal effect on spittlebug nymphs and the choice of spittlebugs adults can be altered for elephant grass, by attraction or repellency, in olfactometry tests.

REFERENCES

ABO-EL-SAAD, M. M.; AL AJLAN, A. M.; AL-EID, M. A.; BOU-KHOWH, I. A. Repellent and fumigant effects of essential oil from clove buds *Syzygium aromaticum* L. against *Tribolium castaneum* (Herbest) (Coleoptera: Tenebrionidae). **Journal of Agricultural Science and Technology A.**, v. 1, p. 613-620, 2011.

ALVARENGA, R.; AUAD, A. M.; MORAES, J. C.; DA SILVA, S. E. B.; RODRIGUES, B. S. Tolerance to nymphs and adults of *Mahanarva spectabilis* (Hemiptera: Cercopidae) by forage plants in fertilized soils. **Pest. Manag. Sci.**, v. 75, n. 8, p. 2242-2250, 2019.

AUAD, A. M.; SIMÕES, A. D.; PEREIRA, A. V.; BRAGA, A. L. F.; SOBRINHO, F. S.; LÊDO, F. J. S.; PAULA-MORAES, S. V.; OLIVEIRA, S. A.; FERREIRA, R. B. Seleção de genótipos de capim-elefante quanto à resistência à cigarrinha-das-pastagens. **Pesquisa Agropecuária Brasileira**, v. 42, n. 8, p. 1077-1081, 2007.

AUAD, A. M.; SIMOES, A. D.; LEITE, M. V.; SILVA, S. E. B.; SANTOS, D. R.; MONTEIRO, P. H. Seasonal dynamics of egg diapause in *Mahanarva spectabilis* (Distant, 1909) (Hemiptera: Cercopidae) on elephant Grass. **Arquivos do Instituto Biológico**, v. 78, n. 2, p. 325–330, 2011.

BARBOSA, F. R.; DA SILVA, C. S. B.; CARVALHO, G. K. de L. **Uso de inseticidas alternativos no controle de pragas agrícolas**. Petrolina: Embrapa SemiÁrido, 2006. 47p.

BARBOSA, F. S.; LEITE, G. L. D.; ALVES, S. M.; NASCIMENTO, A. F.; D'ÁVILA, V. de A.; COSTA, C. D. da. Insecticide effects of *Ruta graveolens*, *Copaifera langsdorffii* and *Chenopodium ambrosioides* against pests and natural enemies in commercial tomato plantation. **Acta Sci. Agron. (Online)**, Maringá, v. 33, n. 1, p. 37-43, 2011.

BENELLI, G.; PAVELA, R.; GIORDANI, C.; CASETTARI, L.; CURZI, G.; CAPPELLACCI, L.; PETRELLI, R.; MAGGI, F. Acute and sub-lethal toxicity of eight essential oils of commercial interest against the filariasis mosquito *Culex quinquefasciatus* and the housefly *Musca domestica*. **Industrial Crops and Products**, v. 112, p. 668-680, 2018.

BESBES, B.; MOLINA-FLORES, B. FAO-ICAR Workshop on Animal Identification and Recording Systems for Traceability and Livestock Development in Countries of Latin America and the Caribbean, Santiago, Chile, 5-7 December, 2011. ICAR Technical Series, v.1, n. 15, 2013.

CASTRO, M. C. R. de; FILHO, F. L.; ZINGERTAS, M. R. de B.; MEDEIROS, M. O. Espécies adultas de cigarrinhas (Homoptera: Cercopidae) sobre *Brachiaria dictyoneura*, na região de Rondonópolis, MT. **Biodiversidade**, v. 6, n. 1, 2007.

CHINTALCHERE, J. M.; LAKARE, S.; PANDIT, R. Bioefficacy of essential oils of *Thymus vulgaris* and *Eugenia caryophyllus* against housefly, *Musca domestica* L. **The Bioscan**, v. 8, n. 3, p. 1029-1034, 2013.

CONGIO, G.F. de S. **Caracterização e quantificação dos danos causados por cigarrinhas adultas do gênero *Mahanarva* sp. (Hemiptera: Cercopidae) em *Brachiaria brizantha* cv. Marandu**. 2010. Dissertação (Mestrado em Ciência Animal e Pastagens) – ESALQ, Universidade de São Paulo, Piracicaba, 2010. 87 p.

DIAS, M. L.; AUAD, A. M.; MAGNO, M. C.; RESENDE, T. T.; FONSECA, M. G.; SILVA, S. E. Insecticidal activity of compounds of plant origin on *Mahanarva spectabilis* (Hemiptera: Cercopidae). **Insects**, v. 10, p. 360, 2019.

DUDAREVA, N.; NEGRE, F.; NAGEGOWDA, D. A.; ORLOVA, I. Plant volatiles: recent advances and future perspectives. **Crit. Rev. Plant Sci**., v. 25, p. 417–440, 2007.

FAZOLIN, M.; SANTOS, R. S.; ANDRADE, C. M. S. de; ASSIS, G. M. L. de; VALENTIM, J. F. **Cigarrinha-das-pastagens: como identificar e controlar a principal praga das pastagens**. Rio Branco: Embrapa Acre, 2016.

GONZÁLEZ-GUIÑEZ, R.; SILVA-AGUAYO, G.; URBINA-PARRA, A.; GERDING-GONZÁLEZ, M. Aceite esencial de *Eucalyptus globulus* Labill y *Eucalyptus nitens* H. Deane & Maiden (myrtaceae) para el control de *Sitophilus zeamais* Motschulsky. **Chilean journal of agricultural & animal sciences**, v. 32, n. 3, p. 204-216, 2016.

GORRI, J. E. R. **Prospecção de extratos botânicos para o controle de *Spodoptera frugiperda***. Dissertação (Mestrado em Produção Vegetal), Universidade Federal de Viçosa – Campus Rio Paranaiba, 2018. 29p.

HIKAL, W. M.; BAESHEN, R. S.; SAID-AL AHL, H.A.H. Botanical insecticide as simple extractives for pest control. **Cogent Biology**, v. 3, n. 1, 1404274, 2017.

KARUNAMOORTHI, K.; ILANGO, K. Larvicidal activity of *Cymbopogon citratus* (DC) Stapf. and *Croton macrostachyus* Del. against *Anopheles arabiensis* Patton, a potent malaria vector. **Eur. Ver. Med. Pharmacol. Sci.**, v. 14, n. 1, p. 57–62, 2010.

LANDOLT, P. J.; HOFSTETTER, R. W.; BIDDICK, L. L. Plant essential oils as arrestants and repellents for neonate larvae of the codling moth (Lepidoptera: Tortricidae). **Environmental Entomology**, v. 28, n. 6, p. 954–960, 1999.

LEYVA, M.; DEL CARMEN MARQUETTI, M.; MONTADA, D.; PAYROLL, J.; SCULL, R.; MOREJÓN, G.; PINO, O. Aceites esenciales de *Eucalyptus globulus* (Labill) y *Bursera graveolens* (kunth) Triana & Planch para el control de mosquitos de importancia médica. **The Biologist**, v. 18, n. 2, 2020.

LIMA, R. K.; CARDOSO, M. G.; MORAES, J. C.; VIEIRA, S. S.; MELO, B. A. FILGUEIRAS, C. C. Composição dos óleos essenciais de anis-estrelado *Illicium verum* L. e de capim-limão *Cymbopogon citratus* (DC.) Stapf: Avaliação do efeito repelente sobre *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae). **BioAssay**, v. 3, n. 1, p. 1-6, 2008.

LIU, X.; CHEN, Q.; WANG, Z.; XIE, L.; XU, Z. Allelopathic effects of essential oil from *Eucalyptus grandis* × *E. urophylla* on pathogenic fungi and pest insects. **Frontiers of Forestry in China**, v. 3, n. 2, p. 232-236, 2008.

LOVATTO, P. B.; SCHIEDECK, G.; GARCIA, F. R. M. A interação co-evolutiva entre insetos e plantas como estratégia ao manejo agro- ecológico em agroecossistemas sustentáveis. **Interciencia**. v. 37, n. 9, p. 657-653, 2012.

MARTINS, A. G.; ROSÁRIO, D. L.; BARROS, M. N.; JARDIM, M. A.G. Levantamento etnobotânico de plantas medicinais, alimentares e tóxicas da Ilha do Combu, Município de Belém, Estado do Pará, Brasil. **Revista Brasileira de Farmácia**, v. 86, n. 1, p. 21-30, 2005.

MOBKI, M.; SAFAVI, S. A.; SAFARALIZADEH, M. H.; PANAHI, O. Toxicity and repellency of garlic (*Allium sativum* L.) extract grown in Iran against *Tribolium castaneum* (Herbst) larvae and adults. **Archives of Phytopathology and Plant Protection**, v. 47, n. 1, p. 59-68, 2014.

MOREIRA, M. D.; PICANÇO, M. C.; SILVA, M. E.; MORENO, S. C.; MARTINS, J. C. Uso de inseticidas botânicos no controle de pragas. In: VENZON, M.; PAULA JÚNIOR, T. J.; PALLINI, A. (Ed.). **Controle alternativo de pragas e doenças**. Viçosa: Epamig/CTZM, 2006. p. 89-120.

PAVELA, R. History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects – a review. **Plant Protect.** Sci., v. 52, p. 229-241, 2016..

PEREIRA, R.B. **Extrato de casca de café e óleo de tomilho no controle de *Cercospora coffeicola* Berk & Cooke em cafeeiro**. Dissertação (Mestrado em Agronomia/Fitopatologia). Universidade Federal de Lavras, 2006. 79p.

PEREIRA, L. F.; FERREIRA, C. F. C.; GUIMARÃES, R. M. F. Manejo, qualidade e dinâmica da degradação de pastagens na Mata Atlântica de Minas Gerais – Brasil. **Nativa**, Sinop, v. 6, n. 4, p. 370-379, 2018.

PROWSE, G. M., GALLOWAY, T. S., FOGGO, A. Insecticidal activity of garlic juice in two dipteran pests. **Agricultural and Forest Entomology**, v. 8, n. 1, p. 1-6, 2006.

RIBEIRO, L. do P.; CAZAROTTO, A. R. Cigarrinhas-das-pastagens em Santa Catarina: avaliação do complexo de espécies e da incidência natural de fungos entomopatogênicos. **Agropecuária Catarinense**, Florianópolis, v. 32, n. 2, p. 73-79, 2019.

RIFFEL, A.; DA COSTA, J. G. **Os voláteis de plantas e o seu potencial para a agricultura**. Aracaju, SE: Embrapa Tabuleiros Costeiros. 2015. 48 p.

RIZVI, S. A. H.; HUSSAIN, S.; REHMAN, S. U.; JAFFAR, S.; REHMAN, M. F. U. Efficacy of ecofriendly botanical extracts of Ginger (*Zingiber officinale*), Garlic (*Allium sativum*) and Tobacco (*Nicotiana tabacum* L) for the control of cabbage looper (*Trichoplusia binotalis*) under agroecological conditions of Peshawar, Pakistan. **Journal of Entomology and Zoology Studies**, v. 4, n. 1, p. 88-90, 2016.

ROEL, A.R. Utilização de plantas com propriedades inseticidas: uma contribuição para o desenvolvimento rural sustentável. **Revista Internacional de Desenvolvimento Local**, v. 1, n. 2, p. 43-50, 2001.

SABONARO, D. Z.; CARMO, J. B. do. A transferência de tecnologia para o produtor rural: um caminho para a sustentabilidade. Revista Gestão & Sustentabilidade Ambiental, v. 9, p. 120-132, 2020.

SAGHEER, M.; ALI, K.; RASHID, A.; SAGHEER, U.; ALVI, A. Repellent and toxicological impact of acetone extracts of *Nicotiana tabacum*, *Pegnum hermala*, *Saussurea costus* and *Salsola baryosma* against red flour beetle, *Tribolium castaneum* (Herbst). **Pakistan Journal of Zoology**, v. 45, n. 6, p. 1735-1739, 2013.

SAMARASEKERA, R.; KALHARI, K. S.; WEERASINGHE, I. S. Mosquitocidal activity of leaf and bark essential oils of ceylon *Cinnamomum zeylanicum*. **Journal of Essential Oil Research**, v. 17, n. 3, p. 301–303, 2005.

SAMPAIO, A. A.; FONTES, L. da S.; BARBOSA, D. R. S.; LIMA, V. M. G da C. Bioatividade dos extratos aquosos de folhas de pimenta malagueta e eucalipto sobre *Zabrotes subfasciatus* em grãos de fava. **Revista Verde de Agroecologia e Desenvolvimento Sustentável**, v. 12, n. 2, p. 198-203, 2018.

SANTIN, J. R.; LEMOS, M.; KLEIN, L. C.; MACHADO, I. D.; COSTA, P.; OLIVEIRA, A. P.; TILIA, C.; SOUZA, J. P.; BARRETO, J. P.; BASTOS, J. K.; FALONI, S. Gastroprotective activity of essential oil of the *Syzygium aromaticum* and its major component eugenol in different animal models. **Naunyn Schmied Arch Pharmacol**, v. 383, p. 149-158, 2011.

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

SILVA, E. Y. Y. da; MORETTI, C. L.; MATTOS, L. M. Compostos funcionais presentes em bulbilhos de alhos armazenados sob refrigeração, provenientes de cultivos no Brasil e na China. **Cienc. Rural**, Santa Maria, v. 40, n. 12, p. 2580-2587, 2010.

SILVA, L. M; CALDAS, A. P.; RODRIGUES, A. L. M.; OLIVEIRA, J. S.; SIMONETTI, E. R. de S. O uso do extrato de fumo (*Nicotina tabacum*) como alternativa para o controle de pragas em hortaliças, 2017. In: CONGRESSO INTERNACIONAL DAS CIÊNCIAS AGRÁRIAS, 2., 2017, Natal. **Anais** [...]. Natal, 2017.

SINGH, D.; SINGH, A. K.. Repellent and insecticidal properties of essential oils against housefly, *Musca Domestica* L. **Int. J. Trop. Insect Sci.**, v. 12, p. 487–491, 1991.

SOUZA, J. C.; SILVA, R. A.; REIS, P. R.; QUEIROZ, D. S.; SILVA, D. B. **Cigarrinhas-das-pastagens: histórico, bioecologia, prejuízos, monitoramento e medidas de controle**. Belo Horizonte: Circular técnica EPAMIG, v. 42, 2008. p. 8.

TAYOUB, G.; SULAIMAN, H.; ALORF, M. Determination of nicotine levels in the leaves of some *Nicotiana tabacum* varieties cultivated in Syria. **Herba Polonica**, v. 61, n. 4, 2016.

TEIXEIRA, C. A. D.; COSTA, J. N.; CRUZ, P. G. da. **Cigarrinhas-das-pastagens: manejo para controlar**. Porto Velho, RO: Embrapa Rondônia, 2017, 4p.

TEIXEIRA, C. A. D.; JÚNIOR, J. R. V.; COSTA, J. N. M. Pragas e doenças em pastagens na Amazônia. n: SALMAN, A. K. D.; PFEIFER, L. F. M. (Ed.). **Pecuária leiteira na Amazônia**. Brasília, DF: Embrapa, 2020. p. 345-370.

THOMPSON, V. Associative nitrogen fixation, C 4 photosynthesis, and the evolution of spittlebugs (Hemiptera: Cercopidae) as major pests of neotropical sugarcane and forage grasses. **Bulletin of entomological research**, v. 94, n. 3, p. 189-200, 2004.

VALÉRIO, J. R. Manejo de insetos-praga. In: REIS, R. A.; BERNARDES, T. F.; SIQUEIRA, G. R. (Org.) **Forragicultura: ciência, tecnologia e gestão dos recursos forrageiros**. São Paulo: FUNEP, 2013. p. 317-331.

VALERIO, J. R.; JELLER, H.; PEIXER, J. Seleção de introduções do gênero *Brachiaria* (Griseb) resistentes à cigarrinha *Zulia entreriana* (Berg) (Homoptera: Cercopidae). **An. Soc. Entomol. Bras.**, Londrina, v. 26, n. 2, p. 383-387, 1997.

CHAPTER 2: INSECTICIDAL ACTIVITY OF AQUEOUS EXTRACTS OF PLANT ORIGIN ON *Mahanarva spectabilis* (Distant, 1909) (HEMIPTERA: CERCOPIDAE) [1]

ABSTRACT

The research on the botanical compounds with insecticidal potential is justified by the need to create the tactics that are less aggressive to the environment, socially viable and, of low cost for the small-scale farmers to use in the pest control. Spittlebug, *Mahanarva spectabilis* is considered as the main pest and most limiting one in the elephant grass, which has caused economic losses to the Brazilian farmers. Thus, the objective of this work was to evaluate the insecticidal effects of the aqueous extracts of garlic, star anise, rue, cinnamon, lemongrass, cloves, eucalyptus, tobacco and thyme on the nymphs of *M. spectabilis*. The extracts were made, using ultrasound-assisted extraction. The results suggested that the tobacco extract was the most effective among all the nine tested. At a concentration of 20%, after 48 hours, it reached an efficiency of 76%. Then, bioassays were carried out by comparing 5 concentrations of the tobacco extract (5%, 10%, 15%, 20% and, 25%), where the extract in the concentration of 25% reached an efficiency that was greater than 92%. As for the extraction methods, realized in the comparative bioassays, the infusion and decoction methods came out to be an equivalent to the UAE, which showed that these are the methods, easy and effective, to be practiced by the small producers. In summary, among the nine aqueous plant extracts tested in the laboratory, tobacco extract was the only one recommended against the *M. spectabilis*, as it had the highest insecticidal bioactivity at a concentration of 25%, with extraction by infusion and decoction.

Keywords: spittlebug, mortality, plant active ingredients, extraction methods, agroecology.

---

[1] This article was written according to the standards of the Bulletin of Entomological Research, to which it was submitted. In this dissertation, the article was adapted to ABNT/UFJF standards.

1 INTRODUCTION

Spittlebugs (Hemiptera: Cercopidae) are pests of great economic importance in Tropical America, they attack sugar cane fields, pastures and, weeds and cause damage to the dairy and cattle farming. Losses of up to $ 2.1 billion per year are estimated due to the action of spittlebugs worldwide (THOMPSON, 2004). The greatest losses are caused by the adults of this insect pest, which when feeds on the sap of the plant, injects toxins that cause burning of the leaves (VALÉRIO, 2009). Besides, this pest especially in its young stage, suck the xylem content in the roots, interfering with the transport of water and nutrients to the shoot of the plant (BYERS and WELLS, 1966).

Among the species, *Mahanarva spectabilis* (Distant, 1909) (Hemiptera: Cercopidae) is the main and most limiting pest in the elephant grass (*Pennisetum purpureum* Schum.) (AUAD *et al*., 2007). To avoid losses, research has been carried out in search of strategies that are economically and ecologically viable for producers. Embrapa Gado de Leite (Embrapa Dairy Cattle), a Brazilian agricultural research agency, has been constantly publishing results of research with resistant and tolerant plants (PEREZ, *et al*., 2019; SOBRINHO *et al*., 2010), in addition to making partnerships with researchers from Brazilian public universities, for research with biological control (VERISSIMO *et al*., 2020), chemical (DIAS *et al*., 2019) and on pasture diversification (ALVARENGA *et al*., 2019). It should be noted that, all of these techniques are part of the integration of control methods to combat this pest.

Among the mentioned strategies, chemical control with synthetic molecules increases the costs of control, mainly affecting family farmers. In Brazil, for example, according to the 2017 Census of Agriculture, conducted by the Brazilian Institute of Geography and Statistics (IBGE), there are about 1.17 million establishments producing milk, with about 60% of the milk produced in the country. comes from properties that fall into the category of family farming, described in Law no. 11,326 / 2006 (BRASIL, 2006; IBGE, 2017). From the social point of view, the creation of alternative forms of pest control, of low cost and easy acquisition, are fundamental measures for the maintenance of family farming (ROEL, 2001).

In another perspective, pest control with synthetic molecules is responsible for generalized environmental pollution, generating negative effects on non-target organisms and human health, in addition to being counterproductive, as it leads to the development of resistance by insects (PAVUNRAJ *et al*., 2016; SOSA *et al*., 2019). In this sense, considering social and environmental aspects, botanical insecticides are alternatives to replace and / or reduce synthetic insecticides, in chemical control, since their correct use can prove to be ecological, cheap and versatile (AHMAD *et al*., 2019).

The use of botanical insecticides is widespread in organic and family farming, especially in low-income countries. Based mainly on the traditional knowledge and secular empirical observations, farmers use homemade extracts from numerous plant species, which have active ingredients with insecticidal activity, to combat pests in different cultures (CORREA and SALGADO, 2011; DOUGOUD *et al*., 2019). Garlic (*Allium sativum* L.) is a plant in the Alliaceae family, which presents significant amounts of bioactive organosulfur compounds, mainly concentrated in the bulbs. Its botanical potential against the pests has been proven, for example, on *Aedes aegypti* (Diptera: Culicidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (AHMAD *et al*., 2019; SARMA *et al*., 2019). Tobacco (*Nicotiana tabacum* L.), which is widely used in agriculture in the form of extract, owes its insecticidal properties to nicotine and other alkaloids (DOUGOUD *et al*., 2019). Researchers have demonstrated its efficiency against hemiptera, coleoptera, and lepidopterans (ALI *et al*., 2017; JAVED *et al*., 2018; PRISHANTHINI and VINOBABA, 2014).

The essential oil (OE) of thyme (*Thymus vulgaris* L.), is rich in thymol and carvacrol, substances tested and described in the literature as potent ovicides and insecticides (DIAS *et al*., 2019; MUDRONČEKOVÁ *et al*., 2019). (E)-cinamaldehyde is the main aromatic component present in the OE of cinnamon (*Cinnamomum verum* J. Presl), that has demonstrated high insecticidal potential against two species of dipterans (BENELLI *et al*. 2018). The most abundant active compound in star anise (*Illicium verum* Hook f.) is trans-anethole, whose insecticidal effect has already been experienced on *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and *M. spectabilis*, with promising results (DIAS *et al*., 2019; WEI *et al*., 2014). The clove (*Syzygium aromaticum* L.) has the greatest bioactive constituents, such as Eugenol and E-caryophyllene, where, the first one is a compound tested by many researchers against pests, with good results (DIAS *et al*., 2019; GHAFOOR *et al*., 2019; ZENG *et al*., 2010). Eucalyptus (*Eucalyptus globulus* Labill) has its insecticidal properties due to the presence of the component 1,8- cineole in its essential oil (ADAK *et al*., 2020; MOSSI *et al*., 2011). The lemongrass OE (*Cymbopogon citratus* Stapf) has shown efficiency as a repellent and / or insecticide in different studies (ALVES *et al*., 2019; DIABATE *et al*., 2019; FURTADO *et al*. 2005). The rue (*Ruta graveolens* L.), in the form of powder, OE and, aqueous extract, has active ingredients that provoke its insecticidal effects (BARBOSA *et al*., 2011; CHAABAN *et al*., 2019; DA SILVA *et al*., 2016).

Few published studies that tested the insecticidal effects of the compounds of plant origin against the spittlebugs of the genus *Mahanarva*. Garcia *et al*. (2006), proved the potential of neem-based products and extracts (*Azadiractha indica* A. Juss), for the control of *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae). Likewise, Pistori *et al*. (2013), demonstrated that the aqueous extract of *Anacardium humile* St. Hill caused significant reductions in the nymphal survival rate of *M. fimbriolata*. Dias *et al*. (2019) tested the insecticidal potential of different compounds of plant origin, acquired in their standard chemical form, on the spittlebug *M. spectabilis*. The researchers obtained expressive results in the control of nymphs and adults with the compound transanethole, which is also present in star anise. These works demonstrate that the extracts and compounds of botanical origin can be alternatives to the control of spittlebugs with synthetic chemical pesticides and that more research is needed, which can take into account the factors as plants, concentrations, and forms of extraction that could serve the small producers.

Thus, with the need for viable and low-cost tactics for the spittlebugs and by considering that the botanical insecticides are social and environmental alternatives, the objective was to verify the insecticidal effect of the aqueous extracts of *A. sativum*, *R. graveolens*, *C. verum*, *C. citratus*, *S. aromaticum*, *I. verum*, *E. globulus*, *N. Tabacum* and *T. vulgaris* on the nymphs of the spittlebug, *Mahanarva spectabilis*.

2 MATERIALS AND METHODS

2.1 BOTANICAL MATERIAL

For the formation of elephant grass seedlings (*Pennisetum purpureum* Schum) stakes of approximately 10 cm, with a single node, were obtained from plants in the Experimental Field of Embrapa Dairy Cattle in Coronel Pacheco, MG, Brazil. The cuttings were propagated in plastic pots (500 mL) that contained the substrate (soil/manure in the proportion 1: 1), to form the seedlings. The seedlings were kept in a greenhouse for about 60 days, until they were used in the experiments as feeding substrates for the nymphs.

Seedlings of lemongrass, rue and, thyme were planted and irrigated daily in plastic pots of 1 L and stored in the greenhouse at the Embrapa Dairy Cattle for 3 months. These seedlings and the dried parts of cinnamon, cloves, star anise and, tobacco were purchased from the traders of the Municipal Market of Juiz de Fora, MG, Brazil. Organic garlic was purchased from the street fair at Juiz de Fora. Eucalyptus leaves were collected from the 12-year-old trees planted in Maripá de Minas, MG, Brazil. The vegetables used for making the extracts (Table 1) were obtained from different sources.

Table 1 - List of the species of plants and their parts used to make the aqueous extracts.

Plant species	**Family**	**Parts used**	**Active ingredients**	**References**
Garlic (*Allium sativum* L.).	Alliaceae	Bulbs	Methyl allyl disulfide and Diallyl trisulfide	Huang *et al*. (2000)
Tobacco (*Nicotiana tabacum* L.)	Solanaceae	Leaves	Nicotine	Dougoud *et al*. (2019)
Lemongrass (*Cymbopogon citratus* Stapf.)	Poaceae	Leaves	Geranial; Neral	Olivero-Verbel *et al*. (2010)
Eucalyptus (*Eucalyptus globulus* Labill.)	Myrtaceae	Leaves	1,8-cineole	Mossi *et al*. (2011)
Rue (*Ruta graveolens* L.)	Rutaceae	Leaves	2-Nonanone; 2-Undecanone	Orlanda and Nascimento (2015)
Thyme (*Thymus vulgaris* L.)	Lamiaceae	Leaves	Thymol; Carvacrol	Park *et al*. (2017)
Cinnamon (*Cinnamomum verum* J. Presl)	Lauraceae	Bark	Cinnamaldehyde	Benelli *et al*. (2018)
Star anise (*Illicium verum* Hook.f)	Illiciaceae	Fruits	Trans-anethole	Wei *et al*. (2014)
Clove (*Syzygium aromaticum*, L.)	Myrtaceae	Flower bud	Eugenol and E-caryophyllene	Zeng *et al*. (2010)

Source: Elaborated by the author (2020).

2.2 PROCESSING OF BOTANICAL MATERIAL

To obtain the botanical extracts, the selected parts of each plant (Table 1) were washed with distilled water and then distributed on the sheets of paper in metal trays. Consequently, they were placed in an oven of forced ventilation (Binder FD115) at an average temperature of 40 °C for 72 hours.

The kiln-dried materials were milled in a basic analytical IKA® mill, model A11, and placed in beckers. At that point, the plant extracts were prepared by mixing, the milled and, dry material with the distilled water, in different proportions for each bioassay with the aid of an Ultrasound shaker (ELMA E60H). The resulting solutions were filtered, using a voile fabric, that gave rise to aqueous extracts. The extracts were then stored in the bottles of amber glass, protected from light, until the time of the bioassays.

2.3 INSECTS

For the realization of the bioassays, nymphs of *M. spectabilis* of fourth and / or fifth instar were collected from the elephant grass cv. Kurumi at the Embrapa Dairy Cattle Experimental Field and taken to the Laboratory of Entomology. In the collection procedure, the nymphs were removed from the base of the plant with the help of a brush, placed in beakers containing roots, and kept in plants in the laboratory until use in the experiment. The collection of approximately 1000 nymphs was performed on different dates, in the mornings before each bioassay.

2.4 ASSESSMENT OF INSECTICIDAL ACTIVITY

2.4.1 Comparative Bioassays between Extracts

For the development of the bioassay, the methodology of Dias et al., (2019) was used. Two bioassays were carried out with different concentrations, 3%, and 20%, to compare the extracts against their insecticidal potential on the *M. spectabilis* nymphs. In the tests, nine extracts (Table 1), positive controls (Tiametoxam + Lambda-cyhalotrin), and negative controls (distilled water) were used, totaling in 11 treatments with 10 repetitions.

In each repetition, 10 nymphs were distributed over Petri dishes, and each nymph received 10 μL of the solution on its back, through a micropipette (V3-PLUS 0.5-10 μL). Consequently, the insects were transferred to the plastic pots (500 mL) with elephant grass plants, arranged in random blocks. The plants used had their roots previously exposed to facilitate nymphal feeding. The cups were wrapped in the bags of "voil" fabric to prevent the nymphs from escaping. The experiment was maintained in a Fitotron at 25 ± 2 °C, at the humidity of 70% ± 10%, and with the photophase of 12 hours, during the tests. The insecticidal activity of each extract was evaluated after 24 and 48 hours of its application, and subsequently, the number of alive and killed nymphs by the insecticidal action was counted.

2.4.2 Comparative Bioassay between Tobacco Concentrations

The tobacco extract showed satisfactory results in previous bioassays. Based on this information, it was decided to continue the tests with a bioassay comparing different concentrations of the tobacco extract.

Five concentrations (5%, 10%, 15%, 20% and 25%) of aqueous tobacco extract were prepared according to item 2.2. In the bioassay, each concentration was considered as a treatment, and distilled water was used as a control. Ten repetitions were performed. The test methodology was the same as previously described in the comparative bioassay between the extracts. The insecticidal activity of each concentration of the extract was evaluated after 24 and 48 hours of its application, and the number of the nymphs alive and killed by the insecticidal action was counted.

2.4.3 Comparative Bioassay between Extraction Methods

In this bioassay, four forms of extraction were tested and compared in relation to the efficiency of extraction in tobacco, comparing the results through the nymph survival of *M. spectabilis*, they are Static Maceration, Infusion, Decoction and Ultrasound Assisted Extraction (UAE). The UAE was the basis for comparisons because it is the methodology adopted in the other bioassays of the present research.

The method of extraction by static maceration consisted of placing the pressed and shredded tobacco leaves in contact with distilled water for 24 hours, and then separating the liquid part from the solid with the help of filtration using the voile fabric. In the decoction process, the pressed and shredded sheets of tobacco were placed in a container with distilled water. The container was taken to a magnetic stirrer with temperature control (Tecnal® model TE-0852) and kept until it boiled (~15 minutes). At the end of the process, the extract was filtered through the voile fabric. In the infusion process, the tobacco was dried in an oven of forced ventilation (Binder FD115) at an average temperature of 40 °C, for 72 hours, and milled in a basic analytical IKA® mill, model A11. The resulting powder was placed in contact with the boiling distilled water (100 °C) and left until it was cooled (~20 minutes). The extract was filtered through the voile fabric.

In the bioassay, all the extracts were prepared in the concentration of 25%, and distilled water was used as a control, totaling in five treatments. Ten repetitions were performed. In each repetition, 10 nymphs were distributed on the Petri dishes. The test and evaluation methodology was the same as the one previously described in the comparative bioassay between the extracts.

2.5 STATISTICAL ANALYSIS

The mortality data for *M. spectabilis* obtained by the bioassays were submitted to the Abbot formula (ABBOTT, 1925) to obtain the efficacy of the treatments. Mortality values were subjected to the analysis of variance (ANOVA) and means were compared by using Scott Knott test ($p < 0.05$). To evaluate the effect of different tobacco concentrations on the nymphal mortality, regression analysis was used with the data transformed into sine arc root x + 1. All the analysis was performed using the free software Sisvar, version 5,6, build 90 (FERREIRA, 2019).

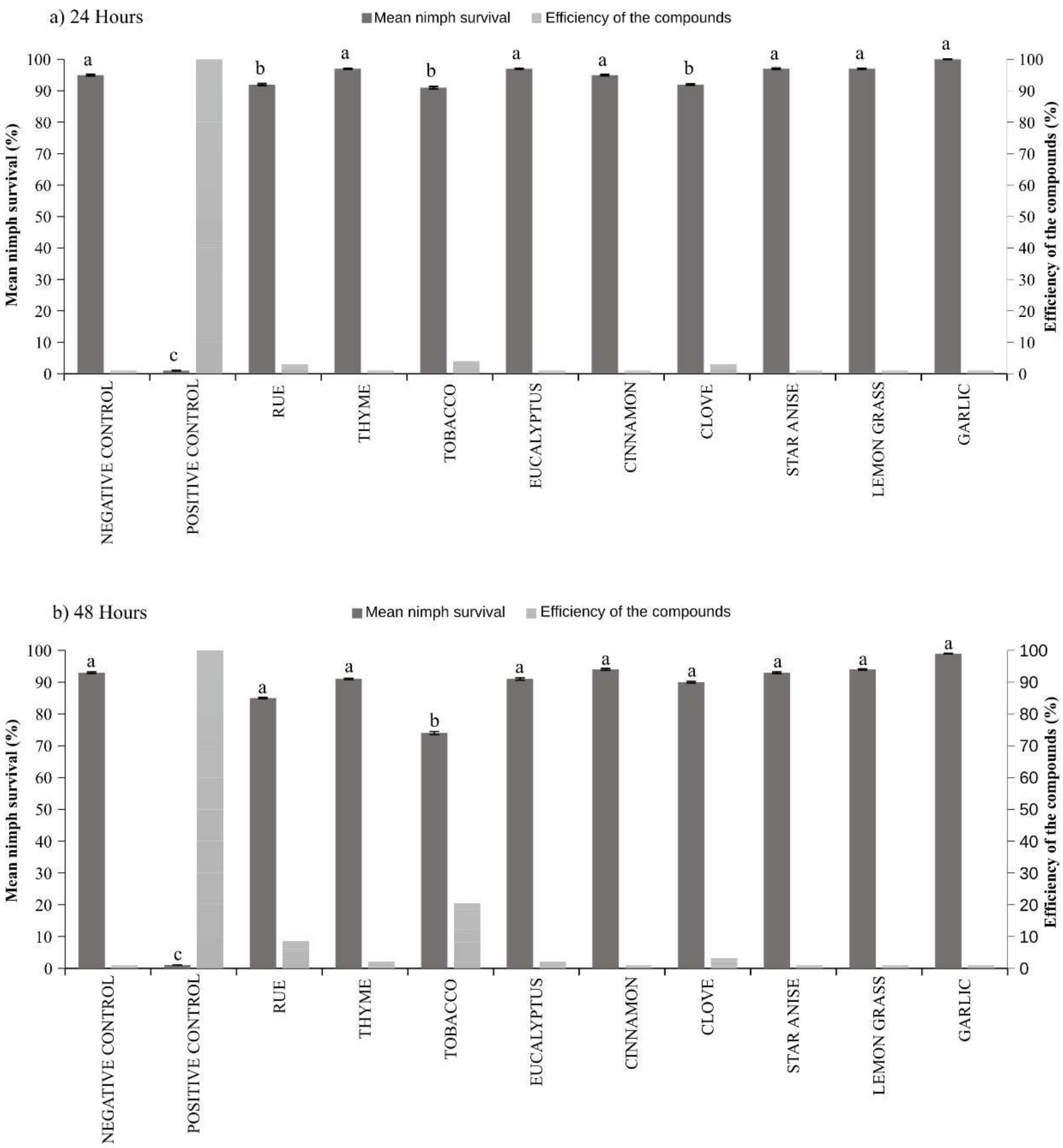
3 RESULTS

3.1 COMPARATIVE BIOASSAYS BETWEEN EXTRACTS

A significant difference was found in the survival of nymphs ($F = 199.98$; $p < 0.0001$) between the negative control and the aqueous extracts of tobacco, rue and cloves, at a concentration of 3%, 24 hours after application. Although they differ significantly, the efficiency of these extracts was less than 4%. The insecticide used (positive control) promoted 0% survival of nymphs of M. spectabilis (Figure 1a). In the evaluation after 48 hours, only the tobacco extract differed significantly ($F = 108.39$; $p < 0.0001$) from the negative control, with an efficiency of 20% (Figure 1b).

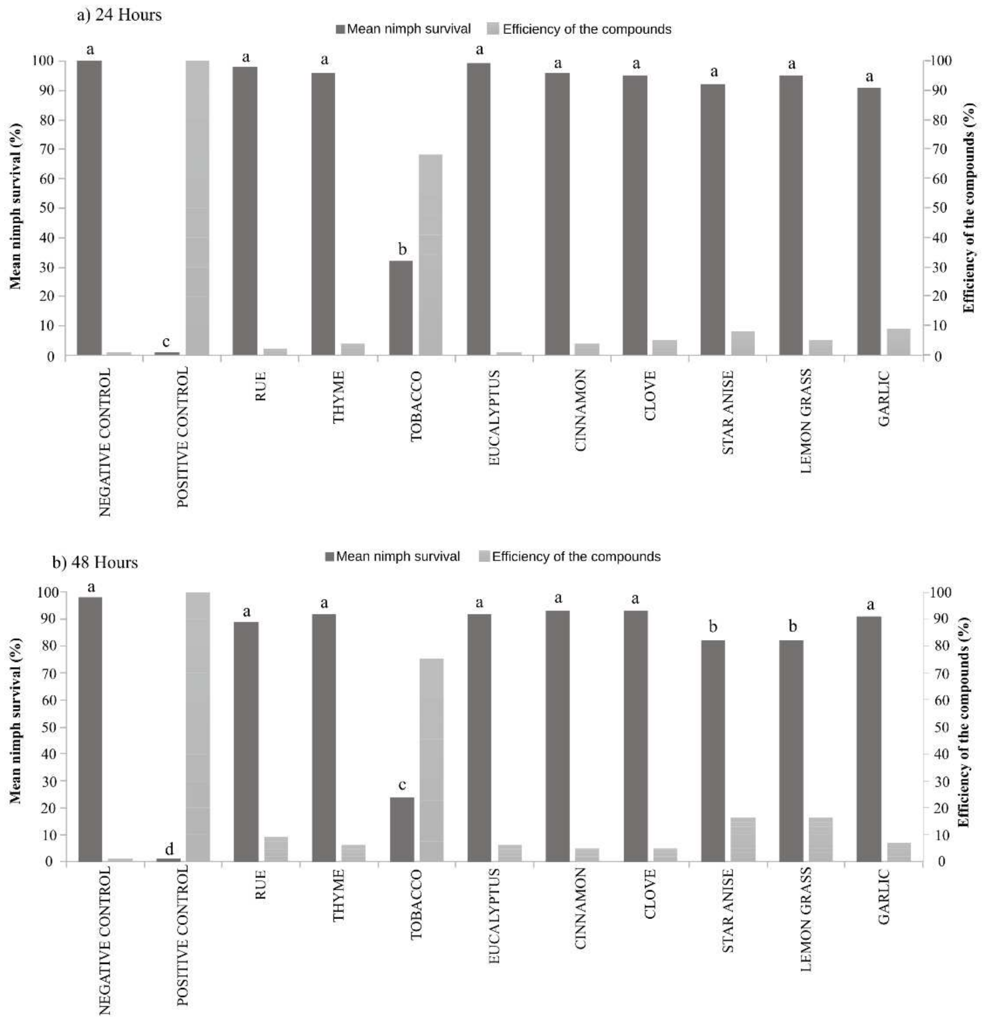
At a concentration of 20%, the tobacco extract promoted significant lethal effects in the nymphs of *M. spectabilis*, after 24 hours ($F = 150.78$; $p < 0.0001$) and 48 hours ($F = 140.36$; $p < 0 , 0001$) of its application, in comparison with the negative control and the other extracts. The efficacy of the tobacco extract was 68% and 76% in the evaluations performed after 24 and 48 hours, respectively (Figures 2a and 2b). It can also be observed that in the evaluation carried out at 48 hours of application, in addition to the tobacco extract, the extracts of star anise and lemongrass differed significantly from the negative control, but obtained a greater survival of nymphs of the insect pest in relation to the tobacco extract (Figure 2b).

Figure 1 - Insecticidal activity of botanical extracts (concentration of 3%) against *Mahanarva 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 ($p < 0.05$).



Source: Elaborated by the author (2020).

Figure 2 - Insecticidal activity of botanical extracts (concentration of 20%) against *Mahanarva 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 ($p < 0.05$).



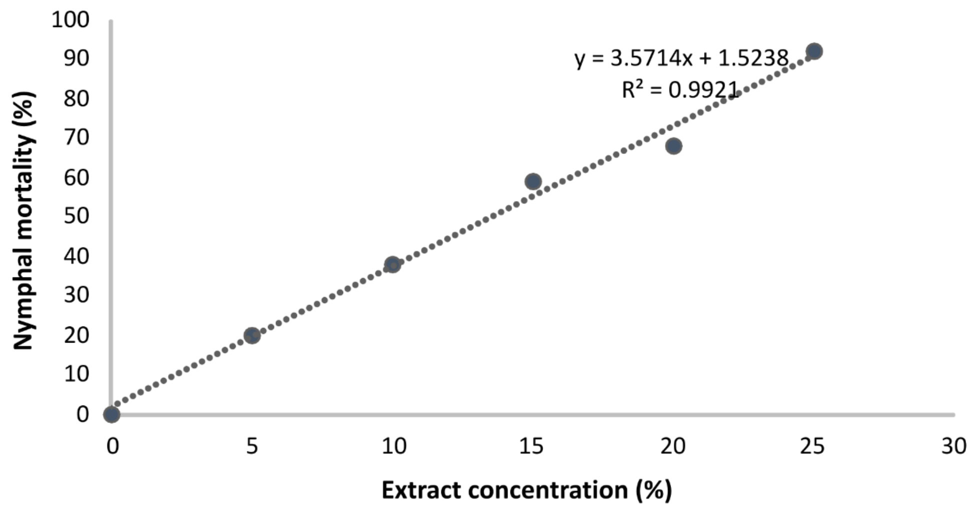
Source: Elaborated by the author (2020).

3.2 COMPARATIVE BIOASSAY BETWEEN TOBACCO CONCENTRATIONS

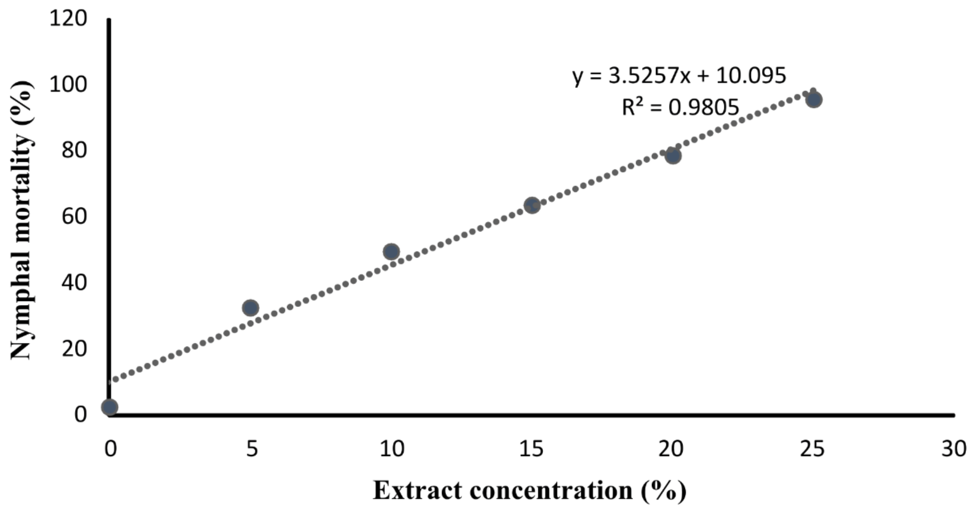
The graphical representation of the regression showed a linear increase in the nymphal morality, as the aqueous tobacco extract concentrations increased, 24 and 48 hours after its application (Figures 3a and 3b). A significant nymphal decrease in *M. spectabilis* was observed due to the increase in the concentration of tobacco extract after 24 hours ($F = 173.73$; $p < 0.0001$) and 48 hours ($F = 211.05$; $p < 0.001$). Efficiency above 92% was found with the tobacco extract, applied at a concentration of 25%. Intermediate efficiencies (63 and 78%) were obtained at concentrations of 15 and 20% of tobacco extracts after 48 hours of application. In the other concentrations, the extracts differed significantly from the negative control, but reached an efficacy equal to or less than 48% (Figure 4).

Figure 3 - Graphical representation and regression equation between tobacco extract concentrations and percentage of mortality of *Mahanarva spectabilis* nymphs after 24 (a) and 48 h (b) of application.

a) 24 hours

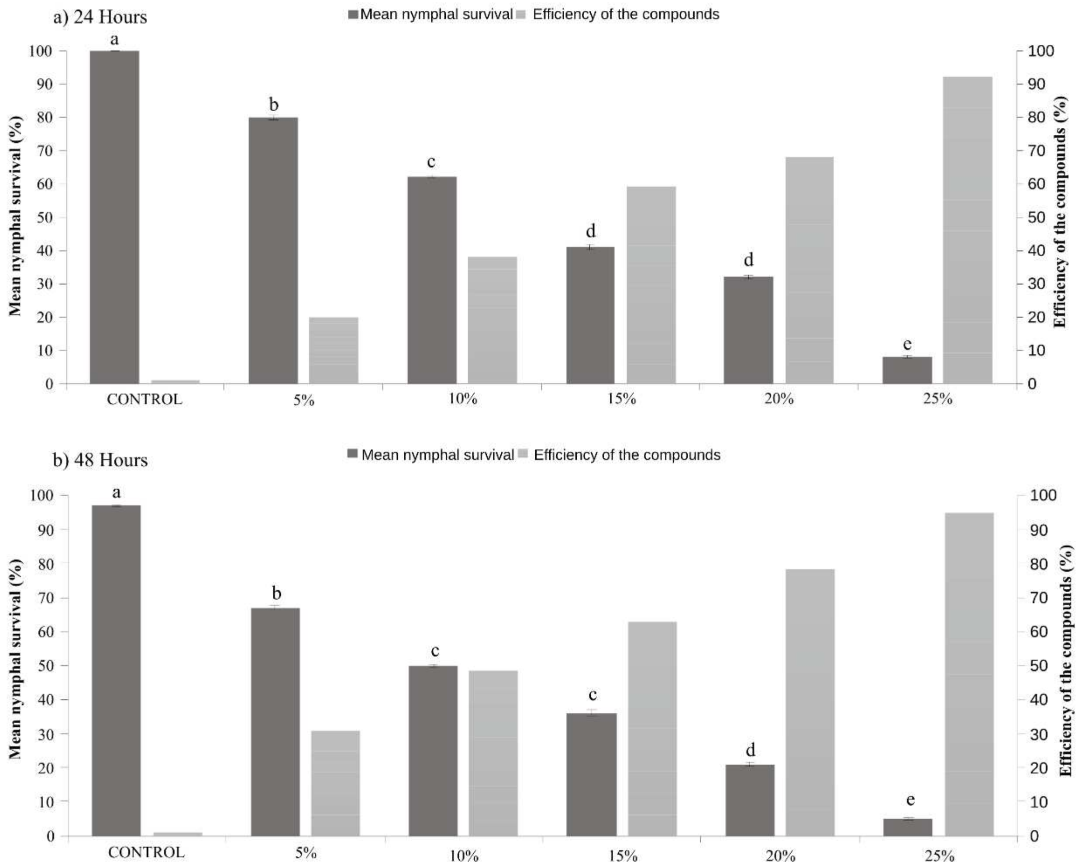


b) 48 hours



Source: Elaborated by the author (2020).

Figure 4 - Insecticidal activity of tobacco extracts produced in different concentrations (5%, 10%, 15%, 20% and 25%) against *Mahanarva 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 ($p < 0.05$).



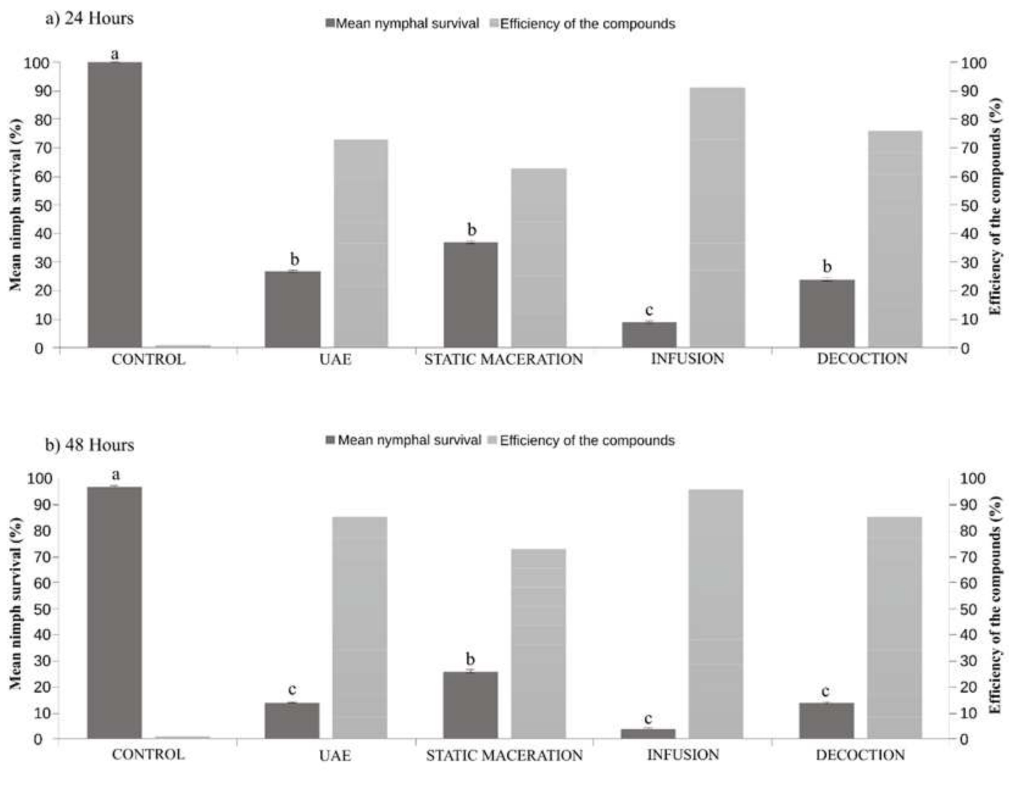
Source: Elaborated by the author (2020).

3.3 COMPARATIVE BIOASSAY BETWEEN EXTRACTION METHODS

In the first evaluation, performed 24 hours after the application of the tobacco extracts, the survival of the *M. spectabilis* nymphs was significantly lower ($F = 52.57$; $p < 0.0001$) in relation to the negative control in all treatments. The efficiency of the treatments ranged from 63% to 91% depending on the extraction method. Static maceration, UAE and decoction did not differ significantly from each other and promoted efficiencies lower than the infusion method, which was the most effective in reducing the nymph survival of *M. spectabilis* (Figure 5a).

After 48 hours of application, all extracts promoted significant differences ($F = 116.34$; $p < 0.0001$) in the survival of insect pest nymphs, compared to the negative control treatment. The efficiency of the treatments ranged from 73% to 96% (Figure 5b). In evaluating treatments, UAE and decoction corresponded significantly to the infusion that was more effective in 24 hours, but differed from the static maceration treatment that was less effective (Figure 5b).

Figure 5 - Insecticidal activity of tobacco extracts produced by different extraction methods against *Mahanarva 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 ($p < 0.05$).



Source: Elaborated by the author (2020).

4. DISCUSSION

In nature, many chemical compounds from the secondary metabolism of plant species have properties that inhibit the action of herbivorous insects (ISMAN, 2006). The action can occur in terms of mortality, inhibition of food, reduction of food consumption, delay in the development, deformations and, sterility (DEQUECH *et al*., 2008). Thus, many plant species provide potential sources of compounds for the control of insect pests, which can be obtained by making the extracts in different solvents.

This research revealed the effect of aqueous extracts obtained from nine different plants, on the nymphs of the spittlebug *Mahanarva spectabilis*. The low efficiency observed for extracts of garlic, rue, cinnamon, lemongrass, clove, eucalyptus and thyme may be associated with differences in the concentration of active ingredients in extracts and plants, and also with low residual effects (MACHADO *et al*., 2007). Another association that can be made is that, according to Dias *et al*. (2019) the foam produced by the nymphs, after applying the treatments, can act in the partial elimination of the irritant. At a concentration of 20%, treatments with aqueous extracts of lemongrass and star anise differed significantly from the negative control, but registered low efficiency. Other authors have obtained expressive results with the extracts of lemongrass and star anise. KARUNAMOORTHI and ILANGO (2010) used the methanolic extract of lemongrass and observed a high larvicidal efficacy against *Anopheles arabiensis* Patton (Diptera: Culicidae), which is among the main vectors of malaria in Africa. And in the work of Zhou *et al*. (2016), the star anise extract, in different solvents, was highly toxic to the adults of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). Thus, considering the results obtained, it is proposed that the difference in toxicity observed between the present research and that of other authors, may have occurred due to the particularities of the species used in each study, such as physiology and resistance mechanisms.

In Brazil, the National Health Surveillance Agency (ANVISA) establishes that tests with insecticides are considered satisfactory when the survival of the treatment reaches an average value of 10% (± 10%) in relation to the control (BRASIL, 2009). None of the extracts observed in the first bioassay promoted satisfactory survival results for ANVISA parameters, however, the aqueous tobacco extract showed results close to the satisfactory value. Other studies have obtained satisfactory results with the tobacco extract, as in that of Sarker and Lim (2018), in which the tobacco extract reduced the survival of the first instar and adult caterpillars of Grapholita molesta (Busck) (Lepidoptera: Tortricidae) among the 32 plant extracts tested. One explanation for why this extract stands out as the most efficient is due to the presence of nicotine, the main active substance present in tobacco plants (RANDO *et al*. 2009). Nicotine causes nerve impulses that lead to hyperexcitation of the insect's nervous system, and consequently its death (SILVA *et al*. 2017). In general, it is known that the tobacco extract acts as a contact insecticide, which has a fast action and degradation in the environment, low phytotoxicity, little harmful to the soil and has a low cost (MENDES *et al*., 2016; SILVA *et al*., 2017). Thus, based on the preliminary results, we opted to perform an assay to compare the effects of different concentrations of tobacco extract on *M. spectabilis*.

Defining the best concentration is important, which should be neither high enough to get wasted, causing costs and environmental problems, and nor scarce, to be inefficient to lead the insects to develop resistance (DENT, 2000). In other words, the appropriate concentration allows to determine the intensity of the field treatment after the preliminary screening in the laboratory. The results of this bioassay confirmed that all tested concentrations of aqueous tobacco extract showed significant insecticidal activity against nymphs of *M. spectabilis*. Similar observations about the effect of *N. tabacum* extract on other insect species have been reported. Rizvi *et al*. (2016) showed that the tobacco extract significantly reduced the infestation levels of *Trichoplusia binotalis* Hiibner (Lepidoptera: Noctuidae) in cabbage plants (*Brassica oleracea* L.), as compared to the controls. The researchers Lokesh *et al*. (2017), reported 100% mortality of the adults of *Cylas formicarius* Fabricius (Coleoptera: Brentidae) after 72 hours, which were submitted to the extract of chloroform and acetone from the tobacco leaves. Natural tobacco-based insecticides have been used by man since the 18th century, but these insecticides were gradually replaced in the last century with the synthetic ones, with substances that are highly aggressive to man and to the environment (COSTA *et al*., 2004; NUNES and RIBEIRO, 1999). However, due to the health and environmental problems generated by the largescale use of synthetic insecticides and the market's interest in more sustainable products, there is a growing interest by the farmers in the alternative products that can be used for pest management (CORREA and SALGADO, 2011).

However, for the adoption of alternative technologies such as plant extracts, it is necessary that their preparation, extraction processes, be easy and viable for farmers on their properties. The methods of extracting hot compounds, such as decoction and infusion, consist of extracting active ingredients from the plants. Since plants are degraded by the combined action of water and heat, so the methods become simple, fast, and feasible for the farmers (LAMEIRA and PINTO, 2008). One of the first mentions of insecticides and forms of extraction, in 1763, already reports the use of the infusion of tobacco leaves for the control of lice (NIU and YU, 2009). Currently, methods of extracting plant compounds by infusion and decoction are still widely used, researchers Cuevas-Salgado *et al*. (2015) obtained significant mortality rates of the eggs and caterpillars of *Leptophobia aripa* Boisduval (Lepidoptera: Pieridae) by using botanical tobacco infusion. In their research, 48 hours after the application of the extracts, the methods of infusion and decoction statically matched with the UAE method (laboratory control). The infusion method was more effective than the UAE. Therefore, it is the most recommended method for the extraction of the bioactive compounds of tobacco by the farmers. The results reported in this work, open the possibility of further investigations on the efficacy of the tobacco extract, and its insecticidal properties on *M. Spectabilis*, in field conditions, and for the future recommendations in the programs of integrated pest management on small farms.

5 CONCLUSIONS

Among the nine aqueous extracts of plants tested in the laboratory, the tobacco extract against the spittlebug *M. spectabilis* is recommended, since it showed the highest insecticidal bioactivity at the concentration of 25%, with its extraction using infusion and decoction.

REFERENCES

ABBOTT, W. S. A method of computing the effectiveness, of an insecticide. **J. Econ. Entomol.**, v. 18, n. 2, p. 265–267, 1925.

ADAK, T.; BARIK, N.; PATIL, N. B.; GADRATAGI, B. G.; ANNAMALAI, M.; MUKHERJEE, A. K.; RATH, P. C. Nanoemulsion of eucalyptus oil: An alternative to synthetic pesticides against two major storage insects (*Sitophilus oryzae* L.) and *Tribolium castaneum* (Herbst) of rice. **Ind. Crop. Prod.**, v. 143, 111849, 2020.

AHMAD, F.; IQBAL, N.; ZAKA, S. M.; QURESHI, M. K.; SAEED, Q.; KHAN, K. A.; GHRAMH, H. A.; ANSARI, M. V.; JALEEL, W.; AASIM, M.; AWAR, M. B. Comparative insecticidal activity of different plant materials from six common plant species against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). **Saudi J. Biol. Sci.**, v. 26, n. 7, p. 1804-1808, 2019.

ALI, K.; SAGHEER, M.; HASAN, M.; HANIF, C. M. S.; MALIK, S.; RIZWAN, M.; RASHID, A. Medicinal Response of *Moringa oleifera* and *Nicotiana tabacum* as repellent and toxicant against *Trogoderma granarrium* and *Rhyzopertha dominica*. **Z. Arznei-gewurzpfla**. v. 22, n. 3, p. 132-135, 2017.

ALVES, M. DE S.; CAMPOS, I. V.; DE BRITO, D. DE M. C.; CARDOSO, C. M.; PONTES, E.G., DE SOUZA, M. A. A. Efficacy of lemongrass essential oil and citral in controlling *Callosobruchus maculatus* (Coleoptera: Chrysomelidae), a post-harvest cowpea insect pest. **Crop. Prot.**, v. 119, p. 191-196, 2019.

AUAD, A. M.; SIMÕES, A. D.; PEREIRA, A. V.; BRAGA, A. L. F.; SOBRINHO, F. S.; LÊDO, F. J. S.; PAULA-MORAES, S. V.; OLIVEIRA, S. A.; FERREIRA, R. B. Seleção de genótipos de capim-elefante quanto à resistência à cigarrinha-das-pastagens. **Pesquisa Agropecuária Brasileira**, v. 42, n. 8, p. 1077-1081, 2007.

BARBOSA, F. S.; LEITE, G. L. D.; ALVES, S. M.; NASCIMENTO, A. F.; D'ÁVILA, V. de A.; COSTA, C. D. da. Insecticide effects of *Ruta graveolens*, *Copaifera langsdorffii* and *Chenopodium ambrosioides* against pests and natural enemies in commercial tomato plantation. **Acta Sci. Agron. (Online)**, Maringá, v. 33, n. 1, p. 37-43, 2011.

BENELLI, G.; PAVELA, R.; GIORDANI, C.; CASETTARI, L.; CURZI, G.; CAPPELLACCI, L.; PETRELLI, R.; MAGGI, F. Acute and sub-lethal toxicity of eight essential oils of commercial interest against the filariasis mosquito *Culex quinquefasciatus* and the housefly *Musca domestica*. **Industrial Crops and Products**, v. 112, p. 668-680, 2018.

BESBES, B.; MOLINA-FLORES, B. FAO-ICAR Workshop on Animal Identification and Recording Systems for Traceability and Livestock Development in Countries of Latin America and the Caribbean, Santiago, Chile, 5-7 December, 2011. **ICAR Technical Series**, v.1, n. 15, 2013.

BRASIL. **Lei n. 11.326, de 24 de julho de 2006**. Políticas direcionadas à Agricultura Familiar e Empreendimentos Familiares Rurais. Presidência da República, Brasília, Brasil, 2006.

BRASIL. **Manual de Testes de Eficácia em Produtos Desinfestantes**. Brasília, DF: Anvisa, 2009.

BYERS, R.; WELLS, H. D. Phytotoxemia of Coastal bermudagrass caused by the two-lined spittlebug, *Prosapia bicincta* (Homoptera: Cercopidae). **Ann. Entomol. Soc. Am. Amer.**, v. 59, p. 1067–1071, 1966

CARDONA, C.; FORY, P.; SOTELO, G.; PABÓN, A.; DIAZ, G.; MILES, J. W. Antibiosis and tolerance to five species of spittlebug (Homoptera: Cercopidae) in *Brachiaria* spp.: implications for breeding for resistance. **J. Econ. Entomol.**, v. 97, n. 2, p. 635–645, 2004.

CHAABAN, S. B.; HAMDI, S. H.; MAHJOUBI, K.; JEMÂA, J. M. B. Composition and insecticidal activity of essential oil from *Ruta graveolens*, *Mentha pulegium* and *Ocimum basilicum* against *Ectomyelois ceratoniae* Zeller and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). **J. Plant. Dis. Prot.**, v. 126, n. 3, p. 237-246, 2019.

CORREA, J. C. R.; SALGADO, H. R. N. Atividade inseticida das plantas e aplicações: revisão. **Rev. Bras. Plantas Med. (online)**, v. 13, n. 4, p. 500-506, 2011.

COSTA, E. L. N.; SILVA, R. F. P.; FIUZA, L. M. Efeitos, aplicações e limitações de extratos de plantas inseticidas. **Acta Biol. Leopoldensia**, v. 26, n. 2, p. 173-85, 2004.

CUEVAS-SALGADO, M. I.; RODRÍGUEZ-MORALES, M. P.; ROMERO-NÁPOLES, C. Infusiones botánicas para el control de *Leptophobia aripa elodia* Boisduval (Lepidoptera: Pieridae) en brócoli (*Brassica oleracea* var. italica) bajo condiciones de laboratorio. **Bol. Soc. Mex. Ento. (nueva serie),** v. 1, p. 71-77, 2015.

DA SILVA, K. F.; BALDIN, E. L. L.; PANNUTI, L. E. DA R. Use of botanical insecticides as an alternative for the management of the mexican bean weevil. **Rev. Caatinga**, v. 29, n. 2, p. 348-357, 2016.

DENT, D., **Insect Pest Management**. Wallingford, UK: Cabi, 2000.

DEQUECH, S. T. B.; SAUSEN, C. D.; LIMA, C. G.; EGEWARTH, R. Efeito de extratos de plantas com atividade inseticida no controle de *Microtheca ochroloma* Stal (Col.: Chrysomelidae), em laboratório. **Biotemas**, v. 21, n. 1, p. 41-46, 2008.

DIABATE, S.; MARTIN, T.; MURUNGI, L. K.; FIABOE, K. K. M.; SUBRAMANIANA, S.; WESONGA, J.; DELETRE, E. Repellent activity of *Cymbopogon citratus* and *Tagetes minuta* and their specific volatiles against *Megalurothrips sjostedti*. **J. Appl. Entomol.**, v. 143, n. 8, p. 855-866, 2019.

DIAS, M. L.; AUAD, A. M.; MAGNO, M. C.; RESENDE, T. T.; FONSECA, M. G.; SILVA, S. E. Insecticidal activity of compounds of plant origin on *Mahanarva spectabilis* (Hemiptera: Cercopidae). **Insects**, v. 10, p. 360, 2019.

DOUGOUD, J.; TOEPFER, S.; BATEMAN, M.; JENNER, W. H. Efficacy of homemade botanical insecticides based on traditional knowledge. A review. **Agron. Sustentar. Dev.**, v. 39, p. 37, 2019.

FERREIRA, D.F. SISVAR: A computer analysis system to fixed effects split plot type designs. **Rev. Bras. Biom.**, v. 37, n. 4, p. 529-535, 2019.

FURTADO, R. F.; LIMA, M. G. A.; ANDRADE NETO, M.; BEZERRA, J. N. S.; SILVA. M.G.V. Atividade larvicida de óleos essenciais contra *Aedes aegypti* L. (Díptera: Culicidae). **Neotrop. Entomol.**, v. 34, p. 843-847, 2005.

GARCIA, J. F.; GRISOTO, E.; VENDRAMIM, J.D.; BOTELHO, P. S. M. Bioactivity of neem, *Azadirachta indica*, against spittlebug *Mahanarva fimbriolata* (Hemiptera: Cercopidae) on sugarcane. **J. Econ. Entomol.**, v. 99, n. 6, p. 2010–2014, 2006.

GHAFOOR, H. A.; AFZAL, M.; RIAZ, M. A.; MAJEED, M. Z. In-vitro toxicity evaluation of some phytoextracts against mealybug *Drosicha mangiferae* (Hemiptera: Pseudococcidae) infesting citrus orchards in Pakistan. **Pakistan J. Zool**., v. 51, n. 5, p. 1815-1822, 2019.

HUANG, Y.; CHEN, S. X.; HO, S. H. Bioactivities of methyl allyl disulfide and diallyl trisulfide from essential oil of garlic to two species of stored-product pests, *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). **J. Econ. Entomol.**, v. 93, n. 2, p. 537-543, 2000.

IBGE, 2019. **Censo agropecuário: resultados definitivos**. Rio de Janeiro: IBGE, 2017.

ISMAN, M. B. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. **Annu. Rev. Entomol.**, v. 51, n. 1, p. 45–66, 2006.

JAVED, M.; MAJEED, M. Z.; SUFYAN, M.; ALI, S.; AFZAL, M. Field efficacy of selected synthetic and botanical insecticides against *Lepidopterous Borers*, *Earias vittella* and *Helicoverpa armigera* (Lepidoptera: Noctuidae), on Okra (*Abelmoschus esculentus* (L.) Moench). **Pakistan J. Zool.**, v. 50, n. 6, p. 1999-2398, 2018.

KARUNAMOORTHI, K.; ILANGO, K. Larvicidal activity of *Cymbopogon citratus* (DC) Stapf. and *Croton macrostachyus* Del. against *Anopheles arabiensis* Patton, a potent malaria vector. **Eur. Ver. Med. Pharmacol. Sci.**, v. 14, n. 1, p. 57–62, 2010.

LAMEIRA, O. A.; PINTO, J. E. B. P. **Plantas medicinais: do cultivo, manipulação e uso à recomendação popular**. Belém, PA: Embrapa Amazônia Oriental, 2008. 264 p.

LOKESH, K. V.; KANMANI, S.; ADLINE, J. D.; RAVEEN, R.; SAMUEL, T.; ARIVOLI, S.; JAYAKUMAR, M. Adulticidal activity of *Nicotiana tabacum* Linnaeus (Solanaceae) leaf extracts against the sweet potato weevil *Cylas formicarius* Fabricius 1798 (Coleoptera: Brentidae). **J. Entomol. Zool. Stud.**, v. 5, n. 5, p. 518–524, 2017.

MACHADO, L. A.; BARBOSA E SILVA, V.; OLIVEIRA, M. M. de. Uso de extratos vegetais no controle de pragas em horticultura. **Biológico**, v. 69, n. 2, p. 103-106, 2007.

MENDES, A. M. S.; SOUZA, M. M. S.; FONSECA, A. M.; COLARES, R. P.; CARNEIRO, J. B.; SANTOS, F. G. N. Inseticidas naturais: uma alternativa sustentável para o controle de pragas na agricultura familiar, 2016. In: Congresso Brasileiro de Química (Online), 56, Belém. **ABQ**, Rio de Janeiro, 2016.

MOSSI, A. J.; ASTOLFI, V.; KUBIAK, G.; LERIN, L.; ZANELLA, C.; TONIAZZO, G.; OLIVEIRA, D.; TREICHEL, H.; DEVILLA, I. A.; CANSIAN, R.; RESTELLO, R. Insecticidal and repellency activity of essential oil of *Eucalyptus* sp. against *Sitophilus zeamais* Motschulsky (Coleoptera, Curculionidae). **J. Sci. Food. Agric.**, v. 91, n. 2, p. 273-277, 2011.

MUDRONČEKOVÁ, S.; FERENČÍK, J.; GRUĽOVÁ, D.; BARTA, M. Insecticidal and repellent effects of plant essential oils against *Ips typographus*. **J. Pest Sci**., v. 92, n. 2, p. 595–608, 2019.

NIU, J.; YU, G., 2009. Agricultural chemicals. In: YI, Q. (Ed.). **Point sources of pollution: Local effects and their control**. Oxford, UK: EOLSS Publications/UNESCO, 2009. p. 43-60.

NUNES, G. S.; RIBEIRO, M .L. Pesticidas: Uso, legislação e controle. **Pesticidas: Rev. Ecotox. Meio Amb.**, v. 9, p. 31-44, 1999.

OLIVERO-VERBEL, J.; NERIO, L. S.; STASHENKO, E. E. Bioactivity against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) of *Cymbopogon citratus* and *Eucalyptus citriodora* essential oils grown in Colombia. **Pest Manag. Sci.**, v. 66, n. 6, p. 664-668, 2010.

ORLANDA, J. F. F; NASCIMENTO, A. R. Chemical composition and antibacterial activity of *Ruta graveolens* L. (Rutaceae) volatile oils, from São Luís, Maranhão, Brazil. **S. Afr. J. Bot.**, v. 99, p. 103-106, 2015.

PARK, J. H.; JEON, Y. J.; LEE, C. H.; CHUNG, N.; LEE, H. S. Insecticidal toxicities of carvacrol and thymol derived from *Thymus vulgaris* Lin. against *Pochazia shantungensis* Chou & Lu., newly recorded pest. **Sci. Rep.**, v. 7, 40902. 2017.

PAVUNRAJ, M.; BASKAR, K.; PAULKUMAR, K.; JANARTHANAN, S.; RAJENDRAN, P. Antifeedant activity of crude extracts and fractions isolated from *Catharanthus roseus* leaf against spotted bollworm, *Earias vittella*. **Phytoparasitica**, v. 44, p. 419-422, 2016.

PISTORI, M. G. B.; ROEL, A. R.; VALÉRIO, J. R.; OLIVEIRA, M. C. M.; GRISOTO, E.; MATIAS, R. Effect of *Anacardium humile* St. Hill (Anacardiaceae) aqueous extract on *Mahanarva fimbriolata* (Stal, 1854) (Hemiptera: Cercopidae). **Acta Sci. Agron**., v. 35, n. 4, p. 413-417, 2013.

PRISHANTHINI, M.; VINOBABA, M. Efficacy of some selected botanical extracts against the Cotton mealybug *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae). **IJSRP**, v. 4, n. 3, p. 1-6, 2014.

RANDO, J. S. S.; LIMA, C. B.; LOURENÇO, C. C.; FELDHAUS, D. C.; MALANOTTE, M. L.; AVILA, R. R. Extratos de *Nicotiana tabacum*, *Ocimum gratissimum* e *Equisetum* sp.

no controle do pulgão da couve *Brevicoryne brassicae* (L.) **Rev. Bras. Agroecol.**, v. 4, n. 2, p. 204-208, 2009.

RIZVI, S. A. H.; HUSSAIN, S.; REHMAN, S. U.; JAFFAR, S.; REHMAN, M. F. U. Efficacy of ecofriendly botanical extracts of Ginger (*Zingiber officinale*), Garlic (*Allium sativum*) and Tobacco (*Nicotiana tabacum* L) for the control of cabbage looper (*Trichoplusia binotalis*) under agroecological conditions of Peshawar, Pakistan. **Journal of Entomology and Zoology Studies**, v. 4, n. 1, p. 88-90, 2016.

ROEL, A.R. Utilização de plantas com propriedades inseticidas: uma contribuição para o desenvolvimento rural sustentável. **Revista Internacional de Desenvolvimento Local**, v. 1, n. 2, p. 43-50, 2001.

SARKER, S.; LIM, U. T. Extract of *Nicotiana tabacum* as a potential control agent of *Grapholita molesta* (Lepidoptera: Tortricidae). **PLoS One**, v. 13, n. 8, e0198302, 2018.

SARMA, R., ADHIKARI, K., MAHANTA, S., KHANIKOR, B. Combinations of plant essential oil based terpene compounds as larvicidal and adulticidal agent against *Aedes aegypti* (Diptera: Culicidae). **Sci. Rep.**, v. 9, n. 9471, p. 1-12, 2019.

SILVA, L. M; CALDAS, A. P.; RODRIGUES, A. L. M.; OLIVEIRA, J. S.; SIMONETTI, E. R. de S. O uso do extrato de fumo (*Nicotina tabacum*) como alternativa para o controle de pragas em hortaliças, 2017. In: CONGRESSO INTERNACIONAL DAS CIÊNCIAS AGRÁRIAS, 2., 2017, Natal. **Anais** [...]. Natal, 2017.

SOSA, A.; DIAZ, M.; SALVATORE, A.; BARDON, A.; BORKOSKY, S.; VERA, N. Insecticidal effects of *Vernonanthura nebularum* against two economically important pest insects. **Saudi J. Biol. Sci.**, v. 26, n. 5, p. 881–889, 2019.

THOMPSON, V. Associative nitrogen fixation, C 4 photosynthesis, and the evolution of spittlebugs (Hemiptera: Cercopidae) as major pests of neotropical sugarcane and forage grasses. **Bulletin of entomological research**, v. 94, n. 3, p. 189-200, 2004.

VALÉRIO. J.R., 2009. **Cigarrinhas-das-pastagens**. Campo Grande, MS: Embrapa Gado de Corte, 2009. 51 p.

WEI, L.; HUA, R.; LI, M.; HUANG, Y.; LI, S.; HE, Y.; SHEN, Z. Chemical composition and biological activity of star anise *Illicium verum* extracts against maize weevil, *Sitophilus zeamais* adults. **J. Insect Sci.**, v. 14, n. 1, p. 80, 2014.

ZENG, L.; LAO, C. Z.; CEN, Y. J.; LIANG, G. W. Study on the insecticidal activity compounds of the essential oil from *Syzygium aromaticum* against stored grain insect pests. **Julius-Kühn-Archiv**., v. 425, p. 766-771, 2010.

ZHOU, B.-G.; WANG, S.; DOU, T.-T.; LIU, S.; LI, M.-Y.; HUA, R.-M.; LI, S.-G.; LIN, H.-F. Aphicidal activity of *Illicium verum* fruit extracts and their effects on the Acetylcholinesterase and Glutathione S-transferases activities in *Myzus persicae* (Hemiptera: Aphididae). **J. Insect Sci**., v. 16, p. 11. 2016.

CHAPTER 3: OLFACTORY RESPONSE OF *Mahanarva spectabilis* (Distant, 1909) (Hemiptera: Cercopidae) TO VOLATILE AQUEOUS EXTRACTS OF PLANT ORIGIN APPLIED TO ELEPHANT GRASS PLANTS (*Pennisetum purpureum* Schum)[2]

ABSTRACT

In this study, we evaluate the olfactory responses of *Mahanarva spectabilis* adults to aqueous extracts from the following non-host plants for the pest insect: garlic (*Allium sativum* L.), rue (*Ruta graveolens* L.), cinnamon (*Cinnamomum verum* J. Presl), lemongrass (*Cymbopogon citratus* Stapf.), clove (*Syzygium aromaticum* L.), star anise (*Illicium verum* Hook.f), eucalyptus (*Eucalyptus globulus* Labill.), tobacco (*Nicotiana tabacum* L.), and thyme (*Thymus vulgaris* L.) applied to the host plant, elephant grass (*Pennisetum purpureum* Schum.). The bioassays were performed using a Y olfactometer, and the combinations of the plant extracts applied to the host plant were tested against fresh air and against the host plant without the extract. The results show that the extracts of tobacco and star anise were non-attractive to *M. spectabilis* adults. The extracts from the remaining plants did not alter the response of *M. spectabilis* to the host plant.

Keywords: agroecology; repellency; spittlebugs; volatile organic compounds.

---

[2] This article was written according to ABNT/UFJF standards. It was submitted for publication in the special edition "Semiochemicals in Pest Management" of the scientific journal Agronomy.

1 INTRODUCTION

In Latin America, most animal production is based on grazing, which is often planned in those areas where the soil is unsuitable for other agricultural activities. Consequently, serious levels of degradation occur in considerable parts of the grazing areas, which proves to be a limiting factor in the production of meat and milk (LIRA *et al*., 2017) Additionally, besides the complex agroecological process, biotic factors such as insect pests contribute substantially to the degradation of the pastures (HOHNWALD *et al*., 2019).

Among the most important insect pests associated with the pastures are spittlebugs. The attack of these insects occurs mainly in the rainy season of the year, which causes a fall in the production of forage, precisely in the period of high demand by cattle; thus, it thoroughly damages the chains of beef and milk production (CONGIO, 2010; RIBEIRO and CAZAROTTO, 2019). According to Thompson (2004), damage by spittlebugs on pastures generates losses of up to the USD 2.1 billion per year worldwide. In Brazil, the spittlebug *Mahanarva spectabilis* (Distant, 1909) (Hemiptera: Cercopidae) is considered an obstacle to forage production and is the main and most limiting pest associated with the production of elephant grass (*Pennisetum purpureum* Schum. (Poales: Poaceae)) (AUAD *et al*., 2007).

Female spittlebugs *M. spectabilis* lay eggs close to the forage base, and the eggs are elongated and resemble the shape of a yellow-colored rice grain (BATISTA *et al*., 2010; VALÉRIO, 2009). After hatching, the nymphs immediately fix themselves on the roots and synthesize a foam, in which they are immersed for five instars until they reach adulthood. The foam protects them against natural enemies and provides a microclimate that is favorable for their development (BATISTA *et al*., 2010; SOUZA *et al*., 2008). Adults, unlike nymphs, have wings and reproductive organs and move around in low jumps and short flights (BATISTA *et al*., 2010; MENEZES *et al*., 1983). They have an average longevity rate of 9 days (±3) in females and 10 days (±4) in males (GUIMARÃES *et al*., 2007). They prefer to stay fixed in the sheaths of forage leaves, sucking the sap and injecting toxins that cause yellowing and death of the plant tissues (BYERS and WELLS, 1966), it is important to have necessary strategies for the control of such pests.

Spittlebug management strategies must be used together so that the control of this pest is sustainable and efficient. Among the management strategies used in Brazil, we highlight the diversification of pastures (ALVARENGA *et al*., 2019), chemical control (SUTIL *et al*., 2020), biological control (CAMPAGNANI *et al*., 2017; VERÍSIMO *et al*., 2020), and the use of resistant plants (SOBRINHO *et al*., 2010). The use of insecticides and botanical repellents is a widespread strategy, and studies of this nature are necessary for the management of forage pests.

Dias *et al*. (2020) demonstrated that compounds of plant origin are effective against the spittlebug *M. spectabilis*. It must be considered that plants present phytochemicals in their constitution with unique biological characteristics that play different structural roles and act as larvicides (OTABOR *et al*., 2019), insecticides (DIAS *et al*., 2020), repellents (CHILUWAL *et al*., 2017), ovipositional regulators (WAGNER and CARD, 2020), and growth regulators (GAUR and KUMAR, 2020), which partially help them avoid the attacks of the phytophagous insects. The unattractive or attractive action of the plants is closely related to the volatile organic compounds (VOCs) released by the plants. VOCs act to defend the plant by promoting protection against abiotic and biotic stresses. Moreover, they also play crucial roles in the ecological interactions of the plants with the other organisms (DUDAREVA *et al*., 2007; RIFFEL and DA COSTA, 2015). These com-pounds have great potential for their use in integrated pest management once they are obtained through the manufacturing of plant extracts.

The push–pull technique uses the principles of attraction and non-attractiveness to manipulate the behavior of the insect pests through the integration of the stimuli that act to repel or drive the pests away from the main crop (push) while attracting them towards a source crop (pull) by using highly apparent and attractive stimuli. Hence, the pests are subsequently removed away from the desired crop. The attractive or unattractive action of a plant can be natural when generated by the aromatic compounds present in the host plant; however, if this is not the case, it can also be induced externally by the application of stimuli that can mask the host appearance in terms of repellency, deterrence, and attraction (COOK *et al*., 2007). As an example, researchers demonstrated that the choice of a host plant by *M. spectabilis* can be attributed to the specific odors released by forage, such as *Pennisetum purpureum* cv. Pioneiro and *Brachiaria decumbens* cv. Basilisk (SILVA *et al*., 2019). They also demonstrated that damaged plants of *Brachiaria brizantha* cv. Marandú unattracted the adults of *M. spectabilis*.

The aforementioned research results served to hypothesize that the aqueous extracts of certain aromatic non-host plants after their application to elephant grass may alter the attractive response of elephant grass to *M. spectabilis*. The present research is an effort to arrive and identify those suitable non-host plant extracts. Therefore, the objective of this research is to evaluate the olfactory response of the adults of the spittlebug *M. spectabilis* towards the aqueous extracts of the aromatic plants (non-hosts to these insects), such as *Allium sativum* L. (Amaryllidaceae), *Ruta graveolens* L. (Rutaceae), *Cinnamomum verum* J. Presl. (Lauraceae), *Cymbopogon citratus* Stapf. (Poaceae), *Syzygium aromaticum* L. and *Eucalyptus globulus*

Labill. (Myrtaceae), *Illicium verum* Hook.f (Schisandraceae), *Nicotiana tabacum* L. (Solanaceae), and *Thymus vulgaris* L. (Lamiaceae), when they are applied to the host plant, the elephant grass (*Pennisetum purpureum* Schum. (Poaceae)).

2 MATERIALS AND METHODS

2.1 BOTANICAL MATERIAL AND PLANT EXTRACTS

For the olfactometry tests, elephant grass (*Pennisetum purpureum* Schum) was used. It was obtained by using cuttings (≅10 cm, single knot), from the Experimental Field of Embrapa Dairy Cattle, Coronel Pacheco, Brazil. The cuttings were propagated in plastic pots (500 mL) that contained the substrate (soil/manure in a 1:1 ratio) to form the seedlings. The seedlings were kept in a greenhouse at Embrapa Dairy Cattle in Juiz de Fora, MG, Brazil, until they reached an approximate height of 30 cm and were used in the experiments.

The botanical materials for making the plant extracts were obtained from different sources (Table 2). With the exception of eucalyptus leaves, the other materials were purchased from the commercial establishments described in Table 2. For lemon grass, rue, and thyme, seedlings were purchased, and they were planted in 1 L plastic pots and stored in an Embrapa Dairy Cattle greenhouse for 3 months, with daily irrigation, before use. Garlic, cinnamon, cloves, tobacco, and anise star were purchased in their whole form, ready for use. Eucalyptus leaves were collected from trees at the Experimental Field of Embrapa Dairy Cattle.

Nine plant extracts (Table 2) were prepared at the Entomology Laboratory of Embrapa Dairy Cattle, Juiz de Fora, Brazil. Each plant extract was made separately on the day before the olfactometry tests were performed. For the preparation of the extracts, parts from the selected plants (Table 2) were washed with distilled water and then placed in a forced ventilation oven (Binder FD115) for drying at an average temperature of 40 °C for 72 h.

After drying, the materials were ground in a basic analytical mill IKA® model A11 and placed in the beakers, which were filled with distilled water at a concentration of 20% and taken to an ultrasound shaker (ELMA E60H) to extract the compounds. The resulting solutions were filtered through a voile fabric, which gave rise to the aqueous plant extracts. The plant extracts were stored in amber glass bottles and protected from the light until the olfactometry tests.

Table 2 - List of plant species, their sources, forms of acquisition, and parts used to make the aqueous extracts.

Extract	Plant Species	Sources	Forms of Acquisition	Parts Used
Garlic	*Allium sativum* L.	Street Fair, Juiz de Fora, MG, Brazil	Whole product	Bulbs
Star anise	*Illicium verum* Hook.f	Municipal Market of Juiz de Fora, MG, Brazil	Dry parts	Fruits
Rue	*Ruta graveolens* L.	Municipal Market of Juiz de Fora, MG, Brazil	Seedlings	Leaves
Cinnamon	*Cinnamomum verum* J. Presl	Municipal Market of Juiz de Fora, MG, Brazil	Dry parts	Bark
Lemongrass	*Cymbopogon citratus Stapf.*	Municipal Market of Juiz de Fora, MG, Brazil	Seedlings	Leaves
Clove	*Syzygium aromaticum* L.	Municipal Market of Juiz de Fora, MG, Brazil	Dry parts	Flower bud
Eucalyptus	*Eucalyptus globulus* Labill.	Embrapa Dairy Cattle, Coronel Pacheco, MG, Brazil	Collected leaves	Leaves
Tobacco	*Nicotiana tabacum* L.	Municipal Market of Juiz de Fora, MG, Brazil	Dry parts	Leaves
Thyme	*Thymus vulgaris* L.	Municipal Market of Juiz de Fora, MG, Brazil	Seedlings	Leaves

Source: Elaborated by the author (2021).

2.2 INSECTS

For the performance of the olfactometry tests, adult *M. spectabilis* specimens were collected from the elephant grass plants in the Experimental Field and greenhouses of Embrapa Dairy Cattle. Later, they were taken to the entomology laboratory, where they were kept in acrylic cages (30 × 30 × 60 cm) at room temperature (25 ± 2 °C). Females and males of this insect were used in undefined proportions. Adult collections were performed on different dates in the morning before each trial.

2.3 BIOASSAYS OF OLFACTOMETRY

The olfactometry bioassays were carried out at the Embrapa Dairy Cattle Entomology Laboratory. The experimental procedure by Silva *et al*. (2019) was used with adaptations. A Y-type glass olfactometer was used, with the following dimensions: the main arm, 30 cm; the side arms, 23 cm; diameter, 3.5 cm; with an angle of 120° between the arms. The airflow was continuous at 1.0 L/min, and the air was humidified with distilled water, filtered with activated carbon, and calibrated with the help of a flow meter. For the insertion of the plants, a glass dome with 42 cm height and with a diameter of 16 cm was attached to each arm of the olfactometer with the help of silicone tubes. The plastic cups that contained the elephant grass plants were wrapped in the aluminum foils to reduce the volatile effects of the substrate.

The tests were carried out between 9 a.m. and 4 p.m. from December 2019 to March 2020. The average temperature during the test period was 27 ± 2 °C, the relative humidity was 60% ± 10%, and the photophase was 12:12 h. Before performing the bioassays, the olfactometer was calibrated, noting the *M. spectabilis* responses to the combination clean air vs. clean air. In the tests, the extracts of the non-host plants were applied directly to the leaves of the host plants (elephant grass) through a plastic sprayer of 500 mL, 10 sprays for each plant extract (≅8 mL), forming a combination of the aqueous plant extract + elephant grass. All aqueous plant extracts were tested against fresh air and host plants (without extract). The tests proceeded with the following combinations: (1) garlic extract + elephant grass vs. clean air; (2) garlic extract + elephant grass vs. elephant grass; (3) star anise extract + elephant grass vs. clean air; (4) star anise extract + elephant grass vs. elephant grass; (5) rue extract + elephant grass vs. clean air; (6) rue extract + elephant grass vs. elephant grass; (7) cinnamon extract + elephant grass vs. clean air; (8) cinnamon extract + elephant grass vs. elephant grass; (9) lemongrass extract + elephant grass vs. clean air; (10) lemongrass extract + elephant grass vs. elephant grass; (11) clove extract + elephant grass vs. clean air; (12) clove extract + elephant grass vs. elephant grass; (13) eucalyptus extract + elephant grass vs. clean air; (14) eucalyptus extract + elephant grass vs. elephant grass; (15) thyme extract + elephant grass vs. clean air; (16) thyme extract + elephant grass vs. elephant grass; (17) tobacco extract + elephant grass vs. clean air; (18) tobacco extract + elephant grass vs. elephant grass.

M. spectabillis adults were removed from the acrylic cages and placed in the voile fabric cages before each bioassay. The tests with the insects were carried out individually, where each adult was placed on the base of the olfactometer. The responses were counted and considered positive only for the insects that reached the end of one of the lateral arms of the Y within 10 min. The insects that did not reach the lateral arms in this period were quantified, but they were not included in the analysis. For each bioassay, 40 positive responses were evaluated.

To maintain the equipment and to avoid any external interference in the responses of the insects, the following operations were performed: After 5 insects were tested, the olfactometer was washed with 96° ethyl alcohol and dried in an oven at 100 °C. The olfactometer was also rotated 180° to avoid any positional bias. After 10 tests, the olfactometer and the domes were washed in distilled water with neutral detergent and alcohol and dried in an oven at 100°. Additionally, after every 10 tests, the plants from the dome were changed, and aqueous plant extracts were reapplied. At each odor source change, that is, after 40 positive responses, the silicone hoses were replaced and all the equipment and the olfactometer and its parts were washed in distilled water with neutral detergent and alcohol and dried in an oven at 100 °C.

2.5 STATISTICAL ANALYSIS

The results were analyzed using the Chi-square test ($\chi2$) in the BioEstat 5.4 software (AYRES *et al*., 2011). Insects that chose neither arm were excluded from the statistical analysis.

3. RESULTS

M. spectabilis had no significant difference ($\chi2 = 0$; DF = 1; p = 0.9999) when clean air was tested in both arms, which indicated that there was no positional bias in the olfactometer.

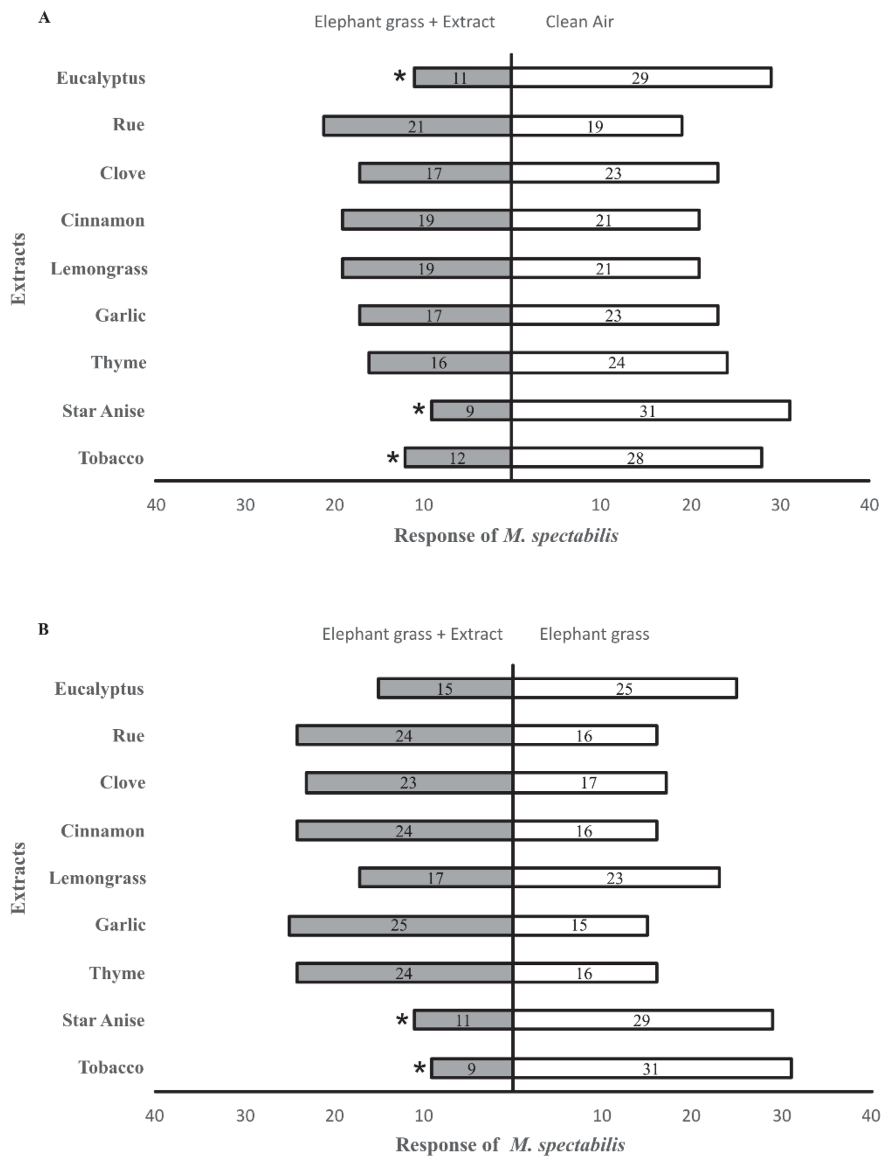
The combination of the host plant with the tobacco extract resulted in non-attractiveness. In this adjustment, 70% of the *M. spectabilis* went to fresh air ($\chi2 = 6.4$; DF = 1; p = 0.0114) and 77.5% to the elephant grass without the extract ($\chi2 = 12.1$; DF = 1; p = 0.0005) (Figure 6 A, B).

The star anise extract combination was also deprecated in relation to the clean air ($\chi2 = 12.1$; DF = 1; p = 0.0005) and elephant grass ($\chi2 = 8.1$; DF = 1; p = 0.0044), presenting non-attractiveness to the adults of *M. spectabillis* respectively, with indexes of 77.5% and 72.5% (Figure 6 A, B).

The combination with the aqueous eucalyptus extract was non-attractive in relation to the fresh air ($\chi2 = 8.1$; DF = 1; p = 0.0044), indicating a non-attractive response of 72.5%; however, it was not significantly attractive or unattractive to the adults of *M. spectabillis*, when tested against the elephant grass plants ($\chi2 = 2.5$; DF = 1; p = 0.1138) (Figure 6 A, B).

Trials using thyme, garlic, cinnamon, lemongrass, rue, and clove extracts, in combination with the elephant grass, were not significantly attractive or unattractive to the *M. spectabilis* when they were tested against the clean air or the elephant grass plants (Figure 6 A, B).

Figure 6 - Behavioral response of *M. spectabilis* adults to the volatiles emitted by the combinations of the extracts with the elephant grass, against clean air (A) and host plant (elephant grass) (B) and olfactometry bioassays. The numbers inside the bars are the total number of spittlebugs that responded to each treatment. (*) There was a significant difference in this treatment.



Source: Elaborated by the author (2021)

4 DISCUSSION

As plants are confronted daily with herbivores, throughout history, they have developed several mechanisms that act in resistance to these herbivores, such as the ability to recognize attacks and reconfigure their responses to produce, among other compounds, volatile organics

(VOCs) (RIFFEL and DA COSTA, 2015; SÃO JOÃO and RAGA, 2016). In this study, we investigated whether the volatile com-pounds present in the aqueous extracts of the nine species of plants with insecticidal potential, applied on the forage host, would be able to alter the adult spittlebugs (*M. spectabilis*) choice of pastures, influencing their behavior of attraction or non-attraction. Elephant grass was chosen since it can release some specific odors that have the potential to attract *M. spectabilis* (SILVA *et al*., 2019).

The *M. spectabilis* responded significantly to the olfactory cues released by the combinations of the host plant with the extracts of the star anise, tobacco, and eucalyptus in the bioassays against the clean air (bioassay A) and the combinations of the host plant with the star anise and tobacco extracts in relation to the host plant without plant extract (bioassay B). The observed non-attraction of *M. spectabilis* to the volatile compounds of the star anise, tobacco, and eucalyptus extracts is added to the observations of Silva *et al*. (2019) on the attraction of adult *M. spectabilis* elephant grass and reveals a change in the behavior of *M. spectabilis* by the application of the plant extracts. Similarly, Cook *et al*. (2007) mention that the behavior of non-attractiveness can occur if the odor reveals a low-quality host, and non-attraction can occur after the application of non-host plant odors.

Some authors have observed that the extracts and oils made from star anise have non-attractive effects on the insects. Singh and Singh (1991) obtained 100% non-attractiveness from *Musca domestica* L. (Diptera: Muscidae) by using the essential oil of star anise. The researchers Wei *et al*. (2014) noted that the non-attractiveness of the star anise extracts increased as the doses increased. The highest percentage of non-attractiveness observed by the authors on the adults of *Sitophilus zeamais* Motschulsky (Coleoptera, Curculionidae) was 76.9%. In the research by Matos *et al*. (2020), with a concentration of 32.78 μL/20 g, the star anise essential oil repelled the adults of *Callosobruchus maculatus* (Fabr.) (Coleoptera: Bruchidae). The main bioactive compound present in star anise is trans-anethole, which is an aromatic compound responsible for the aroma and flavor of anise (SINGH *et al*., 2005; WEI *et al*., 2014). This substance is not attractive for different species of pests, as also noted for *M. spectabilis* in the present research.

Tobacco has been used by humans to control insect pests since the 17th century, mainly due to its high efficiency, low phytotoxicity, and low cost (BRAIBANTE and ZAPPE, 2012; MENDES *et al*., 2016; SILVA *et al*., 2017). Given its properties, tobacco extract is also attributed with being non-attractive to pests. The main bioactive compound present in the tobacco extract is nicotine, which in its volatile form is possibly responsible for the non-attractive action against *M. spectabilis* as observed by Sagheer *et al*. (2013). The acetonic tobacco extract obtained the highest non-attractiveness index (93.33%) among the four extracts tested against *Tribolium castaneum* (Herbst) (DOUGOUD *et al*., 2019; TAYOUB *et al*., 2016).

The volatile compounds present in the species of the Eucalyptus genus have shown promising results in the control of several insects. González-Guiñez *et al*. (2016) proved that the essential oil of *E. globulus* is non-attractive for *S. zeamais* adults. Descamps *et al*. (2019) found that the essential oil of *E. globulus*, at a concentration of 7% w/v, was non-attractive for *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) adults. The main bioactive com-pounds present in *E. globulus* are ρ-cymene, γ-terpinene, and 1.8 cineol (eucalyptol), and the synergistic activity of these and other compounds are responsible for the elaborated action of the *E. globulus* plants on different species of the insects (LEVYA *et al*., 2020). In the present work, the aqueous extract of the *E. globulus* was not attractive to *M. spectabilis* as compared to clean air; however, when compared to the host plant combination, there was no change.

In the olfactometer tests, the combinations of the host plant with the aqueous extracts of garlic, rue, cinnamon, lemongrass, cloves, and thyme did not alter the behavior of the *M. spectabilis* adults significantly. A similar situation was reported by Silva *et al*. (2019), who observed that VOCs from *P. purpureum* cv. Roxo Botucatu, *Panicum maximum* cvs. Makueni and Tanzania, *Hyparrhenia rufa* cv. Jaraguá, *Melinis minutiflora*, *Cynodon dactylon* cv. Tifton, and *Brachiaria brizantha* cv. Marandú were not significantly attractive or repellent for *M. spectabilis*. Nonetheless, previous research shows that the application of the extracts pre-pared from garlic, rue, cinnamon, lemongrass, cloves, and thyme revealed behavioral changes of other insect species. Mobki *et al*. (2014) reported that the garlic extract had a strong non-attractive activity for *T. castaneum*. Perera and Karunaratne (2015) identified strong non-attractiveness (91%) of the aqueous extract of rue against *Sitophilus oryzae* L adults (Coleoptera: Curculionidae). Prajapati *et al*. (2005) demonstrated that the cinnamon essential oil has a high degree of repellency on *Anopheles stephensi* Liston, *Aedes aegypti* (L.), and *Culex quinquefasciatus* (Say) (Diptera: Culicidae). Kimutai et al. reported in their research that lemongrass essential oil showed high non-attractiveness for *Phlebotomus duboscqi* Neveu-Lemaire (Diptera: Psychodidae), which is one of the main vectors of zoonotic cutaneous leishmaniasis in East Africa. Abo-El-Saad *et al*. (2011) used the essential oil of clove for *Tribolium castaneum* (Herbest) (Coleoptera: Tenebrionidae) adults and obtained 100% repellency at concentrations of 1.0%, 0.8%, and 0.2%. Picard *et al*. (2012) observed that the essential oil of thyme, in a concentration of 0.5%, was highly non-attractive for the females of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Despite these compounds inducing a clear behavioral change in some other pest species, as mentioned above, the results obtained in the present study showed that *M. spectabilis* remained unchanged in its comportment in terms of attraction or non-attraction to the host plant upon exposure to the extracts of these plants.

The current analysis is the first to show that plant extracts can alter the behavior of *M. spectabilis* in relation to their host plant. It has been shown that the application of aqueous extracts of tobacco and star anise can mask specific odors of elephant grass. The non-attractive action of eucalyptus in one of the bioassays is a topic for further research.

The results are encouraging against farmers' growing demand for environmentally safe and non-toxic products (KUMAR and SINGH, 2015). The extracts of tobacco and star anise can complement the techniques of handling *M. spectabilis* in small properties, such as by the push–pull pest management technique (COOK, *et al*, 2017).

5 CONCLUSIONS

Aqueous extracts of tobacco, star anise, and eucalyptus were not attractive for *M. spectabilis*, and therefore can be used as a management tactic for *M. spectabilis* adults in elephant grass. The extracts of thyme, garlic, clove, rue, cinnamon, and lemongrass were not significantly attractive to *M. spectabilis* in the pastures.

REFERENCES

ABO-EL-SAAD, M. M.; AL AJLAN, A. M.; AL-EID, M. A.; BOU-KHOWH, I. A. Repellent and fumigant effects of essential oil from clove buds *Syzygium aromaticum* L. against *Tribolium castaneum* (Herbest) (Coleoptera: Tenebrionidae). **Journal of Agricultural Science and Technology A**., v. 1, p. 613-620, 2011.

ALVARENGA, R.; AUAD, A. M.; MORAES, J. C.; DA SILVA, S. E. B.; RODRIGUES, B. S. Tolerance to nymphs and adults of *Mahanarva spectabilis* (Hemiptera: Cercopidae) by forage plants in fertilized soils. **Pest. Manag. Sci**., v. 75, n. 8, p. 2242-2250, 2019.

AUAD, A. M.; SIMÕES, A. D.; PEREIRA, A. V.; BRAGA, A. L. F.; SOBRINHO, F. S.; LÊDO, F. J. S.; PAULA-MORAES, S. V.; OLIVEIRA, S. A.; FERREIRA, R. B. Seleção de genótipos de capim-elefante quanto à resistência à cigarrinha-das-pastagens. **Pesquisa Agropecuária Brasileira**, v. 42, n. 8, p. 1077-1081, 2007.

AYRES, M.; AYRES, J. R. M.; AYRES, D. L.; SANTOS, A. S. B. **BioEstat 5.3: Aplicações Estatísticas nas Áreas das Ciências Biológicas e Médicas.** Belém: Publicações Avulsas do Mamirauá, 2011, 361 p.

BATISTA, E; AUAD, A. M.; BRAGA, A. L. F.; FERREIRA, R. B.; HALLACK, N.M. DO R. Aspectos do comportamento da cigarrinha-das-pastagens *Mahanarva spectabilis* (Distant) (Hemiptera: Cercopidae) na produção de espuma**. EntomoBrasilis**, v. 3, p. 25-28, 2010.

BYERS, R.; WELLS, H. D. Phytotoxemia of Coastal bermudagrass caused by the two-lined spittlebug, *Prosapia bicincta* (Homoptera: Cercopidae). **Ann. Entomol. Soc. Am. Amer.**, v. 59, p. 1067–1071, 1966

BRAIBANTE, M. E. F., ZAPPE, J. A. A química dos agrotóxicos. **Química nova na escola**, v. 34, n. 1, p. 10-15, 2012.

CAMPAGNANI, M. O.; CAMPOS, W. G.; AMORIM, S. S.; ROSA, L. H.; AUAD, A. M.; CANGUSSÚ, M. A.; MAURÍCIO, R. M. Prospection and fungal virulence associated with *Mahanarva spectabilis* (Hemiptera: Cercopidae) in an Amazon silvopastoral system**. Florida Entomologist**, v. 100, n. 2, p. 426-432, 2017.

CHILUWAL, K.; KIM, J.; DO BAE, S.; PARK, C. G. Essential oils from selected wooden species and their major components as repellents and oviposition deterrents of *Callosobruchus chinensis* (L.). **Journal of Asia-Pacific Entomology**, v. 20, n. 4, p. 1447-1453, 2017.

COOK, S. M.; KHAN, Z. R.; PICKETT, J. A. The use of push-pull strategies in integrated pest management. **Annu. Rev. Entomol.**, v. 52, p. 375-400, 2007.

CONGIO, G.F. de S. **Caracterização e quantificação dos danos causados por cigarrinhas adultas do gênero *Mahanarva* sp. (Hemiptera: Cercopidae) em *Brachiaria brizantha* cv. Marandu**. 2010. Dissertação (Mestrado em Ciência Animal e Pastagens) – ESALQ, Universidade de São Paulo, Piracicaba, 2010. 87 p.

DESCAMPS, L. R.; BIZET TUROVSKY, J. A. J.; BRUSTLE, C. M.; SÁNCHEZ CHOPA, F. Actividad repelente del aceite esencial de *Eucalyptus globulus* Labill. (Myrtaceae) y *Mentha* x *piperita* L. (Lamiaceae) en *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae); Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. **Dominguezia**, v. 35, n. 1, p. 93-96, 2019.

DIAS, M. L.; AUAD, A. M.; MAGNO, M. C.; RESENDE, T. T.; FONSECA, M. G.; SILVA, S. E. Insecticidal activity of compounds of plant origin on *Mahanarva spectabilis* (Hemiptera: Cercopidae). **Insects**, v. 10, p. 360, 2019.

DOUGOUD, J.; TOEPFER, S.; BATEMAN, M.; JENNER, W. H. Efficacy of homemade botanical insecticides based on traditional knowledge. A review. **Agron. Sustentar. Dev.**, v. 39, p. 37, 2019.

DUDAREVA, N.; NEGRE, F.; NAGEGOWDA, D. A.; ORLOVA, I. Plant volatiles: recent advances and future perspectives. **Crit. Rev. Plant Sci**., v. 25, p. 417–440, 2007.

GAUR, S. K.; KUMAR, K. Toxicity and insect growth regulatory effects of root extract from the medicinal plant, *Withania somnifera* (Linnaeus) against red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae). **Archives of Phytopathology and Plant Protection**. V. 53, n. 17-18, p. 856-875, 2020.

GONZÁLEZ-GUIÑEZ, R.; SILVA-AGUAYO, G.; URBINA-PARRA, A.; GERDING-GONZÁLEZ, M. Aceite esencial de *Eucalyptus globulus* Labill y *Eucalyptus nitens* H. Deane & Maiden (myrtaceae) para el control de *Sitophilus zeamais* Motschulsky. **Chilean journal of agricultural & animal sciences**, v. 32, n. 3, p. 204-216, 2016.

GUIMARÃES, F. C.; PAULA-MORAES, S. V.; NUNES, R. V.; PONTES, R. A.; AUAD, A. M.; TAKADA, S. C. S. **Biologia de ovos e adultos de *Mahanarva spectabilis* (Distant, 1909) (Hemiptera: Cercopidae)**. In: FALEIRO, F.G.; DE ANDRADE, S. R. M, (Eds.). III Encontro de Jovens Talentos da Embrapa Cerrados. Planaltina: Embrapa Cerrados, 2007, p. 21.

HOHNWALD, S.; KATO, O.; WALENTOWSKI, H. Accelerating capoeira regeneration on degraded pastures in the northeastern amazon by the use of pigs or cattle. **Sustainability**, v. 11, n. 6, 1729. 2019.

LEYVA, M.; DEL CARMEN MARQUETTI, M.; MONTADA, D.; PAYROLL, J.; SCULL, R.; MOREJÓN, G.; PINO, O. Aceites esenciales de *Eucalyptus globulus* (Labill) y *Bursera graveolens* (kunth) Triana & Planch para el control de mosquitos de importancia médica. **The Biologist**, v. 18, n. 2, 2020.

LIRA, M. DE A.; DE MELLO, A. C. L.; DA CUNHA, M. V.; DOS SANTOS, M. V. F.; DUBEUX, JR, J. C. B.; LIRA JR., M. DE A.; APOLINÁRIO, V. X. DE O. Animal production in tropical pastures of Latin America. **Archivos Latinoamericanos de Producción Animal**, v. 25, p. 1-2. 2017.

MATOS, L. F.; BARBOSA, D. R. S.; LIMA, E. DA C.; DUTRA, K. DE A.; NAVARRO, D. M. DO A. F.; ALVES, J. L. R.; SILVA, G. N. Chemical composition and insecticidal effect of essential oils from *Illicium verum* and *Eugenia caryophyllus* on *Callosobruchus maculatus* in cowpea. **Ind. Crop. Prod.** v. 145, 112088, 2020.

MENDES, A. M. S.; SOUZA, M. M. S.; FONSECA, A. M.; COLARES, R. P.; CARNEIRO, J. B.; SANTOS, F. G. N. Inseticidas naturais: uma alternativa sustentável para o controle de pragas na agricultura familiar, 2016. In: Congresso Brasileiro de Química (Online), 56, Belém. **ABQ**, Rio de Janeiro, 2016.

MENEZES, M.; EL-KADI, M. K.; PEREIRA, J. M.; RUIZ, M. A. M. Bases para o controle integrado das cigarrinhas das pastagens na região sudeste da Bahia. Ilhéus: CEPLAC-CEPEC, Brasil, 1983. 33 p.

MOBKI, M.; SAFAVI, S. A.; SAFARALIZADEH, M. H.; PANAHI, O. Toxicity and repellency of garlic (*Allium sativum* L.) extract grown in Iran against *Tribolium castaneum* (Herbst) larvae and adults. **Archives of Phytopathology and Plant Protection**, v. 47, n. 1, p. 59-68, 2014.

OTABOR, J. I.; ROTIMI, J.; OPOGGEN, L.; EGBON, I. N.; UYI, O. O. Phytochemical constituents and larvicidal efficacy of methanolic extracts of *Cymbopogon citratus*, *Ocimum gratissimum* and *Vernonia amygdalina* against *Culex quinquefasciatus* larvae. **Journal of Applied Sciences and Environmental Management**, v. 23, n. 4, p. 701-709, 2019.

PERERA, A. G. W. U.; KARUNARATNE, M. M. S. C. Eco-Friendly Alternatives for Storage Pest Management: Leaves of *Ruta Graveolens* (Aruda) as a Repellent Against the Rice Weevil, *Sitophilus Oryzae* L. **International Journal of Multidisciplinary Studies**, v. 2, n. 2, p.49–56, 2015.

PICARD, I.; HOLLINGSWORTH, R. G.; SALMIERI, S.; LACROIX, M. Repellency of essential oils to *Frankliniella occidentalis* (Thysanoptera: Thripidae) as affected by type of oil and polymer release. **Journal of Economic Entomology**, v. 105, n. 4, p. 1238–1247, 2012.

PRAJAPATI, V.; TRIPATHI, A. K.; AGGARWAL, K. K.; KHANUJA, S. P. S. Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. **Bioresource technology**. v. 96, n. 16, p. 1749-1757, 2005.

RIBEIRO, L. do P.; CAZAROTTO, A. R. Cigarrinhas-das-pastagens em Santa Catarina: avaliação do complexo de espécies e da incidência natural de fungos entomopatogênicos. **Agropecuária Catarinense**, Florianópolis, v. 32, n. 2, p. 73-79, 2019.

RIFFEL, A.; DA COSTA, J. G. **Os voláteis de plantas e o seu potencial para a agricultura**. Aracaju, SE: Embrapa Tabuleiros Costeiros. 2015. 48 p.

SAGHEER, M.; ALI, K.; RASHID, A.; SAGHEER, U.; ALVI, A. Repellent and toxicological impact of acetone extracts of *Nicotiana tabacum*, *Pegnum hermala*, *Saussurea costus* and *Salsola baryosma* against red flour beetle, *Tribolium castaneum* (Herbst). **Pakistan Journal of Zoology**, v. 45, n. 6, p. 1735-1739, 2013.

SÃO JOÃO, R. E., RAGA, A. **Mecanismo de defesa das plantas contra o ataque de insetos sugadores**. São Paulo, SP: Instituto Biológico- APTA, 2016, 13 p.

SILVA, L. M; CALDAS, A. P.; RODRIGUES, A. L. M.; OLIVEIRA, J. S.; SIMONETTI, E. R. de S. O uso do extrato de fumo (*Nicotina tabacum*) como alternativa para o controle de pragas em hortaliças, 2017. In: CONGRESSO INTERNACIONAL DAS CIÊNCIAS AGRÁRIAS, 2., 2017, Natal. **Anais** [...]. Natal, 2017.

SILVA, S. E.; AUAD, A. M.; MORAES, J. C.; ALVARENGA, R.; FONSECA, M. G.; MARQUES, F. A.; SANTOS, N. C. S.; NAGATA, N. Olfactory response of *Mahanarva spectabilis* (Hemiptera: cercopidae) to volatile organic compounds from forage grasses. **Sci. Rep**, v. 9, 10284, 2019.

SINGH, D.; SINGH, A. K.. Repellent and insecticidal properties of essential oils against housefly, *Musca Domestica* L. **Int. J. Trop. Insect Sci.**, v. 12, p. 487–491, 1991.

SINGH, G.; MAURYA, S.; DELAMPASONA, M. P.; CATALAN, C. Chemical constituents, antimicrobial investigations and antioxidative potential of volatile oil and acetone extract of star anise fruits. **Journal of the Science of Food and Agriculture**, v. 86, n. 1, p. 111-121, 2005.

SOBRINHO, F. S., AUAD, A. M., DA SILVA LÉDO, F. J. Genetic variability in *Brachiaria ruziziensis* for resistance to spittlebugs. Crop Breeding and Applied Biotechnology, v. 10, n. 1, p. 89-94, 2010.

SOUZA, J. C.; SILVA, R. A.; REIS, P. R.; QUEIROZ, D. S.; SILVA, D. B. **Cigarrinhas-das-pastagens: histórico, bioecologia, prejuízos, monitoramento e medidas de controle.** Belo Horizonte: Circular técnica EPAMIG, v. 42, 2008. p. 8.

SUTIL, W. P.; SANTOS, R. S.; DE OLIVEIRA, L. C. Controle químico de cigarrinhas-das-pastagens no estado do Acre. In: SEMINÁRIO DA EMBRAPA ACRE DE INICIAÇÃO CIENTÍFICA E PÓS-GRADUAÇÃO, 2., 2019, Rio Branco, AC. A Contribuição da ciência para a agropecuária no Acre. **Anais**. Rio Branco, AC: Embrapa Acre, 2020.

TAYOUB, G.; SULAIMAN, H.; ALORF, M. Determination of nicotine levels in the leaves of some *Nicotiana tabacum* varieties cultivated in Syria. **Herba Polonica**, v. 61, n. 4, 2016.

THOMPSON, V. Associative nitrogen fixation, C 4 photosynthesis, and the evolution of spittlebugs (Hemiptera: Cercopidae) as major pests of neotropical sugarcane and forage grasses. **Bulletin of entomological research**, v. 94, n. 3, p. 189-200, 2004.

VALÉRIO. J.R., 2009. **Cigarrinhas-das-pastagens.** Campo Grande, MS: Embrapa Gado de Corte, 2009. 51 p.

VERÍSSIMO, B. A.; AUAD, A. M.; OLIVEIRA, C. M.; PAIVA, I. G. Seasonality of predatory insects (Diptera: Syrphidae and Asilidae) in pasture monoculture and silvopastoral systems from Southeast Brazil. **International Journal of Tropical Insect Science (Online)**, v. 40, p. 1-12, 2020.

WAGNER, R. L.; CARD, J. A. *Ailanthus altissima* aqueous extract deters *Spodoptera frugiperda* oviposition. **The Great Lakes Entomologist**, v. 53, n. 1, p. 68-72, 2020.

WEI, L.; HUA, R.; LI, M.; HUANG, Y.; LI, S.; HE, Y.; SHEN, Z. Chemical composition and biological activity of star anise *Illicium verum* extracts against maize weevil, *Sitophilus zeamais* adults. **J. Insect Sci.**, v. 14, n. 1, p. 80, 2014.

FINAL REMARKS

In this dissertation we highlight the importance of researching alternative technologies for the control of insect pests that complement the strategies currently practiced, that are sustainable and accessible. In this context, we used methodologies already established by other researchers and evaluated the response of spittlebug *M. spectabilis* to aqueous plant extracts, by means of insecticidal action tests and olfactometry tests to verify attraction and non-attraction. To evaluate the insecticidal effect of plant extracts on *M. spectabilis* nymphs, the Petri dish method was adopted, where we applied the compounds directly to the insect and evaluated its responses after 24h and 48h. In part of this evaluation step, we verified the potential use of tobacco extract as an alternative substance in the control of spittlebug *M. spectabilis*, thus advancing the research and determining through the same evaluation methodology that the most effective concentration of aqueous tobacco extract is 25 % with extraction by infusion and decoction, and its use may be associated with other integrated pest management techniques.

The second product of this dissertation was an article in which we evaluated the olfactory response of *M. spectabilis* adults to nine aqueous plant extracts applied on elephant grass, using the methodology of olfactometry. According to the literature, elephant grass has volatile compounds attractive to *M. spectabilis*, which justifies research with compounds that may cause non-attractiveness. The research required great effort, as 18 combinations of treatments were evaluated, two for each extract, with at least 40 positive responses for each treatment. As a result of this great effort, we can recommend the aqueous extracts of tobacco, star anise and eucalyptus to be used as a management tactic for *M. spectabilis* adults in elephant grass, as they were not attractive for *M. spectabilis*.

We can determine that this research presented relevant and innovative results for the study area, the aqueous extracts of tobacco, star anise and eucalyptus are alternatives for the control of *M. spectabilis* in elephant grass, and can be used in new research to determine composition and action in the field, with low-cost products, natural origin and low human and environmental impact, which can be manufactured by family farmers on their properties.