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FERNANDA PEREIRA ANDRADE

Varronia curassavica Jacq. (Boraginaceae) AS AN AGROECOLOGICAL TOOL FOR COFFEE PEST MANAGEMENT

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Thesis submitted to the Entomology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Madelaine Venzon

Co-advisers: Angelo Pallini
Maira Christina Marques Fonseca

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Assent:

Fernanda Pereira Andrade
Author

Madelaine Venzon
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To my parents.

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“Entenda os seus medos, mas jamais deixe que eles sufoquem os seus sonhos.”

(Alice no País das Maravilhas)

ABSTRACT

ANDRADE, Fernanda Pereira, D.Sc., Universidade Federal de Viçosa, August, 2022. ***Varronia curassavica*** **Jacq. (Boraginaceae) as an agroecological tool for coffee pest management**. Adviser: Madelaine Venzon. Co-advisers: Angelo Pallini and Maira Christina Marques Fonseca.

Coffee (*Coffea* sp.) is a crop of great relevance in agriculture. However, its productivity can be severely affected by the attack of pests, such as *Leucoptera coffeella* and *Hypothenemus hampei.* The coffee leaf miner, *L. coffeella,* is a key coffee pest in the Neotropics. The larvae feed on the parenchyma of coffee leaves decreasing the photosynthesis capacity and leading to significant losses in yield. The coffee berry borer, *H. hampei*, is the most damaging insect pest of coffee worldwide. This pest lives inside the coffee berry consuming the seeds thus reducing the quantity and quality of the coffee grains. The most common method of control to both pests is the use of synthetic pesticides, which have a low effectiveness due to the selection of resistant populations. Additionally, the excessive use of these products could have a severe impact in the environment and non-target individuals. An alternative for the management of these pests could be the use of specific plants and their secondary metabolites, such as the essential oils. *Varronia curassavica* is a medicinal species that produces an essential oil with a range of biological activities besides attracting natural enemies, such as the Chrysopidae. The specie *Chrysoperla externa* is an important biological control agent of a variety of pests, including *L. coffeella* and *H. hampei.* In this study, I investigate whether *V. curassavica* can be used in the management of *L. coffeella* and *H. hampei* and benefit their natural enemy *C. externa*. I performed a greenhouse experiment to evaluate if the association of *V. curassavica* plants with coffee plants would affect the oviposition of *L. coffeella* and its predator *C. externa* (Chapter I). The association of the *V. curassavica* plants with coffee plants didn't affect *L. coffeella* oviposition, however, it increased the *C. externa* oviposition. I also investigate the effects of the *V. curassavica* essential oil in the oviposition and the *development* of eggs and mines of *L. coffeella* (Chapter II). The *V. curassavica* essential oil inhibited the oviposition of *L. coffeella* but did not affect the development of its eggs and mines. I evaluated the lethal and sublethal effects of *V. curassavica* essential oil on *H. hampei*, assessing the mortality rates, the mobility of H. hampei, and the repellence of the essential oil to this pest (Chapter III). The essential oil of *V. curassavica* is toxic to *H. hampei* and affects its mobility, but it wasn't repellent to the pest in the concentration tested. This study demonstrates that *V. curassavica* plant and its essential oil could be a safer alternative in the management of the key coffee pests.

Keywords: *Coffea* sp. *Leucoptera coffeella*. *Hypothenemus hampei*. *Chrysoperla externa*.

RESUMO

ANDRADE, Fernanda Pereira, D.Sc., Universidade Federal de Viçosa, agosto de 2022. ***Varronia curassavica* Jacq. (Boraginaceae) como uma ferramenta agroecológica para o manejo de pragas do café**. Orientador: Madelaine Venzon. Coorientadores: Angelo Pallini e Maira Christina Marques Fonseca.

O café (*Coffea* sp.) é uma cultura de grande relevância na agricultura, porém sua produtividade pode ser severamente afetada pelo ataque de pragas, como *Leucoptera coffeella* e *Hypothenemus hampei*. O bicho-mineiro do café, *L. coffeella*, é uma praga chave da cultura nos Neotrópicos. As larvas se alimentam do parênquima das folhas de café, diminuindo a capacidade fotossintética e levando a perdas significativas na produtividade. A broca-do-café, *H. hampei*, é a praga do café mais prejudicial em todo o mundo. Essa praga vive dentro do fruto do café consumindo as sementes, o que reduz a quantidade e a qualidade dos grãos. O método mais comum de controle dessas pragas é o uso de inseticidas sintéticos, que possuem baixa eficácia de controle e induzem à seleção de populações resistentes. Além disso, o uso excessivo desses produtos pode ter um grande impacto negativo no meio ambiente e em indivíduos não-alvo. Uma alternativa para o manejo dessas pragas poderia ser o uso de certas plantas e seus metabólitos secundários, como os óleos essenciais. *Varronia curassavica* é uma espécie medicinal, que produz óleo essencial com diversas atividades biológicas e atrai inimigos naturais, como Chrysopidae. A espécie *Chrysoperla externa* é um importante agente de controle biológico de uma variedade de pragas, incluindo *L. coffeella* e *H. hampei*. Neste estudo investigou-se se *V. curassavica* pode ser usada no manejo de *L. coffeella* e *H. hampei* e beneficiar seu inimigo natural *C. externa*. Realizou-se um experimento em casa de vegetação para avaliar se a associação de plantas de *V. curassavica* com plantas de café afetaria a oviposição de *L. coffeella* e do seu predador *C. externa* (Capítulo I). A associação das plantas de *V. curassavica* com plantas de café não afetou a oviposição de *L. coffeella*, porém aumentou a oviposição de *C. externa*. Investigou-se também os efeitos do óleo essencial de *V. curassavica* na oviposição e no desenvolvimento de ovos e minas de *L. coffeella* (Capítulo II). O óleo essencial de *V. curassavica* inibiu a oviposição de *L. coffeella*, mas não afetou o desenvolvimento de seus ovos e minas. Avaliou-se os efeitos letais e subletais do óleo essencial de *V. curassavica* sobre *H. hampei*, as taxas de mortalidade, a mobilidade de *H. hampei* e a repelência do óleo essencial a essa praga (Capítulo III). O óleo essencial de V. *curassavica* é tóxico para *H. hampei* e afeta sua mobilidade, mas não foi repelente à praga na concentração testada. Este estudo demonstra que a planta de *V. curassavica* e seu óleo essencial podem ser alternativas seguras para o manejo das pragas chave do cafeeiro.

Palavras-chave: *Coffea* sp. *Leucoptera coffeella*. *Hypothenemus hampei*. *Chrysoperla externa*.

LIST OF ILLUSTRATIONS

LIST OF TABLES

Chapter II

Chapter III

SUMMARY

GENERAL INTRODUCTION

Coffee (*Coffea* sp.) is a crop of great relevance in agriculture, impacting the lives of thousands of people who are part of its production either directly or indirectly (Santos et al., 2010; Infante, 2018; Soares et al., 2022). Brazil stands out as the world's largest producer and exporter of this product. In the 2021 harvest, more than 47 million bags were produced of which more than 40 million were destined for the foreign market, moving around 6.24 billion dollars (Conab, 2021). However, the productivity and quality of production can be affected by several factors, including the attack of pest insects, such as the coffee leaf miner (*Leucoptera coffeella* Guérin-Méneville (Lepidoptera: Lyonetiidae)) and the coffee berry borer (*Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae: Scolytinae)) (Fragoso et al., 2003; Santos et al., 2010; Gonring et al., 2019).

The coffee leaf miner, *L. Coffeella*, is a key coffee pest in the Neotropics (Souza et al., 1998; Reis et al., 2002; Pantoja-Gomez et al., 2019). The larvae of this pest feed on the cell parenchyma of the coffee leaves, which reduces the plant's photosynthetic capacity and leads to leaf fall, thus affecting the productivity of the coffee crop (Souza & Reis, 1992; Souza et al., 1998). In high population levels the defoliation de rate could be up to 70% reducing up to 50% the coffee yields (Reis & Souza, 1996). Coffee berry borer, *H. hampei,* is considered the leading pest of coffee crops worldwide (Vega et al., 2009; Cure et al., 2020). The larvae and the adult of this pest consume the endosperm of the berries, causing economic damage due to the reduction of the quality and quantity of the marketable coffee (Jaramillo et al., 2006; Vega et al., 2009; Infante, 2018). It is estimated that *H. hampei* causes up to 500 million dollars of losses annually (Vega et al., 2002). In Brazil, these losses are estimated in 215 up to 358 million dollars annually (Oliveira et al., 2013).

The common method of control to *L. coffeella* and *H. hampei* worldwide is the use of synthetic insecticides, but the effectiveness of this products is low mainly because of the development of resistant populations to the main molecules on the market, also because of the high costs due to the increase in the applications per year (Vega et al., 2015; David-Rueda et al., 2016; Leite et al., 2020). In addition to that, the misuse of these products leads to outbreaks of secondary pests and loss of beneficials (Fragoso et al., 2003; Pereira et al., 2007; Guedes et al., 2016, 2017; Leite et al., 2020, 2021). This highlights the need of other strategies for the management of the coffee pests.

An alternative for the management of *L. coffella* and *H. hampei* could be the use of plants and their secondary metabolites. Plants produce a variety of secondary metabolites that have as primary function their defense against herbivores, bacteria, fungi, viruses, and even other competing plants (Wink, 2018; Zaynab et al., 2018; Yang et al., 2018). These secondary metabolites will also act as signals to communicate with symbiotic microorganisms, attract pollinators, natural enemies, and seed dispersers, defending the plant indirectly (Wink, 2018; Yang et al., 2018). Plants of Boraginaceae are widely studied because of their production of secondary metabolites with a range of biological activities (Dresler et al., 2017).

Varronia curassavica Jacq., a member of Boraginaceae family, is a medicinal and aromatic species, perennial and native to Brazil that produces an essential oil (EO) in glandular trichomes located in the surface of their leaves (Lorenzi & Matos, 2008; Feijó et al., 2014). Among the several chemical compounds found in *V. curassavica* EO are α-pinene, trans-caryophyllene, alloaromadendrene and α-humulene (Carvalho Jr. et al., 2004), which are reported for some insecticide properties as repellence and for attracting natural enemies (Chen, 2008). For example, the *V. curassavica* EO was reported to be toxic to two pests of great agricultural importance: the two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) and the green peach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), without affect the survival of their natural enemy *Ceraeochrysa cubana* Hagen (Neuroptera: Chrysopidae) (Andrade et al., 2021). Among the natural enemies attracted by *V. curassavica* are predators such as Formicidae, Vespidae and Chrysopidae (Brandão et al., 2015; Hoeltgebaum et al., 2018; Martins, 2021).

Chrysopidae is an important biological control agent of a variety of pests found in many agricultural systems (Ecole et al., 2002; Venzon et al., 2006; Barbosa et al., 2019). The species *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) is native from the Neotropical Region that is widely distributed (Albuquerque et al., 1994). Adults feed on pollen and sugary foods, such as nectar and honeydew and the larvae are generalist predators feeding on a variety of prey including *L. coffeella and H. hampei* (Carvalho & Souza, 2000; Ecole et al., 2002; Venzon et al., 2006; Botti et al., 2021).

Thereby in this thesis, we investigate whether *V. curassavica* can be used in the management of *L. coffeella* and *H. hampei* and benefit the natural enemy *C. externa*. We performed a greenhouse experiment to evaluate if the association of *V. curassavica* plants with coffee plants would affect the oviposition of *L. coffeella* and its predator *C. externa* (Chapter I). In Chapter II, we investigate the effects of the *V. curassavica* EO in the oviposition and the development of eggs and mines of *L. coffeella* with tests in laboratory. We also evaluated the lethal and sublethal effects of *V. curassavica* EO on *H. hampei.* For all of this, we performed laboratory experiments to assess the mortality rates and the mobility of *H. hampei*, and the repellence of the EO to this pest (Chapter III).

References

Andrade, F. P., Venzon, M., das Dôres, R. G. R., Franzin, M. L., Martins, E. F., de Araújo, G. J., & Fonseca, M. C. M. (2021). Toxicity of *Varronia curassavica* Jacq. Essential Oil to Two Arthropod Pests and Their Natural Enemy. *Neotropical Entomology*, 50(5), 835–845. https://doi.org/10.1007/S13744-021-00906-X

Albuquerque, G. S., Tauber, C. A., & Tauber, M. J. (1994). *Chrysoperla externa* (Neuroptera: Chrysopidae): life history and potential for biological control in Central and South America. *Biological control*, 4(1), 8-13.

Barbosa, L. R., Campos, J. M., Wilcken, C. F., & Zanuncio, J. C. (2019). Forests. In: Souza, B., Vázquez, L. L., Marucci, R. C. (Eds). *Natural Enemies of Insect Pests in Neotropical Agroecosystems*, Springer, Cham. 305-317.

Botti, J. M. C., Martins, E. F., Franzin, M. L., & Venzon, M. (2021). Predation of Coffee Berry Borer by a Green Lacewing. *Neotropical Entomology* 51(1),160–163. https://doi.org/10.1007/s13744-021-00884-0.

Brandão, D. S., Mendes, A. D. R., Santos, R. R., Rocha, S. M. G., Leite, G. L. D., & Martins, E. R. (2015). Biologia floral e sistema reprodutivo da erva-baleeira (*Varronia curassavica* Jacq.). *Revista Brasileira de Plantas Medicinais*, 17, 562-569.

Carvalho, C. F. & Souza, B. (2000). Métodos de criação e produção de crisopídeos. In: Bueno, V. H. P. (Ed) *Controle biológico de pragas: produção massal e controle de qualidade*. 2. ed. Lavras: UFLA, 91-109.

Carvalho Jr., P. M., Rodrigues, R. F. O., Sawaya, A. C. H. F., Marques, M. O. M., & Shimizu, M. T. (2004). Chemical composition and antimicrobial activity of the essential oil of *Cordia verbenacea* DC. *Journal of Ethnopharmacology,* 95(2-3), 297-301. https://doi.org/10.1016/j.jep.2004.07.028

Chen, M. S. (2008). Inducible direct plant defense against insect herbivores: a review. *Insect Science*, 15(2), 101-114.

CONAB. Acompanhamento da safra brasileira de café safra 2021 - quarto levantamento. Disponível em: https://www.conab.gov.br/info-agro/safras/cafe>. Acessed: 19 June 2022

Cure, J. R., Rodríguez, D., Gutierrez, A. P., & Ponti, L. (2020). The coffee agroecosystem: bio-economic analysis of coffee berry borer control (*Hypothenemus hampei*). *Scientific Reports*, 10(1), 1-12. https://doi.org/10.1038/s41598-020-68989-x

David-Rueda, G., Constantino, C. L. M., Montoya, E. C., Ortega, M. O. E., Gil, Z. N., & Benavides-Machado, P. (2016). Diagnóstico de *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) y sus parasitoides en el departamento de Antioquia, Colombia. *Revista Colombiana de Entomologia,* 42(1), 4–11. https://doi.org/10.25100/socolen.v42i1.6662

Dresler, S., Szymczak, G., & Wójcik, M. (2017). Comparison of some secondary metabolite content in the seventeen species of the boraginaceae family. *Pharmaceutical Biology*, 55(1), 691–695. https://doi.org/10.1080/13880209.2016.1265986

Ecole, C. C., Silva, R. A., Louzada, J. N., Moraes, J. C., Barbosa, L. R., & Ambrogi, B. G. (2002). Predação de ovos, larvas e pupas do bicho-mineiro-do-cafeeiro, *Leucoptera coffeella* (Guérin-Menèville & Perrottet, 1842) (Lepidoptera: Lyonetiidae) por *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae). *Ciências e Agrotecnologia,* 26, 318-324.

Fernandes, F. L., Mantovani, E. C., Neto, H. B., & Nunes, V. D. V. (2009). Efeitos de variáveis ambientais, irrigação e vespas predadoras sobre *Leucoptera coffeella* (Guérin-Méneville) (Lepidoptera: Lyonetiidae) no cafeeiro. *Neotropical Entomology* 38(3), 410-417. Doi: 10.1590/S1519-566X2009000300018.

Feijó, E. V. R. S., Oliveira, R. A. D., & Costa, L. C. B. (2014). Light affects *Varronia curassavica* essential oil yield by increasing trichomes frequency. *Revista Brasileira de Farmacognosia*, 24(5), 516-523. https://doi.org/10.1016/j.bjp.2014.10.005

Fragoso, D. B., Guedes, R. N. C., & Ladeira, J. A. (2003). Seleção na evolução de resistência a organofosforados em *Leucoptera coffeella* (Guérin-Mèneville) (Lepidoptera: Lyonetiidae). *Neotropical Entomology*, 32(2), 329–334. https://doi.org/10.1590/S1519-566X2003000200020

Gonring, A. H. R., Silva, F. M. A., Picelli, E. C. M., Plata-Rueda, R. A., Gorri, J. E. R., & Fernandes, F. L. (2019). Comparative bioassay methods to determine diamide susceptibility for two coffee pests. *Crop Protection*, 121, 34 – 38. https://doi.org/10.1016/j.cropro.2019.03.010

Guedes, R.N.C., Smagghe, G., Stark, J.D., & Desneux, N. (2016). Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. *Annual Review of Entomology,* 61, 43- 62. https://doi.org/10.1146/annurev-ento-010715-023646.

Guedes, R.N.C., Walse, S.S., & Throne, J.E. (2017). Sublethal exposure, insecticide resistance, and community stress. *Current Opinion in Insect Science* 21, 47-53. https://doi.org/10.1016/j.cois.2017.04.010.

Hoeltgebaum, M. P., Montagna, T., Lando, A. P., Puttkammer, C., Orth, A. I., Guerra, M. P., & Reis, M. S. (2018). Reproductive Biology of *Varronia curassavica* Jacq. (Boraginaceae). *Anais da Academia Brasileira de Ciências,* 90, 59-71. http://dx.doi.org/10.1590/0001-3765201820160273

Infante, F. (2018). Pest Management Strategies Against the Coffee Berry Borer (Coleoptera: Curculionidae: Scolytinae). *Journal of Agricultural and Food Chemistry*, 66(21), 5275-5280. https://doi.org/10.1021/acs.jafc.7b04875

Jaramillo, J., Borgemeister, C., & Baker, P. (2006). Coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae): searching for sustainable control strategies. *Bulletin of Entomological Research,* 96(3), 223-233.https://doi.org/10.1079/BER2006434

Leite, S. A., dos Santos, M. P., Resende-Silva, G. A., da Costa, D. R., Moreira, A. A., Lemos, O. L., Guedes, R. N. C., & Castellani, M. A. (2020). Area-Wide Survey of Chlorantraniliprole Resistance and Control Failure Likelihood of the Neotropical Coffee Leaf Miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae). *Journal of Economic Entomology,* 113(3), 1399–1410. https://doi.org/10.1093/jee/toaa017

Leite, S. A., dos Santos, M. P., Costa, D. R., Moreira, A. A., Guedes, R. N. C, & Castellani, M. A. (2021). Time-concentration interplay in insecticide resistance among populations of the Neotropical coffee leaf miner, *Leucoptera coffeella*. *Agricultural and Forest Entomology*, 23(2), 232-241. https://doi.org/10.1111/afe.12425

Lorenzi, H., & Matos, F. J. A. (2008). Plantas medicinais no Brasil: nativas e exóticas, 2nd ed. Nova Odessa: Instituto Plantarum, 544p.

Martins, E. F. (2021). Conservation biological control of coffee leaf miner: Role of green lacewings and parasitoids. Tese de Doutorado, Universidade Federal de Viçosa, Viçosa

Oliveira, C., Auad, A., Mendes, S. M., & Frizzas, M. R. (2013). Economic impact of exotic insect pests in Brazilian agriculture. *Journal of Applied Entomology*, *137*(1–2), 1–15. https://doi.org/10.1111/jen.12018

Pantoja-Gomez, L. M., Corrêa, A. S., de Oliveira, L. O., & Guedes, R. N. C. (2019). Common Origin of Brazilian and Colombian Populations of the Neotropical Coffee Leaf Miner, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae). *Journal of Economic Entomology*, 112(2), 924–931. https://doi.org/10.1093/JEE/TOY416

Pereira, E. J. G., Picanço, M. C., Bacci, L., Della Lucia, T. M. C., Silva, E. M., Fernandes, F. L. (2007). Natural mortality factors of *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) on Coffea arabica. *Biocontrol Science and Technology*, 17(5), 441-455. https://doi.org/10.1080/09583150701309337.

Reis, P. R., & Souza, J. C. (1996). Manejo integrado do bicho-mineiro, *Perileucoptera coffeella* (Guérin-Mèneville) (Lepidoptera: Lyonetiidae) e seus reflexos na produção de café. *Anais da Sociedade Entomológica do Brasil* 25(1), 77–82.

Reis, P. R., Souza, J. C., & Venzon, M. (2002). Manejo ecológico das principais pragas do cafeeiro. *Informe Agropecuário,* 23(214/215), 83–99.

Santos, M. R. A., Lima, R. A., Silva, A. G., Lima, D. K. S., Sallet, L. A. P., Teixeira, C. A. D., & Facundo, V. A. (2010). Composição química e atividade inseticida do óleo essencial de *Schinus terebinthifolius* Raddi (Anacardiaceae) sobre a broca-do-café (*Hypothenemus hampei*). *Revista Brasileira de Plantas Medicinais,* 15 (4), 757-762.

Soares, W. P., Costa, J. N. M., Vieira Júnior, J. R., Cipriani, H. N., Souza, J. G., Fernandes, C. F. (2022). Atividade inseticida de extratos botânicos sobre a Broca-do-café *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae). *Revista em Agronegócio e Meio Ambiente, 15(1),* 8064. https://doi.org/10.17765/2176-9168.2022v15n1e8064

Souza, J. C.; & Reis, P. R. (1992). Bicho mineiro: Biologia, danos e manejo integrado. Boletim técnico, 37. Belo Horizonte, Epamig. 28p.

Souza, J. C., Reis, P. R., & Rigitano, R. L. O. (1998). Bicho-mineiro do cafeeiro: biologia, danos e manejo integrado. Belo Horizonte, Brasil: EPAMIG, 48 p.

Vega, F. E., Franqui, R. A., & Benavides, P. (2002). The presence of the coffee berry borer, *Hypothenemus hampei*. *Puerto Rico: fact or fiction*, 1-3.

Vega, F. E., Infante, F., Castillo, A., & Jaramillo, J. (2009). The coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae): a short review, with recent findings and future research directions. *Terrestrial Arthropod Reviews,* 22, 129–147.

Vega, F. E., Infante, F., & Johnson, A. J. (2015). The Genus *Hypothenemus*, with emphasis on *H. hampei*, the Coffee Berry Borer, in: Vega, F.E., Hofstetter, R.W. (eds) *Bark Beetles*: Biology and Ecology of Native and Invasive Species, Academic Press: San Diego, CA, USA, pp. 427–494. http://dx.doi.org/10.1016/B978-0-12-417156-5.00011-3

Venzon, M., Rosado, M. C., Euzébio, D. E., Souza, B., & Schoereder, J. H. (2006). Suitability of leguminous cover crop pollens as food source for the green lacewing *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae). *Neotropical Entomology,* 35 (3), 371–376, https://doi.org/10.1590/S1519-566X2006000300012

Wink, M. (2018). Plant secondary metabolites modulate insect behavior-steps towards addiction?. *Frontiers in Physiology*, 9, 364.

Yang, L., Wen, K. S., Ruan, X., Zhao, Y. X., Wei, F., & Wang, Q. (2018). Response of plant secondary metabolites to environmental factors. *Molecules*, 23(4), 762.

Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Umair, M., Zafar, M. H., & Bahadar, K. (2018). Role of secondary metabolites in plant defense against pathogens. *Microbial pathogenesis*, 124, 198–202.

CHAPTER I

Interference of *Varronia curassavica* Jacq. (Boraginaceae) in the coffee leaf miner and *Chrysoperla externa* Hagen (Neuroptera: Chrysopidae) oviposition

ABSTRACT

Plants developed a variety of strategies to defense themselves against pest attacks such as the production and releasing of volatile metabolites, which can repel herbivores and/or attract natural enemies. *Varronia curassavica* is a medicinal aromatic plant that produces an essential oil with a range of biological activities. It has also been studied to be associated to coffee agroecosystems to manage pests, such as the coffee leaf miner. The coffee leaf miner, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae), is a key coffee pest in neotropical production areas. Control of this pest is done through synthetic pesticide but there are a large number of natural enemies reported for its control, such as the green lacewing *Chrysoperla externa* (Neuroptera: Chrysopidae), a generalist predator often found in coffee crops in Brazil. Here, we investigated whether the association of *V. curassavica* plants with coffee interferes in the oviposition site by *L. coffeella* and *C. externa*. We conducted two separate greenhouse experiments, one for each insect, releasing 100 couples of *L. coffeella* and *C. externa* in the greenhouse in each experiment. Treatments consisted of (1) coffee plants with *V. curassavica* plants; (2) coffee plants solo. The total number of laid eggs by the insects was counted for seven days after their releasing. The presence of *V. curassavica* plants did not interfere in the oviposition of *L. coffeella* on coffee plants, however the presence of *V. curassavica* plants stimulated oviposition of the natural enemy *C. externa*. Therefore, the association of *V. curassavica* plants with coffee can be a new strategy that could be applied for the management of *L. coffeella* by increasing the predator's, *C. externa*, population in the coffee crops.

Keywords: Association, *Coffea* sp., *Leucoptera coffeella*, green lacewing.

1. INTRODUCTION

Plant defense systems are intricate and dynamic, can recognize threat signals and trigger responses through various chemical and physical traits (War et al., 2012; Belete, 2018). These traits can be classified in constitutive or induced defense. Constitutive defense is already present in the plant's tissues without the need of a trigger for its expression, and induced defense is activated by herbivore damage (Aljbory & Chen, 2018; Pereira et al., 2021). Both can be observed in two types: direct and indirect defense mechanisms. Direct defenses are related to plant characteristics that will increase resistance against the herbivore such as the production of toxic secondary metabolites (e.g., terpenoids, alkaloids, anthocyanins, phenols, and quinones) that could kill, repel or affect the herbivore development (Hanley et al., 2007; Chen, 2008; Belete, 2018). Indirect defense is related to mechanisms that will not affect the herbivore directly but will attract their natural enemies, such as the release of attractive volatile organic compounds and/or the provision of food and shelter. (Arimura et al., 2009; Aljbory & Chen, 2018; Belete, 2018). The Boraginaceae family is known for holding a group of plants that produce secondary metabolites, such as naphthaquinones, flavonoids, terpenoids and phenol that are related with many biological activities (Dresler et al., 2017).

Varronia curassavica Jacq., a member of Boraginaceae family, is an aromatic species, perennial and native to Brazil (Lorenzi & Matos, 2008). This shrub can reach more than 2.0 m in height, has sessile leaves, inflorescences with white flowers and red and sub globular fruits (Marques et al., 2019). It is a plant with therapeutic activity, being used in popular medicine as an anti-inflammatory and analgesic (Lorenzi & Matos, 2008; Matias et al., 2013). The surface of *V. curassavica* leaves has glandular trichomes, where an essential oil (EO) is produced, stored, and released (Feijó et al., 2014). The volatile chemical compounds present in the *V. curassavica* EO are reported to act in the repellence of some pests and attraction of natural enemies (Chen, 2008). Pollinators and predators, such as Formicidae and Vespidae, are some of the insects attracted by *V. curassavica* (Brandão et al., 2015; Hoeltgebaum et al., 2018). In coffee fields where *V. curassavica* was associated as a companion plant, it was noted the constant and abundant presence of predatory green lacewings (Neuroptera: Chrysopidae) (Martins, 2021; Venzon, 2021).

The green lacewing, *Chrysoperla externa* Hagen (Neuroptera: Chrysopidae) is a species native from the Neotropical Region that is widely distributed and can be found in different agroecosystems (Albuquerque et al., 1994). Adults of this species feed on pollen, nectar, and honeydew and the larvae are generalist predators, feeding on a variety of preys (Carvalho & Souza, 2000; Venzon et al., 2006) such as the coffee leaf miner (Ecole et al., 2002).

The coffee leaf miner, *Leucoptera coffeella* Guérin-Méneville (Lepidoptera: Lyonetiidae), is a microlepidoptera that is considered a key coffee pest in the Neotropics (Fragoso et al., 2003; Pereira et al., 2007; Pantoja-Gomez et al., 2019). The larvae of *L. coffeella* attacks the coffee leaves feeding on the leaf palisade parenchyma, resulting in high losses in yield, weight, and quality of coffee (Pereira et al., 2007; Giraldo-Jaramillo et al., 2019). This pest is usually controlled with synthetic insecticides, but their effectiveness is low, mainly because the coffee leaf miner has already developed resistant populations to the main molecules on the market (David-Rueda et al., 2016; Leite et al., 2020).

Although the association of *V. curassavica* in coffee fields showed an increase of Chrysopidae populations, little is known about the influence in their oviposition and of the pest *L. coffeella.* Therefore, here we evaluated if the association of *V. curassavica* plants with coffee has interference in oviposition site by *L. coffeella* and the green lacewing *C. externa*.

2. MATERIAL AND METHODS

2.1 Plants

Coffee seedlings used in the tests were *Coffea arabica,* variety “Catucaí amarelo 785/15”, obtained from the nursery kept in Universidade Federal de Viçosa, Viçosa – MG. *Varronia curassavica* seedlings were produced from seeds harvested at Experimental Research

Station of Agricultural and Livestock Research Enterprise of Minas Gerais (EPAMIG) in Oratórios-MG, Brazil. The seedlings of both species were transplanted to plastic pots of 5L to develop until the ideal height for the tests (approximately 30 cm).

2.2 Insect rearings

2.2.1 *Leucoptera coffeella*

To establish the rearing of *L. coffeella*, we collected coffee leaves with active mines (containing live larvae) in an experimental area located on the campus of the Universidade Federal de Viçosa, Viçosa – MG. The leaves were inserted through the petiole in a sponge soaked in water inside a plastic tray (20.0 x 10.0 cm) and placed in transparent acrylic cages (40.0 x 40.0 x 40.0 cm). The rearing was kept at a temperature of 27 ± 2°C, relative humidity of 60 ± 10% and a photoperiod of 12:12 h (L:D). Daily, the newly emerged adults were transferred to a new cage containing clean coffee leaves for oviposition (According to Martins et al. (2021) methodology).

2.2.2 *Chrysoperla externa*

We obtained the *C. externa* specimens from a rearing maintained in the Laboratory of Entomology of Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG), cultured following Venzon et al. (2006) methodology. Adults were kept in PVC cages, consisting of a tube of PVC (8 x 11 cm) covered with nylon gauze and placed in a plastic tray. We provided water on a piece of cotton soaked and placed inside a 10 mL vial. Food consisted of a 1:1 mixture of yeast and honey, offered on a parafilm strip. Food and water were replaced twice a week. We transferred the newly emerged larvae to plastic tubes (7 cm high x 2.5 cm in diameter) and fed them with eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) until pupation. The rearing was kept at a temperature of 25 ± 2°C, relative humidity of 60 ± 10% and a photoperiod of 14:10 h (L:D).

2.3 Interference of *V. curassavica* plants on oviposition site by *L. coffeella*

To test whether the association of *V. curassavica* plants with coffee plants would interfere in the oviposition site by *L. coffeella*, we carried out a bioassay in a greenhouse (14 x 5 m) at a temperature of 28 ± 5°C and relative humidity of 70 ± 20%. Clean coffee and *V. curassavica* plants, without inflorescences, were arranged in two treatments and placed on six metal benches (4.5 x 1.5 m) spaced 90 cm apart from each other. Treatments consisted of: (1) five coffee plants and three *V. curassavica* plants; (2) five coffee plants. Each bench contained one repetition of each treatment, with 2.5 m of space between them. The experiment was conducted in four sets of six replicates over time, totaling 24 replicates (Fig. 1). To obtain the *L. coffeella* individuals, laboratory reared pupae were transferred to individual microtubes (1.5 mL) until adult emergence. The newly emerged adults were sexed based on the shape of the terminal abdominal segment as described by Motta (2021) and paired in couples to mating for two days. Afterwards, 100 couples of *L. coffeella* were released in the greenhouse. The releasing was made between 8:00 AM and 10:00 AM, releasing approximately the same number of couples between each bench. After 24h from releasing, the number of *L. coffeella* eggs on coffee leaves was daily counted, for seven days, using a magnifying glass (30x). To the statistical analysis was considered the total number of eggs oviposit in the seven days.

2.4 Interference of *V. curassavica* plants on oviposition site by *C. externa*

To test whether the association of *V. curassavica* plants with coffee plants would interfere in the oviposition site by *C. externa*, we carried out a bioassay in a greenhouse (14 x 5 m) at a temperature of 28 ± 5°C and relative humidity of 70 ± 20%. Clean coffee and *V. curassavica* plants, without inflorescences, were arranged in two treatments and placed on six metal benches (4.5 x 1.5 m) spaced 90 cm apart from each other. Treatments consisted of: (1) five coffee plants and three *V. curassavica* plants; (2) five coffee plants. Each bench contained one repetition of each treatment, with 2.5 m of space between them. The experiment was conducted in four sets of six replicates over time, totaling 24 replicates (Fig.1). To obtain the *C. externa* females, laboratory reared pupae were kept in individual plastic tubes (7 cm high x 2.5 cm in diameter) until adult emergence. The newly emerged adults were sexed based on the shape of external genitalia in the terminal abdominal segment as described by New (2001) and grouped in cages to mating (proportion of 1:1 of males and females). Afterwards, 100 mated females of *C. externa* were released in the greenhouse. The releasing was made between 8:00 AM and 10:00 AM, releasing one cage with approximately the same number of females between each bench. After 24h from releasing, the number of *C. externa* eggs deposited either on coffee or *V. curassavica* plants leaves was counted daily during seven days. To the statistical analysis was considered the total number of eggs oviposit in the seven days.

2.5 Data analyses

For the analysis, we used a model simplification process by 'AICcmodavg' package (Mazerolle & Linden, 2019) and we determined the minimum adequate model(s) by comparing Akaike Information Criterion corrected (AICc) values to all the analysis. We used Generalized linear mixed models (GLMMs) with a negative binomial error distribution to evaluate the oviposition of *L. coffeella* and *C. externa* in the greenhouse experiments. We defined the treatments (coffee and coffee with *V. curassavica*) as a fixed effect and the time as a random effect. For both analyses, we compared the GLMM against null models to attest possible random patterns in the predictor variables. We compared the oviposition of *L. coffeella* and *C. externa* means by χ^2 test of Analysis of Variance (ANOVA). All analyses were performed using R 4.2.0 software (R Development Core Team, 2022).

3. RESULTS

3.1 Interference of *V. curassavica* plants on oviposition site by *L. coffeella*

There was no significant difference in the total number of eggs laid by *L. coffeella* when coffee plants were solo or when they were associated with *V. curassavica* in the greenhouse experiment ($\chi^2 = 1.584$, DF= 1, $p = 0.208$, fig. 2).

3.2 Interference of *V. curassavica* plants on oviposition site by *C. externa*

The number of eggs laid by *C. externa* was significantly higher when coffee plants were associated with *V. curassavica* than when they were solo in the greenhouse experiment ($\chi^2 = 16.205$, DF= 1, $p < 0.05$, fig. 3). The oviposition occurred both in coffee and *V. curassavica* plants in the associated plot, however the number of eggs laid was higher in *V. curassavica* plants, approximately 66,4% of the total for the associated plot.

4. DISCUSSION

Our results suggest that *V. curassavica* is a promising plant for improving conservation biological control of *L. coffeella* due to the increasing of eggs laid by the predator *C. externa* in more than 60 percent in the associated plot. Insects use odors for mediate almost all their vital functions, for example feeding, defense, host plant selection, mating and oviposition (Tegoni et al., 2004). The correct host choice for oviposition is of great importance for insect fitness, since this will impact in the survival and, consequently in the population growth (Mayhew, 2001). Oviposition site usually is selected based on what is most suitable for offspring development until adult stage (Jones et al., 2019). This choice is mediated by a complex collection of stimuli and responses that probably occurs in the sensory system. The receptors sense the metabolites released by the plant mechanisms of defense and trigger a response in the insect that can be an increase or a decrease in the number of eggs oviposited by the females (Navarro-Silva et al., 2009; Städler & Reifenrath, 2008).

The association of *V. curassavica* with coffee plants had no negative effect in the oviposition of *L. coffeella.* The interactions between insects and metabolites are intricated and might work in multiple trophic levels and some of the responses could be related to the co-evolution between the plant and the insect (Bento, 2001; Arab & Bento, 2006). This is observed to *L. coffeella,* the levels of caffeine in coffee leaves favor the production and release of the volatiles, principally *p*-cymene, favoring the attraction and stimulating the oviposition on the coffee leaves (Magalhães et al., 2008). That interaction could be one explanation to our results to *L. coffeella* oviposition, even that other volatiles (from *V. curassavica)* were present in the environment did not affect that specific and close response that was established by the high level of specialization of *L. coffeella* with his host plant.

Another explanation for the *L. coffeella* results could be related to the density of *V. curassavica* plants. The number of plants used in the experiment may not release sufficient volatiles to interfere in the *L. coffeella* oviposition. The production of these volatiles has a cost to the plant, so each plant produces a small amount of these compounds (Chen, 2008). Their production can also vary according to the developmental stage and condition of the plants and the insects related (Arimura et al., 2009). The volatile blend is specific for a particular insect-plant system; this includes natural enemies and the neighboring plants (War et al., 2012).

V. curassavica plants associated with coffee favored the oviposition of the predator *C. externa.* This result indicates that *V. curassavica* have the potential to attract and increase the populations of *C. externa* in coffee crops. The attraction of herbivore and predators could happen because of the volatile organic compounds released by aromatic plants, like *V. curassavica* (Beizhou et al., 2012; Togni et al., 2016). Terpenoids, which are the main constituent chemical group of the *V. curassavica* oil, are among the main component of the volatiles released by plants and their role in the attraction of natural enemies is reported in various systems (Kessler & Baldwin, 2001; Kost & Heil, 2006; Chen, 2008). Caryophillene and

β-caryophillene, specifically are reported to attract adults of the green lacewing *Chrysopa carnea* Stephens ((Neuroptera: Chrysopidae) in cotton fields (Flint et al., 1979). Therefore, it is prossible that the same compound or the combination of them were responsible for the increase in the number of eggs laid by *C. externa* in the presence of *V. curassavica*.

Even though the *V. curassavica* presence didn't affect the oviposition of *L. coffeella,* the increase in *C. externa* oviposition could help suppress the pest population in coffee crops. Natural enemies have been estimated to account for at least 50% of pest control occurring in crop fields (Pimentel, 2005). Generalist predators specially can reduce pest populations in a significant degree and in some cases below economic threshold even (Symondson et al., 2002).

Green lacewings are usually found naturally in coffee crops, but their population levels may not be enough to significantly reduce the *L. coffeella* population (Botti et al., 2021; Venzon, 2021). Coffee monoculture systems don't hold enough resources to keep green lacewings constantly in the areas. In the adult stage, green lacewings feed on plant-derived food, such as nectar and pollen and the larval stage is the predator phase (Venzon et al., 2006). Coffee blooms for a limited period and the availability of the flowers are short, so there are no food resources for adult green lacewings throughout the year (Peters et al., 2016). The association of plants in the main crop could be an alternative to provide these resources and these plants may also provide shelter and oviposition sites (Togni et al., 2016; Batista et al., 2017; Gurr et al., 2017). Recently, Martins (2021) presented the value of *V. curassavica* as a plant that provide food and alternative prey for *C. cubana*, the larvae of this green lacewing species had its survival increased by the presence of *V. curassavica* inflorescences and the prey that inhabit them.

The knowledge of the multiple plant-insect interactions in an agricultural system is a key to ensure success to conservation biological control programs. Here, we demonstrate how a plant could be evaluated for this purpose. And in view of these perspectives, our results support that *V. curassavica* plants can be used by coffee farmers to attract and increase the population of the predator *C. externa* as a promising tool for the management and maintenance of *L. coffeella* below the economic injure levels.

References

Albuquerque, G. S., Tauber, C. A., & Tauber, M. J. (1994). *Chrysoperla externa* (Neuroptera: Chrysopidae): life history and potential for biological control in Central and South America. *Biological control*, 4(1), 8-13.

Aljbory, Z., & Chen, M. S. (2018). Indirect plant defense against insect herbivores: a review. *Insect Science*, 25(1), 2-23.

Arab, A., & Bento, J. M. S. (2006). Plant volatiles: new perspectives for research in Brazil. *Neotropical Entomology*, *35*(2), 151–158. https://doi.org/10.1590/S1519-566X2006000200001

Arimura, G. I., Matsui, K., & Takabayashi, J. (2009). Chemical and molecular ecology of herbivore-induced plant volatiles: proximate factors and their ultimate functions. *Plant and Cell Physiology*, 50(5), 911-923.

Batista, M. C., Fonseca, M. C. M., Teodoro, A. V., Martins, E. F., Pallini, A., & Venzon, M. (2017). Basil (*Ocimum basilicum* L.) attracts and benefits the green lacewing *Ceraeochrysa cubana* Hagen. *Biological Control*, 110, 98-106. https://doi.org/10.1016/j.biocontrol.2017.04.013

Belete, T. (2018). Defense mechanisms of plants to insect pests: From morphological to biochemical approach. *Trends in Technichal & Scientific Research*, 2, 30-38.

Beizhou, S., Jie, Z., Wiggins, N. L., Yuncong, Y., Guangbo, T., & Xusheng, S. (2012). Intercropping with aromatic plants decreases herbivore abundance, species richness, and shifts arthropod community trophic structure. *Environmental Entomology*, *41*(4), 872-879.

Bento, J. M. S. (2001). Voláteis de tricomas glandulares de *Cordia curassavica* (Jacq.) R. & S. *(*Boraginaceae) como atraentes de *Cratosomus flavofasciatus* Guérin, 1844 (Col., Curculionidae). Tese de Doutorado, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba. doi:10.11606/T.11.2019.tde-20191220-142631.

Botti, J. M. C., Martins, E. F., Franzin, M. L., & Venzon, M. (2021). Predation of Coffee Berry Borer by a Green Lacewing. *Neotropical Entomology*, 51(1), 160-163. https://doi.org/10.1007/S13744-021-00884-0

Brandão, D. S., Mendes, A. D. R., Santos, R. R., Rocha, S. M. G., Leite, G. L. D., & Martins, E. R. (2015). Biologia floral e sistema reprodutivo da erva-baleeira (*Varronia curassavica* Jacq.). *Revista Brasileira de Plantas Medicinais,* 17, 562-569.

Carvalho, C. F. & Souza, B. (2000). Métodos de criação e produção de crisopídeos. In: Bueno, V. H. P. (Ed) *Controle biológico de pragas: produção massal e controle de qualidade*. 2. ed. Lavras: UFLA, 91-109.Chen, M. S. (2008). Inducible direct plant defense against insect herbivores: a review. *Insect science*, 15(2), 101-114.

David-Rueda, G., Constantino, C. L. M., Montoya, E. C., Ortega, M. O. E., Gil, Z. N., & Benavides-Machado, P. (2016). Diagnóstico de *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) y sus parasitoides en el departamento de Antioquia, Colombia. *Revista Colombiana de Entomologia,* 42(1), 4–11. https://doi.org/10.25100/socolen.v42i1.6662

Dresler, S., Szymczak, G., & Wójcik, M. (2017). Comparison of some secondary metabolite content in the seventeen species of the boraginaceae family. *Pharmaceutical Biology*, 55(1), 691–695. https://doi.org/10.1080/13880209.2016.1265986

Feijó, E. V. R. S., Oliveira, R. A. D., & Costa, L. C. B. (2014). Light affects *Varronia curassavica* essential oil yield by increasing trichomes frequency. *Revista Brasileira de Farmacognosia*, 24(5), 516-523. https://doi.org/10.1016/j.bjp.2014.10.005

Flint, H. M., Salter, S. S., & Walters, S. (1979). Caryophyllene: an attractant for the green lacewing. *Environmental Entomology*, 8(6), 1123-1125.

Fragoso, D. B., Guedes, R. N. C., & Ladeira, J. A. (2003). Seleção na evolução de resistência a organofosforados em *Leucoptera coffeella* (Guérin-Mèneville) (Lepidoptera: Lyonetiidae). *Neotropical Entomology*, 32(2), 329–334. https://doi.org/10.1590/S1519-566X2003000200020

Giraldo-Jaramillo, M., Garcia-Gonzalez, J., & Rugno, J. B. (2019). Fertility Life Table of *Leucoptera coffeella* (Guérin-Mèneville) (Lepidoptera: Lyonetiidae) at Seven Temperatures in Coffee. *American Journal of Entomology*, *3*(4), 70-76. https://doi.org/10.11648/J.AJE.20190304.12

Gurr, G. M., Wratten, S. D., Landis, D. A., & You, M. (2017). Habitat management to suppress pest populations: progress and prospects. *Annual review of entomology*, 62(1), 91-109.

Hanley, M. E., Lamont, B. B., Fairbanks, M. M., & Rafferty, C. M. (2007). Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology, Evolution and Systematics*, 8(4), 157-178.

Hoeltgebaum, M. P., Montagna, T., Lando, A. P., Puttkammer, C., Orth, A. I., Guerra, M. P., & Reis, M. S. (2018). Reproductive Biology of *Varronia curassavica* Jacq. (Boraginaceae). *Anais da Academia Brasileira de Ciências,* 90, 59-71. http://dx.doi.org/10.1590/0001-3765201820160273

Jones, L. C., Rafter, M. A., & Walter, G. H. (2019). Insects allocate eggs adaptively across their native host plants. *Arthropod-Plant Interactions, 13*(2), 181–191. https://doi.org/10.1007/S11829-019-09688-X

Kessler, A. & Baldwin, I. T. (2001) Defensive function of herbivore-induced plant volatile emissions in nature. *Science,* 291(5511), 2141–2144.

Kost, C., & Heil, M. (2006). Herbivore-induced plant volatiles induce an indirect defence in neighbouring plants. *Journal of Ecology*, 94(3), 619–628.

Leite, S. A., dos Santos, M. P., Resende-Silva, G. A., da Costa, D. R., Moreira, A. A., Lemos, O. L., Guedes, R. N. C., & Castellani, M. A. (2020). Area-Wide Survey of Chlorantraniliprole Resistance and Control Failure Likelihood of the Neotropical Coffee Leaf Miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae). *Journal of Economic Entomology,* 113(3), 1399–1410. https://doi.org/10.1093/jee/toaa017

Lorenzi, H., & Matos, F. J. A. (2008). Plantas medicinais no Brasil: nativas e exóticas, 2nd ed. Nova Odessa: Instituto Plantarum, 544p.

Magalhães, S. T. V., Guedes, R. N. C., Lima, E. R., & Demuner, A. J. (2008). Coffee leaf volatiles and egg laying by the coffee leaf miner *Leucoptera coffeella*. *Crop Protection*, 27(6), 1038–1041. https://doi.org/10.1016/J.CROPRO.2007.12.005

Marques, A. P. S., Bonfim, F. P. G., Dantas, W. F. C., Puppi, R. J., & Marques, M. O. M. (2019). Chemical composition of essential oil from *Varronia curassavica* Jacq. accessions in different seasons of the year. *Industrial Crops and Products*, 140, 111656. https://doi.org/10.1016/j.indcrop.2019.111656

Martins, E. F. (2021). Conservation biological control of coffee leaf miner: Role of green lacewings and parasitoids. Tese de Doutorado, Universidade Federal de Viçosa, Viçosa.

Martins, E. F., Franzin, M. L., Perez, A. L., Schmidt, J. M., & Venzon, M. (2021). Is *Ceraeochrysa cubana* a coffee leaf miner predator? *Biological Control*, 160, 104691. https://doi.org/10.1016/j.biocontrol.2021.104691

Matias, E. F. F., Santos, K. K. A., Falcão-Silva, V. S., Siqueira-Júnior, J. P., Costa, J. G. M., & Coutinho, H. D. M. (2013). Modulation of the norfloxacin resistance in *Staphylococcus aureus* by *Cordia verbenaceae* DC. *The Indian Journal of Medical Research*, 137(1), 178.

Mayhew, P. J. (2001). Herbivore host choice and optimal bad motherhood. *Trends in Ecology & Evolution*, 16(4), 165–167. https://doi.org/10.1016/S0169-5347(00)02099-1

Mazerolle, M. J., Linden, D. (2019). Model Selection and Multimodel Inference Based on (Q)AIC(c). R-CRAN. 233p

Motta, I. O., Dantas, J., Vidal, L., Bílio, J., Pujol-Luz, J. R., & Albuquerque, É. V. (2021). The coffee leaf miner, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae): identification of the larval instars and description of male and female genitalia. *Revista Brasileira de Entomologia*, 65.

Navarro-Silva, M. A., Marques, F. A., & Duque L, J. E. (2009). Review of semiochemicals that mediate the oviposition of mosquitoes: a possible sustainable tool for the control and monitoring of Culicidae. *Revista Brasileira de Entomologia*, 53(1), 1–6. https://doi.org/10.1590/S0085-56262009000100002

New, T. R. (2001). Introduction to the Neuroptera: what are they and how do they operate. 3-5. In: McEwen, P. K., New, T. R., & Whittington, A. E. (Eds.). Lacewings in the crop environment. Cambridge University Press.

Pantoja-Gomez, L. M., Corrêa, A. S., de Oliveira, L. O., & Guedes, R. N. C. (2019). Common Origin of Brazilian and Colombian Populations of the Neotropical Coffee Leaf Miner, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae). *Journal of Economic Entomology*, 112(2), 924–931. https://doi.org/10.1093/JEE/TOY416

Pereira, R. V., Filgueiras, C. C., Dória, J., Peñaflor, M. F. G., & Willett, D. S. (2021). The effects of biostimulants on induced plant defense. *Frontiers in Agronomy*, 45.

Pereira, E. J. G., Picanço, M. C., Bacci, L., Della Lucia, T. M. C., Silva, E. M., Fernandes, F. L. (2007). Natural mortality factors of *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) on Coffea arabica. *Biocontrol Science and Technology*, 17(5), 441-455. https://doi.org/10.1080/09583150701309337.

Peters, V. E., Carlo, T. A., Mello, M. A. R., Rice, R. A., Tallamy, D. W., Caudill, S. A., & Fleming, T. H. (2016). Using Plant–Animal Interactions to Inform Tree Selection in Tree-Based Agroecosystems for Enhanced Biodiversity. *BioScience*, 66(12), 1046–1056. https://doi.org/10.1093/BIOSCI/BIW140

Pimentel, D. (2005). Environmental and economic costs of the application of pesticides primarily in the United States., *Environment, Development and Sustainability*, 7, 229–252.

R Development Core Team (2022). A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Städler, E., & Reifenrath, K. (2008). Glucosinolates on the leaf surface perceived by insect herbivores: review of ambiguous results and new investigations. *Phytochemistry Reviews* 8(1), 207–225. https://doi.org/10.1007/S11101-008-9108-2

Symondson, W. O. C., Sunderland, K. D., & Greenstone, M. H. (2002). Can generalist predators be effective biocontrol agents?. *Annual review of entomology*, 47(1), 561-594.

Tegoni, M., Campanacci, V., & Cambillau, C. (2004). Structural aspects of sexual attraction and chemical communication in insects. *Trends in Biochemical Sciences*, 29(5), 257–264. https://doi.org/10.1016/J.TIBS.2004.03.003

Togni, P. H. B., Venzon, M., Muniz, C. A., Martins, E. F., Pallini, A., & Sujii, E. R. (2016). Mechanisms underlying the innate attraction of an aphidophagous coccinellid to coriander plants: Implications for conservation biological control. *Biological Control*, 92, 77–84. https://doi.org/10.1016/J.BIOCONTROL.2015.10.002

Venzon, M. (2021). Agro-Ecological Management of Coffee Pests in Brazil. *Frontiers in Sustainable Food Systems*, 5. https://doi.org/10.3389/FSUFS.2021.721117/FULL

Venzon, M., Rosado, M. C., Euzébio, D. E., Souza, B., & Schoereder, J. H. (2006). Suitability of leguminous cover crop pollens as food source for the green lacewing *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae). *Neotropical Entomology,* 35 (3), 371–376, https://doi.org/10.1590/S1519-566X2006000300012

War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S., & Sharma, H. C. (2012). Mechanisms of plant defense against insect herbivores. *Plant signaling & behavior*, 7(10), 1306-1320.

Figures

Figure 1. View of the plants disposition in the greenhouse (Photo: Fernanda Pereira Andrade).

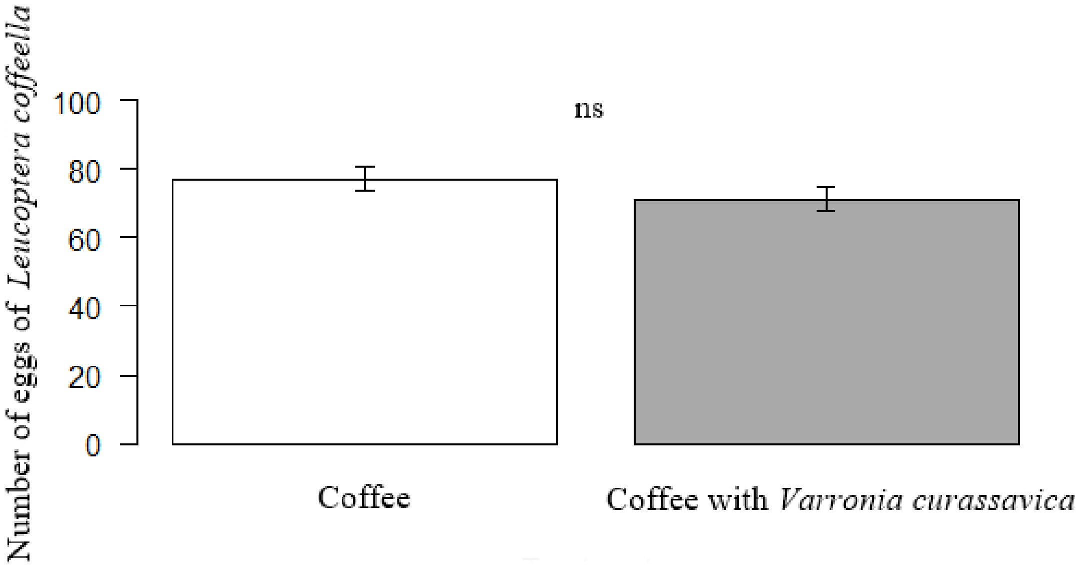

Figure 2. Number of eggs (mean ± standard error) laid by *Leucoptera coffeella* in coffee plants solo and coffee plants associated with *Varronia curassavica* (χ^2 = 1.584, DF= 1, p = 0.208). ns = No significant statistical difference.

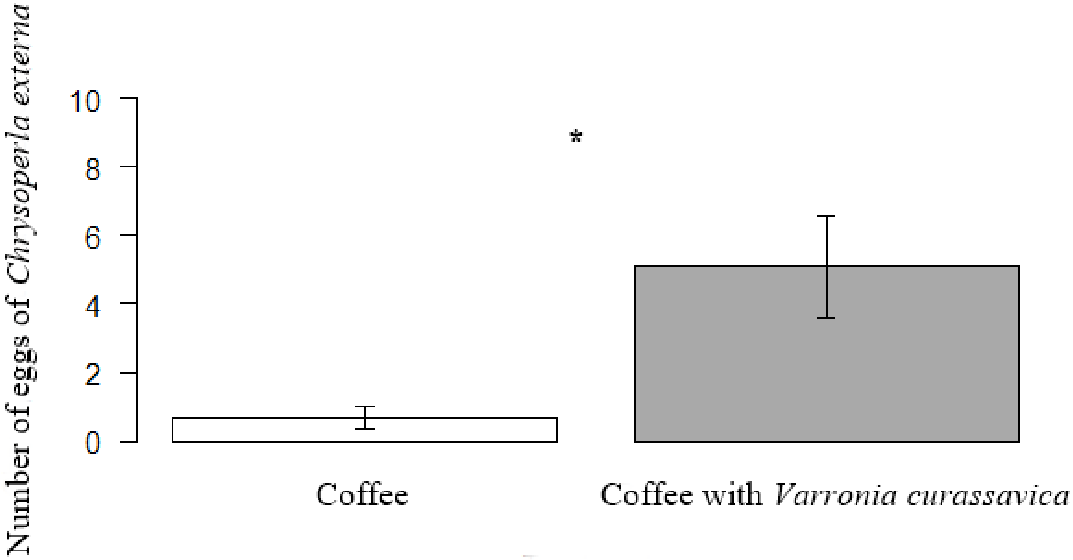

Figure 3. Number of eggs (mean ± standard error) laid by *Chrysoperla externa* in coffee plants solo and coffee plants associated with *Varronia curassavica* (χ^2 = 16.205, DF= 1, p < 0.05). Asterisks on the bars represent the statistical differences.

CHAPTER II

Effects of the *Varronia curassavica* Jacq. (Boraginaceae) essential oil on the oviposition and development of the coffee leaf miner

ABSTRACT

Leucoptera coffeella (Lepidoptera: Lyonetiidae) is a key pest to coffee in neotropical production areas. Larvae of *L. coffeella* feed on cell parenchyma of the leaves reducing the plant's photosynthetic capacity and leading to leaf early senescence, thus affecting the productivity of the coffee plant. Control of this pest is done through synthetic pesticide, but this strategy has low effectiveness due to pest resistance and losses of beneficial insects. An alternative strategy of control could be the use of plant secondary metabolites, like the essential oils (EOs). *Varronia curassavica* is a medicinal aromatic species that produces an essential oil (EO) with a range of biological activities. In this work, we evaluate *V. curassavica* EO effect on the oviposition and development of eggs and mines of *L. coffeella*. We tested four treatments (0.25, 0.5, 0.75 and 1.0 % EO concentration) and the control (distilled water and Tween® 80 (0.05%). To the oviposition test, coffee branches were sprayed with the tested solutions and afterwards a couple of *L. coffeella* were offered to them for ovipositing for two days. After this, the number of eggs was counted. To test the effect in *L. coffeella* eggs, leaves containing four eggs were sprayed with the tested solutions and the number of mines formed was counted. To evaluate the mines development, leaves containing four mines were sprayed with the tested solutions and the size of the mine, the number of pupae and adults were counted. The *V. curassavica* EO inhibited *L. coffeella* oviposition in all concentrations tested. There was no effect in the eggs and mines development. Therefore, our results suggest that *V. curassavica* EO has potential to be used in the management of *L. coffeella* by coffee farmers.

Keywords: Secondary metabolites, *Coffea* sp., *Leucoptera coffeella*, Biopesticide.

1. INTRODUCTION

The coffee leaf miner, *Leucoptera coffeella* Guérin-Méneville (Lepidoptera: Lyonetiidae), is a silver-colored nocturnal microlepidoptera responsible for high losses in coffee crops (Reis et al., 2002; Fernandes et al., 2009; Pantoja-Gomez et al., 2019). Females of *L. coffeella* oviposit in the adaxial surface of the coffee leaves and, when the eggs hatch, the larvae penetrate the leaves where they will live feeding on the leaf palisade parenchyma (Parra, 1985; Pereira et al., 2007; Giraldo-Jaramillo et al., 2019). Lesions caused by larvae, called mines, become necrotic, which reduce the plant's photosynthetic capacity and leads to leaf fall, thus affecting the productivity of the coffee crop (Souza & Reis, 1992; Souza et al., 1998). Their life cycle can vary according to the climatic conditions, but considering a 25 °C temperature, the egg stage lasts around five days, the larval stage lasts around twelve days, and the pupae lasts around five days, a total of nearly 22 days until reaching adulthood (Katiyar & Ferrer, 1968). The pest hits high populations levels in areas with hot and dry weather, which happens in most of the regions of coffee production in Brazil. In such condition, the rate of defoliation could be up to 70%, leading to 50% of losses in the coffee yields (Reis & Souza, 1996; Giraldo-Jaramillo et al., 2019; Leite et al., 2020).

The management of *L. coffeella* is usually with chemical control, but this method presents a series of problems, such as: risks to the environment and human health, the high cost and loss of effectiveness due to development of resistant populations (Sharma, 2008; David-Rueda et al., 2016; Leite et al., 2020). An alternative to the use of chemical control could be the use of plant secondary metabolites. Secondary metabolites are substances produced by plants that are related with their defense mechanisms against herbivores and other microorganisms (Wink, 2018; Yang et al., 2018). Essential oils (EOs) are a secondary metabolite with a variety of biological activities, including insecticide effects, such as: mortality, repellency, sterility, reduced feeding and changes in behavior and development (Isman, 2014; Campos et al., 2019; Isman, 2020). EOs are a complex mixture of volatile compounds with a strong aroma produced by innumerous plants, like the member of Boraginaceae family *Varronia curassavica* Jacq. (Feijó et al., 2014).

Varronia curassavica is an aromatic species with therapeutic activity, being used in popular medicine as an anti-inflammatory and analgesic (Lorenzi & Matos, 2008; Matias et al., 2013). The *V. curassavica* EO is produced in leaves glandular trichomes and has several chemical compounds in its composition, especially: α-pinene, trans-caryophyllene, alloaromadendrene and α-humulene (Carvalho Jr. et al., 2004; Feijó et al., 2014). Andrade et al. (2021) has previously reported the insecticidal activity of *V. curassavica* EO against two pests of great agricultural importance: the two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) and the green peach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphidoidea) without interference in the survival of *Ceraeochrysa cubana* Hagen (Neuroptera: Chrysopidae), a generalist predator that is reported to prey *L. coffeella* (Martins et al., 2021).

Based as previously described, the *V. curassavica* EO could be a potential natural product to *L. coffeella* management, due to its insecticidal activity demonstrated to other pests. However, its effect on coffee leaf miner is still unknown. Thus, here we aim to evaluate the effect of the *V. curassavica* EO on the oviposition and development of eggs and mines of *L. coffeella.*

2. MATERIAL AND METHODS

2.1 Essential oil

Varronia curassavica EO used in the tests was purchased from Legeé Óleos Essenciais (Estiva Gerbi, São Paulo, Brazil). According to the information provided by the supplier, the extraction of the EO was made by hydrodistillation of the leaves and its main constituents are α-pinene (38.82%), β-caryophyllene (21.84%) and alloaromadendrene (4.85%) (Table 1).

2.2 Treatments

A 1% stock solution of essential oil (w/v) was prepared in distilled water plus Tween® 80 (0.05%). This stock solution was then also diluted with distilled water plus Tween® 80 (0.05%) in the other concentrations. Concentrations used in the tests were based in Andrade et al. (2021).

2.3 *Leucoptera coffeella* rearing

To establish the rearing of *L. coffeella*, we collected coffee leaves with active mines (containing larvae) in an experimental area located on the campus of the Universidade Federal de Viçosa, Viçosa – MG. The leaves were inserted through the petiole in a sponge soaked in water inside a plastic tray (20.0 x 10.0 cm) and placed in transparent acrylic cages (40.0 x 40.0 x 40.0 cm). The rearing was kept at a temperature of 27 ± 2°C, relative humidity of 60 ± 10% and a photoperiod of 12:12 h (L:D). Daily, the newly emerged adults were transferred to a new cage containing clean coffee leaves for oviposition (According to Martins et al. (2021) methodology).

2.4 Oviposition of *L. coffeella* on coffee leaves treated with *V. curassavica* essential oil

To evaluate whether *V. curassavica* EO would interfere on the oviposition of *L. coffeella*, coffee branches, with three leaves, were placed individually in plastic pots (500 mL) with their stem inserted in a hole in the pot lid. Pots were filled with water to maintain leaf turgidity. We standardized the number of leaves to facilitate the evaluation of oviposition. The coffee leaves were sprayed with the tested solutions with a hand sprayer until full coverage of the leaves and left to dry at room temperature for 30 min. When the surface of the leaves was completely dry, a transparent plastic cylinder (32 × 12 cm), with two rectangular openings (10 × 6 cm) covered with voile cloth, was placed over the branch to protect them. The cylinder was fixed in the plastic pots with a parafilm strip (Fig. 1). To obtain the *L. coffeella* individuals, laboratory reared pupae were transferred to individual microtubes (1.5 mL) until adult emergence. The newly emerged adults were sexed based on the shape of the terminal abdominal segment as described by Motta (2021) and paired in couples to mating for two days. A couple of *L. coffeella* (one female and one male) was released in the plastic cylinder, where they stayed for 48h to allow females to oviposit. After this period, the leaves were detached from the branches and were examined under a stereomicroscope at a magnification of 40x to count the number of *L. coffeella* eggs. The test was carried out at a temperature of 27 ± 2°C, relative humidity of 60 ± 10% and a photoperiod of 12:12 h (L:D), in a completely randomized design with four treatments (0.25, 0.5, 0.75 and 1.0 % EO concentration) and the control (distilled water and Tween® 80 (0.05%)) and 30 replicates.

2.5 Development of *L. coffeella* eggs treated with *V. curassavica* essential oil

To test whether the *V. curassavica* EO would affect the development of *L. coffeella* eggs, we applied the treatments in leaves containing the eggs. To obtain leaves with enough eggs, coffee leaves were inserted through the petiole in a sponge soaked in water inside a plastic tray (20.0 x 10.0 cm) and placed in cages containing females of *L. coffeella* for oviposition for a period of two days. After this period, the leaves were removed from the cages and analyzed under a stereomicroscope at a magnification of 40x to count the number of eggs and make a standardization of 4 eggs per leaf. The period of two days was established to guarantee that the eggs used in the experiment was in the beginning of the development. The leaves were sprayed with the tested solutions with a hand sprayer until full coverage of the leaves and left to dry at room temperature for 30 min. When the surface of the leaves was completely dry, they were inserted by the petiole into a sponge soaked in water inside a plastic tray (20.0 x 10.0 cm) and placed in transparent acrylic cages (40.0 x 40.0 x 40.0 cm). Leaves were examined daily under a stereomicroscope at a magnification of 40x to count the number of mines formed. The test was carried out at a temperature of 27 ± 2°C, relative humidity of 60 ± 10% and a photoperiod of 12:12 h (L:D), in a completely randomized design with four treatments (0.25, 0.5, 0.75 and

1.0 % EO concentration) and the control (distilled water and Tween® 80 (0.05%)) and 27 replicates. A leaf with 4 eggs constitutes one repetition.

2.6 Development of *L. coffeella* mines treated with *V. curassavica* essential oil

To test whether de *V. curassavica* EO would affect the development of *L. coffeella* mines we applied the treatments in leaves containing mines. To obtain leaves with enough mines, coffee leaves were inserted through the petiole in a sponge soaked in water inside a plastic tray (20.0 x 10.0 cm) and placed in placed in cages containing females of *L. coffeella* for oviposition for a period of two days. After this period, the leaves were removed from the cages and analyzed under a stereomicroscope at a magnification of 40x to count the number of eggs and make a standardization of 4 eggs per leaf. The leaves were kept until the formation of the mine before receiving the treatments. The size of mines from all leaves did not differ significantly when treatments were applied ($F = 1.635$, $p = 0.169$, $DF = 4$, fig. 2). The leaves were sprayed with the tested solutions with a hand sprayer until full coverage of the leaves and left to dry at room temperature for 30 min. When the surface of the leaves was completely dry, they were inserted by the petiole into a sponge soaked in water inside a plastic tray (20.0 x 10.0 cm) and placed in transparent acrylic cages (40.0 x 40.0 x 40.0 cm). In test was evaluated the mine size, number of pupae formed, and number of adults emerged. To evaluate the mines size, we measure the maximum length of the mines in the pictures with the ImageJ software (Abramoff et al., 2004). The test was carried out at a temperature of 27 ± 2°C, relative humidity of 60 ± 10% and a photoperiod of 12:12 h (L:D), in a completely randomized design with four treatments (0.25, 0.5, 0.75 and 1.0 % EO concentration) and the control (distilled water and Tween® 80 (0.05%)) and 27 replicates. A leaf with 4 mines constitutes one repetition.

2.7 Data analyses

We used a model simplification process by 'AICcmodavg' package (Mazerolle & Linden, 2019) and we determined the minimum adequate model(s) by comparing Akaike

Information Criterion corrected (AICc) values to the oviposition, number of mines, pupae, and adults' analysis. We used Generalized linear models (GLMs) with a negative binomial error distribution to evaluate the oviposition of *L. coffeella* analyzed by χ^2 test of Analysis of Variance (ANOVA) and a contrast analysis using the lsmeans function from lsmeans package v.2.30-0 (Lenth, 2016) to identify the levels in which the differences occurred. For number of mines, pupae e adults per leaf we used GLM adjusted to Poisson distribution with a χ^2 test of Analysis of Variance (ANOVA). For those analysis, we compared the GLM against null models to attest possible random patterns in the predictor variables. For mines size we used Analysis of Deviance and pairwise comparisons performed by emmeans. All analyses were performed using R 4.2.0 software (R Development Core Team, 2022).

3. RESULTS

3.1 Oviposition of *L. coffeella* on coffee leaves treated with *V. curassavica* essential oil

We found that the different concentrations of *V. curassavica* EO significantly affected the number of eggs deposited by *L. coffeella* (χ^2 = 87.248, DF = 4, p < 0.001). A lower oviposition was observed on leaves treated with EO compared to the control treatment. However, there was not significant difference among EO concentrations (Fig. 3).

3.2 Development of *L. coffeella* eggs treated with *V. curassavica* essential oil

There was no significant difference in the number of mines of *L. coffeella* originating from eggs on coffee leaves treated with the different concentrations of *V. curassavica* EO and the control treatment (χ^2 = 1.208, DF= 4, p = 0,876, fig. 4).

3.3 Development of *L. coffeella* mines treated with *V. curassavica* essential oil

The size of *L. coffeella* mines treated with the different concentrations of *V. curassavica* EO did not differed significantly from the size of those in the control (F = 0.407, p = 0.803, DF = 4, fig. 5). We also didn't find significant difference in the number of pupae (χ^2 = 4.506, DF =

4, p = 0.341, fig. 6) and the number of adults (χ^2 = 4.010, DF = 4, p = 0.404, fig. 7) originating from mines on coffee leaves treated with the different concentrations of *V. curassavica* EO compared to the control treatment.

4. DISCUSSION

Our results suggest that the use of *V. curassavica* EO in coffee crops is a promising strategy to control *L. coffeella*. In all the tested concentrations the oviposition was lower than on the control treatment. Hence, this highlights the fact that even small amounts of the EO are sufficient to reduce the number of *L. coffeella* eggs in coffee plants. The reason for this reduction may be related to the main compounds present in the *V. curassavica* EO. The EOs, in general, are an aromatic and volatile mixture of multiple compounds of two major chemical groups, phenylpropanoids and the terpenoids (monoterpenes and sesquiterpenes) (Pavela, 2015). In this study, the major constituents' compounds of the *V. curassavica* EO used were the sesquiterpenes β-caryophillene and the monoterpene α-Pinene. Sesquiterpenes are known for their important larvicidal activity and the repelling action (Yajima et al., 2007; Ashour et al., 2018). The β-caryophillene specifically is well-described as a repellent compound (Liu et al., 2010; Pavela, 2015; Ali et al., 2016; Benelli et al., 2018). Monoterpenes are substances with high potential for toxic interference in basic biochemical processes causing physiological and behavioral changes in insects (Ashour et al., 2018; Abdelgaleil et al., 2019). The α-Pinene has also been reported as a repellent (Bedini et al., 2015; Benelli et al., 2018). However, the effect of a EO is not related exclusively to the major compounds. It is reported that are interactions between the compounds, such as synergistic and addictive, leading to a final response (Tak & Isman, 2015; Melo et al., 2020).

The choice of a host plant to oviposition is mediated by a complex of stimuli and responses, that probably occurs in the sensory system, where receptors will recognize the plants metabolites and give a response that will reflect in choice of oviposition and/or the number of eggs oviposited by the females (Navarro-Silva et al., 2009; Städler & Reifenrath, 2008). The correct host choice for oviposition is of great importance for insect fitness, since this will impact the survival and, consequently, the population growth (Mayhew, 2001).

The treatment of *L. coffeella* eggs with the *V. curassavica* EO had no effect on their development. This result is probably due to the eggs protection granted by a lipid or waxy layer inside the chorion, present in lepidopterans eggs. This layer involves the embryonic membrane, which may retain products with ovicidal action protecting the embryo (Smith & Salkeld, 1966).

An alternative hypothesis could be related to the insects' eggs age and the EO volatility. Older eggs could have a lower quantity of wax, which permit that a higher amount of the EO enters in the egg through the micropyle in the chorion (Smith & Salkeld, 1966; Hilker & Meiners, 2008). In this time frame is when the cuticle is been formed, so the larger amount of toxic chemicals could affect that process, leading to an ovicidal effect (Krinski & Foerster, 2016). The eggs utilized in our tests were at maximum 48 hours old, a relatively young egg considering that the egg phase in *L. coffeella* lasts about five days (Katiyar & Ferrer, 1968). It's possible that the diffusion of oxygen through the chorion in younger eggs is lower than in older eggs, due to the requirement of a less amount of oxygen in this stage (Salkeld & Potter, 1953; Krinski & Foerster, 2016). This way younger eggs will be less affected by the EO, since its high volatility will lead to a rapidly degradation to the ambient, consequently lower amounts of the EOs compounds will diffuse into the chorion egg. Taking into consideration that in coffee crops we can find younger and older eggs at the same time, based on our hypothesis, the older eggs could be affected by *V. curassavica* EO, suppressing the pest population.

Mines of *L. coffeella* treated with *V. curassavica oil* developed normally till adult stage. That could be an indication that *V. curassavica* EO lacks translaminar activity and thus is not able to affect the *L. coffeella* larvae. The feeding habit of *L. coffeella* larvae (i.e., penetrating the coffee leaf and feeding on the palisade parenchyma) creates a completely enclosed space for its development, protected from the external environment (Stephenson & Scott, 1992; Dantas et al., 2021). This way to control the *L. coffeella* inside the mines it is necessary a translaminar or systemic effect (Venzon et al., 2005).

EOs are a promising alternative source for pest control. In recent years the number of studies regarding the insecticide activity of EOs has increased (Isman, 2020). This could be explained by the low persistence in the environment, resulting in less chance of resistant population selection, reduced risk of contamination and low toxicity to non-target organisms (Hincapié et al., 2008; Veronez et al., 2012; Isman, 2020). In conclusion, our results indicate that *V. curassavica* EO is promising to be used in the management of *L. coffeella* in coffee crops due to the reduction of *L. coffeella* oviposition, which could lead to lower populations of the pest, reducing this way the damage levels.

References

Abdelgaleil, S. A. M., Badawy, M. E. I., Mahmoud, N. F., & Marei, A. E. S. M. (2019). Acaricidal activity, biochemical effects and molecular docking of some monoterpenes against two-spotted spider mite (*Tetranychus urticae* Koch). *Pesticide Biochemistry and Physiology*, 156, 105–115. https://doi.org/10.1016/j.pestbp.2019.02.006

Ali, A., Tabanca, N., Amin, E., Demirci, B., & Khan, I. A. (2016). Chemical composition and biting deterrent activity of essential oil of *Tagetes patula* (marigold) against *Aedes aegypti*. *Natural Product Communications*, 11(10), 1535–1538. https://doi.org/10.1177/1934578X1601101028

Andrade, F. P., Venzon, M., das Dôres, R. G. R., Franzin, M. L., Martins, E. F., de Araújo, G. J., & Fonseca, M. C. M. (2021). Toxicity of *Varronia curassavica* Jacq. Essential Oil to Two Arthropod Pests and Their Natural Enemy. *Neotropical Entomology*, 50(5), 835–845. https://doi.org/10.1007/S13744-021-00906-X

Ashour, M., Wink, M., & Gershenzon, J. (2018). Biochemistry of Terpenoids: Monoterpenes, Sesquiterpenes and Diterpenes. *Annual plant reviews volume 40: biochemistry of plant secondary metabolism*, 258-303. https://doi.org/10.1002/9781119312994.apr0427

Bedini, S., Flamini, G., Girardi, J., Cosci, F., & Conti, B. (2015). Not just for beer: evaluation of spent hops (*Humulus lupulus* L.) as a source of eco-friendly repellents for insect pests of stored foods. *Journal of Pest Science, 88*(3), 583–592. https://doi.org/10.1007/S10340-015-0647-1

Benelli, G., Pavela, R., Petrelli, R., Cappellacci, L., Santini, G., Fiorini, D., Sut, S., Dall'Acqua, S., Canale, A., & Maggi, F. (2018). The essential oil from industrial hemp (*Cannabis sativa* L.) by-products as an effective tool for insect pest management in organic crops. *Industrial Crops and Products*, 122, 308–315. https://doi.org/10.1016/J.INDCROP.2018.05.032

Campos, E. V. R., Proença, P. L. F., Oliveira, J. L., Bakshi, M., Abhilash, P. C., & Fraceto, L. F. (2019). Use of botanical insecticides for sustainable agriculture: Future perspectives. *Ecological Indicators,* 105, 483-495. https://doi.org/10.1016/j.ecolind.2018.04.038

Carvalho Jr., P. M., Rodrigues, R. F. O., Sawaya, A. C. H. F., Marques, M. O. M., & Shimizu, M. T. (2004). Chemical composition and antimicrobial activity of the essential oil of *Cordia verbenacea* DC. *Journal of Ethnopharmacology,* 95(2-3), 297-301. https://doi.org/10.1016/j.jep.2004.07.028

Dantas, J., Motta, I. O., Vidal, L. A., Nascimento, E. F., Bilio, J., Pupe, J. M., Veiga, A., Carvalho, C., Lopes, R. B., Rocha, T. L., Silva, L. P., Pujol-Luz, J. R., & Albuquerque, É. V. (2021). A comprehensive review of the coffee leaf miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) - a major pest for the coffee crop in Brazil and others neotropical countries. *Insects*, 12(12), 1130.

David-Rueda, G., Constantino, C. L. M., Montoya, E. C., Ortega, M. O. E., Gil, Z. N., & Benavides-Machado, P. (2016). Diagnóstico de *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) y sus parasitoides en el departamento de Antioquia, Colombia. *Revista Colombiana de Entomologia,* 42(1), 4–11. https://doi.org/10.25100/socolen.v42i1.6662

Ecole, C. C., Silva, R. A., Louzada, J. N., Moraes, J. C., Barbosa, L. R., & Ambrogi, B. G. (2002). Predação de ovos, larvas e pupas do bicho-mineiro-do-cafeeiro, *Leucoptera coffeella* (Guérin-Menèville & Perrottet, 1842) (Lepidoptera: Lyonetiidae) por *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae). *Ciências e Agrotecnologia,* 26, 318-324.

Feijó, E. V. R. S., Oliveira, R. A. D., & Costa, L. C. B. (2014). Light affects *Varronia curassavica* essential oil yield by increasing trichomes frequency. *Revista Brasileira de Farmacognosia*, 24(5), 516-523. https://doi.org/10.1016/j.bjp.2014.10.005

Fernandes, F. L., Mantovani, E. C., Neto, H. B., & Nunes, V. D. V. (2009). Efeitos de variáveis ambientais, irrigação e vespas predadoras sobre *Leucoptera coffeella* (Guérin-Méneville) (Lepidoptera: Lyonetiidae) no cafeeiro. *Neotropical Entomology,* 38(3), 410-417. Doi: 10.1590/S1519-566X2009000300018.

Giraldo-Jaramillo, M., Garcia-Gonzalez, J., & Rugno, J. B. (2019). Fertility Life Table of *Leucoptera coffeella* (Guérin-Mèneville) (Lepidoptera: Lyonetiidae) at Seven Temperatures in Coffee. *American Journal of Entomology*, *3*(4), 70-76. https://doi.org/10.11648/J.AJE.20190304.12

Hilker, M., & Meiners, T. (2008). *Chemoecology of insect eggs and egg deposition*. John Wiley & Sons.

Hincapié, C. A., López, G. E., & Torres, R. (2008). Comparison and Characterization of Garlic (*Allium sativum* L.) Bulbs Extracts and Their Effect on Mortality and Repellency of *Tetranychus urticae* Koch (Acari: Tetranychidae). *Chilean Journal of Agricultural Research*, 68(4), 317-327. https://doi.org/10.4067/s0718-58392008000400001

Isman, M. B. (2014). Botanical insecticides: A global perspective. In: *Biopesticides: State of the art and future opportunities,* 21-30. https://doi.org/10.1021/bk-2014-1172.ch002

Isman, M. B. (2020). Botanical insecticides in the twenty-first century - fulfilling their promise?. *Annual Review of Entomology*, 65, 233-249.

Katiyar, K.P., & Ferrer, F. (1968). Rearing Technique, Biology and Sterilization of the Coffee Leaf Miner, *Leucoptera coffeella* Guer. (Lepidoptera: Lyonetiidae). *International Atomic Energy Agency: Vienna IAEA*, 165–175.

Krinski, D., & Foerster, L. A. (2016). Toxicity of essential oils from leaves of Piperaceae species in rice stalk stink bug eggs, *Tibraca limbativentris* (Hemiptera: Pentatomidae). *Ciência e Agrotecnologia*, 40, 676-687.

Kost, C. & Heil, M. (2006). Herbivore-induced plant volatiles induce an indirect defence in neighbouring plants. *Journal of Ecology*, 94(3), 619–628.

Leite, S. A., dos Santos, M. P., Resende-Silva, G. A., da Costa, D. R., Moreira, A. A., Lemos, O. L., Guedes, R. N. C., & Castellani, M. A. (2020). Area-Wide Survey of Chlorantraniliprole Resistance and Control Failure Likelihood of the Neotropical Coffee Leaf Miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae). *Journal of Economic Entomology,* 113(3), 1399–1410. https://doi.org/10.1093/jee/toaa017

Lenth, R.V. (2016). Least-Squares Means: The R Package lsmeans. *Journal of Statistical Software*, 69, 1-33.

Liu, Y., Xue, M., Zhang, Q., Zhou, F., & Wei, J. (2010). Toxicity of β-caryophyllene from *Vitex negundo* (Lamiales: Verbenaceae) to *Aphis gossypii* Glover (Homoptera: Aphididae) and its action mechanism. *Acta Entomologica Sinica*, *53*(4), 396–404.

Lorenzi, H., & Matos, F. J. A. (2008). Plantas medicinais no Brasil: nativas e exóticas, 2nd ed. Nova Odessa: Instituto Plantarum, 544p.

Martins, E. F. (2021). Conservation biological control of coffee leaf miner: Role of green lacewings and parasitoids. Tese de Doutorado, Universidade Federal de Viçosa, Viçosa.

Matias, E. F. F., Santos, K. K. A., Falcão-Silva, V. S., Siqueira-Júnior, J. P., Costa, J. G. M., & Coutinho, H. D. M. (2013). Modulation of the norfloxacin resistance in *Staphylococcus aureus* by *Cordia verbenaceae* DC. *The Indian Journal of Medical Research*, 137(1), 178.

Mayhew, P. J. (2001). Herbivore host choice and optimal bad motherhood. *Trends in Ecology & Evolution*, 16(4), 165–167. https://doi.org/10.1016/S0169-5347(00)02099-1

Mazerolle, M. J., Linden, D. (2019). Model Selection and Multimodel Inference Based on (Q)AIC(c). R-CRAN. 233p

Melo, C. R., Oliveira B. M. S., Santos, A. C. C., Silva, J. E., Ribeiro, G.T., Blank, A.F., Araujo, A. P. A, & Bacci, L. (2020). Synergistic effect of aromatic plant essential oils on the ant *Acromyrmex balzani* (Hymenoptera: Formicidae) and antifungal activity on its symbiotic fungus *Leucoagaricus gongylophorus* (Agaricales: Agaricaceae). *Environmental Science and Pollution Research,* 27(14), 17303–17313. https://doi.org/10.1007/s11356-020-08170-z

Motta, I. O., Dantas, J., Vidal, L., Bílio, J., Pujol-Luz, J. R., & Albuquerque, É. V. (2021). The coffee leaf miner, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae): identification of the larval instars and description of male and female genitalia. *Revista Brasileira de Entomologia*, 65.

Navarro-Silva, M. A., Marques, F. A., & Duque L, J. E. (2009). Review of semiochemicals that mediate the oviposition of mosquitoes: a possible sustainable tool for the control and monitoring of Culicidae. *Revista Brasileira de Entomologia*, 53(1), 1–6. https://doi.org/10.1590/S0085-56262009000100002

Pantoja-Gomez, L. M., Corrêa, A. S., de Oliveira, L. O., & Guedes, R. N. C. (2019). Common Origin of Brazilian and Colombian Populations of the Neotropical Coffee Leaf Miner, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae). *Journal of Economic Entomology*, 112(2), 924–931. https://doi.org/10.1093/JEE/TOY416

Parra, J. R. P. (1985). Biologia comparada de *Perileucoptera coffeella* (Guérin-Méneville, 1842) (Lepidoptera:Lyonetiidae) visando ao seu zoneamento ecológico no Estado de São Paulo. *Revista Brasileira de Entomologia*, 29(1), 45–76.

Pavela, R. (2015). Essential oils for the development of eco-friendly mosquito larvicides: A review. *Industrial Crops and Products*, 76, 174–187. https://doi.org/10.1016/J.INDCROP.2015.06.050

Pereira, E. J. G., Picanço, M. C., Bacci, L., Crespo, A. L. B., & Guedes, R. N. C. (2007). Seasonal mortality factors of the coffee leafminer, *Leucoptera coffeella*. *Bulletin of Entomological Research*, 97(4), 421–432. https://doi.org/10.1017/S0007485307005202

R Development Core Team (2022). A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reis, P. R., & Souza, J. C. (1996). Manejo integrado do bicho-mineiro, *Perileucoptera coffeella* (Guérin-Mèneville) (Lepidoptera: Lyonetiidae) e seus reflexos na produção de café. *Anais da Sociedade Entomológica do Brasil* 25(1), 77–82.

Reis, P. R., Souza, J. C., & Venzon, M. (2002). Manejo ecológico das principais pragas do cafeeiro. *Informe Agropecuário,* 23(214/215), 83–99.

Salkeld, E. H., & Potter, C. (1953). The effect of the age and stage of development of insect eggs on their resistance to insecticides. *Bulletin of Entomological Research*, 44(3), 527-580.

Souza, J. C.; & Reis, P. R. (1992). Bicho mineiro: Biologia, danos e manejo integrado. Boletim técnico, 37. Belo Horizonte, Epamig. 28p.

Souza, J. C., Reis, P. R., & Rigitano, R. L. O. (1998). Bicho-mineiro do cafeeiro: biologia, danos e manejo integrado. Belo Horizonte, Brasil: EPAMIG, 48 p.

Sharma, H. C. (2008) Biotechnological approaches for pest management and ecological sustainability. Florida, CRC Press, 546p.

Smith, E. H., & Salkeld, E. H. (1966). The use and action of ovicides. *Annual review of Entomology*, 11, 331-368.

Städler, E., & Reifenrath, K. (2008). Glucosinolates on the leaf surface perceived by insect herbivores: review of ambiguous results and new investigations. *Phytochemistry Reviews* 8(1), 207–225. https://doi.org/10.1007/S11101-008-9108-2

Stephenson, J., & Scott, A. C. (1992). The geological history of insect-related plant damage. *Terra Nova*, 4(5), 542-552.

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

Venzon, M., Rosado, M. C., Fadini, M. A. M., Ciociola Jr, A. I., & Pallini, A. (2005). The potential of NeemAzal for the control of coffee leaf pests. *Crop protection*, 24(3), 213-219.

Veronez, B., Sato, M. E., & Nicastro, R. L. (2012). Toxicidade de compostos sintéticos e naturais sobre *Tetranychus urticae* e o predador *Phytoseiulus macropilis*. *Pesquisa Agropecuária Brasileira*, 47(4), 511-518. https://doi.org/10.1590/S0100-204X2012000400006

Wink, M. (2018). Plant secondary metabolites modulate insect behavior-steps towards addiction?. *Frontiers in Physiology*, 9, 364.

Yajima, A., Yamaguchi, A., Saitou, F., Nukada, T., & Yabuta, G. (2007). Asymmetric synthesis of abietane diterpenoids via B-alkyl Suzuki-Miyaura coupling. Formal total asymmetric synthesis of 12-deoxyroyleanone and cryptoquinone. *Tetrahedron*, 63(5), 1080-1084. https://doi.org/10.1016/j.tet.2006.11.072

Tables

Table 1. Chemical composition of the essential oil of *Varronia curassavica*. Information provided by the supplier Legeé Óleos Essenciais (Estiva Gerbi, São Paulo, Brazil).

No	Compound	Relative composition (%)
1	α-Tujene	0.57
2	α-Pinene	38.82
3	Campheno	0.15
4	Sabinene	0.56
5	β-Pinene	0.59
6	β-Myrcene	0.34
7	ρ-Cymene	0.13
8	β-Tujene	1.38
9	Eucalyptol	1.06
10	Bornyl Acetate	0.59
11	δ-Elemene	4.80
12	α-Cubebene	0.39
13	Copaene	0.46
14	β-Bourbonene	0.19
15	β-Elemene	2.26
16	7-epi-Sesquithujene	0.98
17	α-Bergamotene	0.86
18	β-Caryophyllene	21.84
19	γ-Elemene	1.29
20	Humulene	4.49
21	Alloaromadendrene	4.85
22	β-Cubebene	2.14
23	α-Zingibereneo	1.62
24	Bicyclogermacrene	1.10
25	β-Bisabolene	2.39
26	δ-Cadinene	2.14
27	γ-Bisabolene	0.55
28	Germacrene B	0.12
29	Caryophyllene oxide	0.42
30	Isospathulenol	0.82
31	α-Santalol	1.01

Figures

Figure 1. Cylinder accoupled to the plastic pots to test the oviposition of *Leucoptera coffeella* on coffee leaves treated with *Varronia curassavica essential* oil (Photo: Fernanda Pereira Andrade)

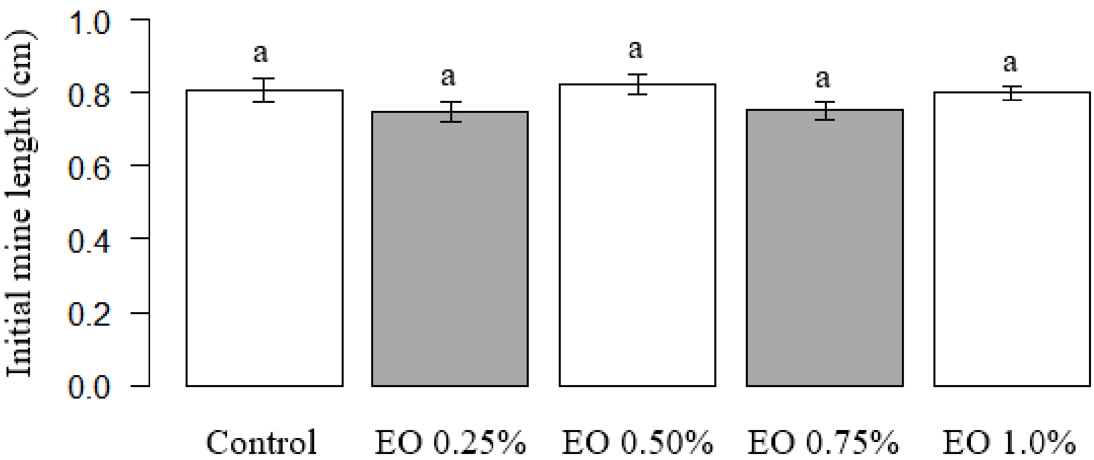

Figure 2. Initial size of *Leucoptera coffeella* mines measured by the maximum length treated with different concentrations of *Varronia curassavica* essential oil (EO) and control (distilled

water plus Tween® 80 (0.05%) (F = 1.635, p = 0.169, DF = 4). Bars with same letters have no statistical differences.

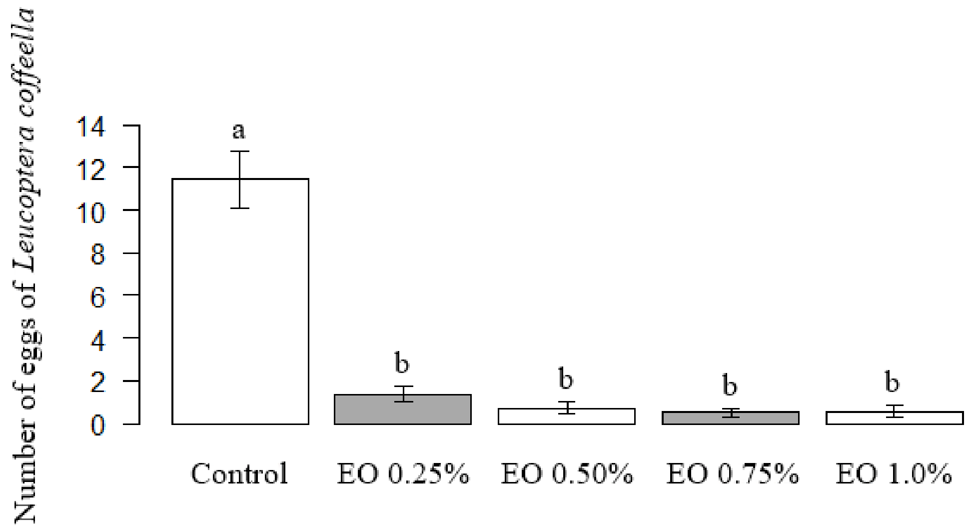

Figure 3. Number of *Leucoptera coffeella* eggs per coffee branch containing three leaves treated with different concentrations of *Varronia curassavica* essential oil (EO) and control (distilled water plus Tween® 80 (0.05%) (χ^2 = 87.248, DF = 4, p < 0.001). Different letters on the bars represent statistical differences.

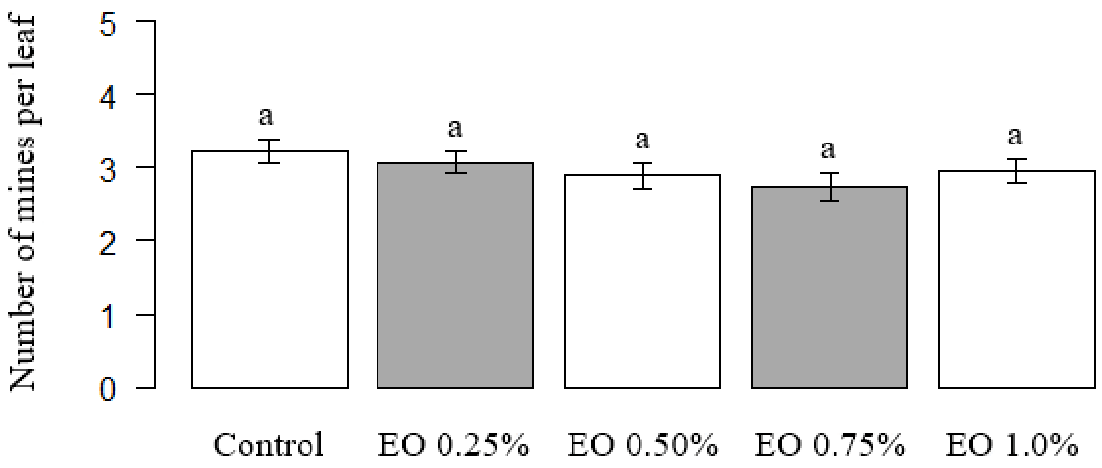

Figure 4. Number of *Leucoptera coffeella* mines originating from eggs on coffee leaves treated with the different concentrations of *Varronia curassavica* essential oil (EO) and control (distilled water plus Tween® 80 (0.05%) (χ^2 = 1.208, DF= 4, p = 0,876). Bars with same letters have no statistical differences.

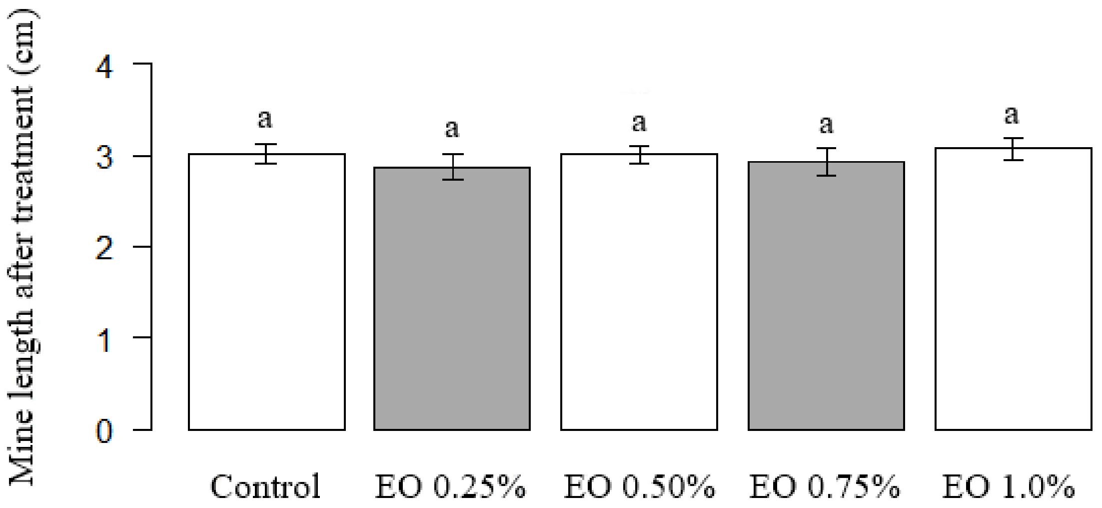

Figure 5. Size of *Leucoptera coffeella* mines measured by the maximum length after treatment with different concentrations of *Varronia curassavica* essential oil (EO) and control (distilled water plus Tween® 80 (0.05%) (F = 0.407, p = 0.803, DF = 4). Bars with same letters have no statistical differences.

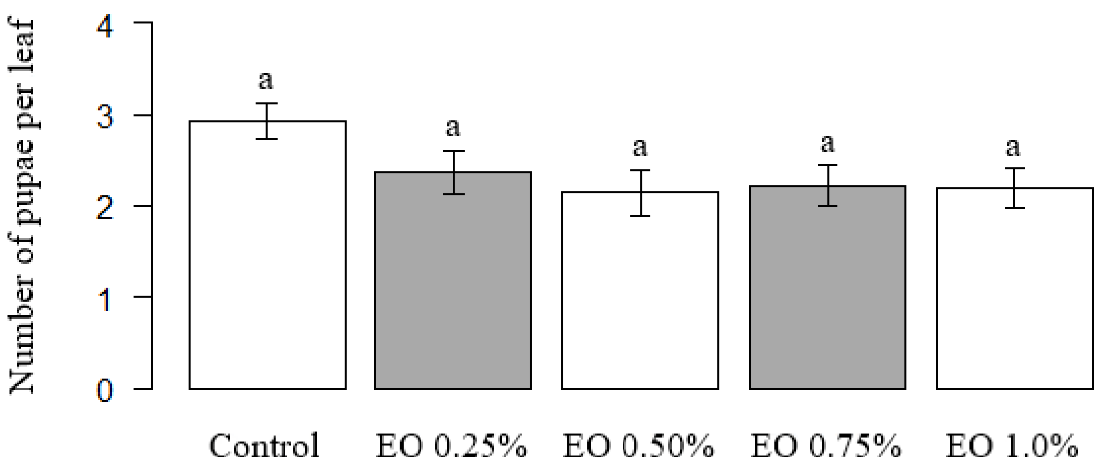

Figure 6. Number of *Leucoptera coffeella* pupae originating from mines on coffee leaves treated with the different concentrations of *Varronia curassavica* essential oil (EO) and control (distilled water plus Tween® 80 (0.05%) ($\chi^2 = 4.506$, DF = 4, p = 0.341). Bars with same letters have no statistical differences.

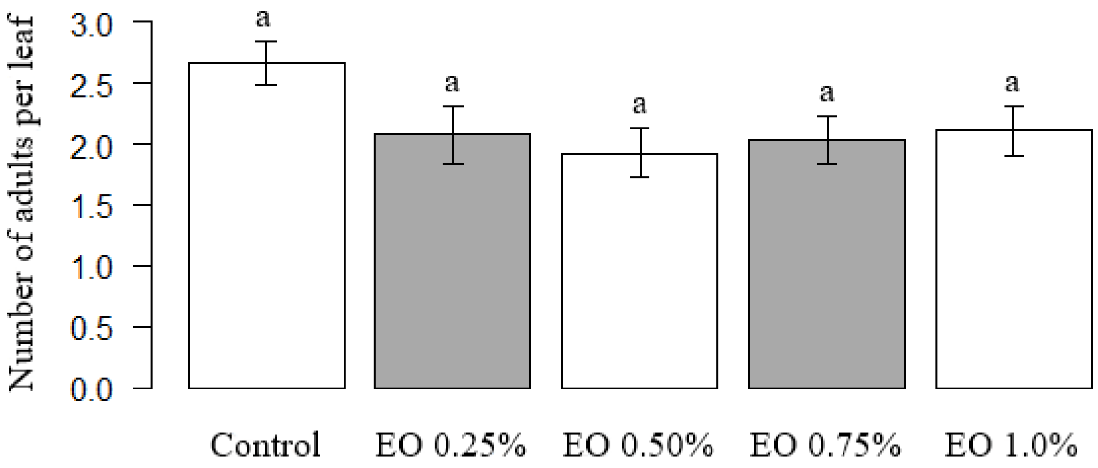

Figure 7. Number of *Leucoptera coffeella* pupae originating from mines on coffee leaves treated with the different concentrations of *Varronia curassavica* essential oil (EO) and control (distilled water plus Tween® 80 (0.05%) ($\chi^2 = 4.010$, DF = 4, p = 0.404). Bars with same letters have no statistical differences.

CHAPTER III

Lethal and sublethal effects of *Varronia curassavica* Jacq. (Boraginaceae) essential oil on the coffee berry borer

ABSTRACT

The coffee berry borer, *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae, Scolytinae) is the most damaging insect pest of coffee worldwide. Adults and larvae live inside coffee berries, where they feed on the endosperm reducing the quality and the economic value of the coffee grains. Due to its cryptic habit of living inside the fruit, the control of coffee berry borer is difficult, but the female transit period of coffee berry borer in the crop enables the control by pesticides, which are still the main strategy of management of this pest. However, the chemical control for the use of these products presents several issues for environment and humans. The use of essential oils (EOs) could be an environmentally safer alternative to control of the coffee berry borer. *Varronia curassavica* is a medicinal aromatic species that produces an essential oil (EO) with a range of biological activities. In this work we evaluate lethal and sublethal effects of *V. curassavica* EO on *H. hampei*. To the mortality test, we used five treatments (0.2, 0.4, 0.6, 0.8, and 1.0 % EO concentration) and the control (distilled water and Tween® 80 (0.05%)). We exposed the *H. hampei* females to a surface contaminated with the tested solutions for two days. After this, the number of dead insects were counted. To evaluate the sublethal effects, we performed repellency and a mobility test using the lower concentration of *V. curassavica* EO (0.2) and the control (distilled water and Tween® 80 (0.05%)). The *V. curassavica* EO was toxic to *H. hampei* in all concentrations tested. There was no repellence effect, but the mobility of *H. hampei* was affected by the *V. curassavica* EO. Thus, our results suggest that *V. curassavica* EO has potential to be used in the management of *H. hampei* in coffee crops.

Keywords: Biopesticide, *Hypothenemus hampei*, *Coffea sp.,* secondary metabolites.

1. INTRODUCTION

The coffee berry borer, *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae, Scolytinae) native from Central Africa, is the most damaging insect pest of coffee worldwide (Reis et al., 2002; Vega et al., 2009; Cure et al., 2020). Females of this pest enters the coffee berries building tunnels to oviposit and feed on the endosperm and their offspring will also feed and develops inside this berry (Reis et al., 2002; Vega et al., 2015). Males of *H. hampei* can't fly, so they will remain in the coffee berries for mating (Reis et al., 2005). Their life cycle can vary between 28 to 34 days, with juvenile stages lasting around 4 (egg), 15 (larva) and 7 (pupa) days, respectively, considering a 27° C temperature (Damon, 2000). The *H. hampei* infestation negatively affects the crop causing senescence of berries, significant losses in yield and quality (Jaramillo et al., 2006; Vega et al. 2015; Infante, 2018). Annually in Brazil, the losses caused by coffee berry borer are estimated at between US$215 and 358 million (Oliveira et al., 2013).

Due to its cryptic habit of living inside the fruit, the control of coffee berry borer is difficult, but the female transit period (80 and 90 days after flowering), that coffee berry borer females leave the fruit to search for food, enables the control by pesticides (Vega et al., 2009; Celestino et al., 2016). Chemical control is the main management strategy used to *H. hampei* however the use of these products presents several issues such as an insufficient number of active ingredients that leads to selection of resistant strains, elimination of non-target populations and contamination of environment and human beings (Vega et al., 2015; Infante, 2018; Campos et al., 2019; Johnson et al., 2020).

The search for safer options to control pest is increasing, with notably attention to products of plants secondary metabolism, such as essential oils (EOs) (Regnault-Roger et al., 2012; Pavela & Sedlák, 2018; Isman, 2020). The insecticide effect of EOs against pests of agricultural importance has been documented (Isman, 2014). For *H. hampei* there are reports of insecticidal activity of EOs of *Aleollanthus pubescens* Benth (Lamiaceae) (Mawussi et al., 2009), *Ocimum canum* Sims (Lamiaceae) (Mawussi et al., 2012), *Schinus terebinthifolius* Raddi

(Anacardiaceae) (Santos et al., 2013), *Corymbia* and *Eucalyptus* species (Myrtaceae) (Reyes et al., 2019), and *Pogostemon cablin* (Benth.) Blanco (Lamiaceae) (Santos et al., 2022). The insecticidal activity of *Varronia curassavica* Jacq. (Boraginaceae) EO against crop pests has been previously reported (Andrade et al., 2021), however its use to control the coffee berry borer is still unknown.

Varronia curassavica, is a medicinal plant species, native to Brazil, traditionally used in popular medicine as anti-inflammatory drug (Lorenzi & Matos, 2008; Matias et al., 2013). It's a shrub that can reach up to 2m high with sessile leaves, dense inflorescence with white flowers and red fruits (Marques et al., 2019). The surface of its leaves has glandular trichomes, where the EO is produced and stored (Feijó et al., 2014). Among the several chemical compounds in *V. curassavica* EO, the majors usually are α-pinene and trans-caryophyllene (Carvalho Jr. et al., 2004). These compounds are reported to their insecticidal activities (Chen, 2008).

Thus, we test the hypothesis that *V. curassavica* EO can control the coffee berry borer. For testing it, we evaluated the lethal and sublethal effects of *V. curassavica* EO against *H. hampei* through mortality, repellency, and mobility bioassays.

2. MATERIAL AND METHODS

2.1 Essential oil

Varronia curassavica EO used in the tests was purchased from Legeé Óleos Essencias (Estiva Gerbi, São Paulo, Brazil). According to information provided by the supplier, the extraction of the EO was made by hydrodistillation of the leaves and its main constituents are α-pinene (38.82%), β-caryophyllene (21.84%), and aloaromadendrene (4.85%) (Table 1).

2.2 Treatments

A 1% stock solution of essential oil (w/v) was prepared in distilled water plus Tween® 80 (0.05%). This stock solution was then diluted, also with distilled water plus Tween® 80 (0.05%), in the other concentrations. Concentrations used in the tests was chosen based in previous tests.

2.3 *Hypothenemus hampei* rearing

To establish the rearing of *H. hampei*, we collected coffee berries infested with the coffee berry border in an experimental area located on the campus of the Universidade Federal de Viçosa, Viçosa – MG. The coffee berries were immersed in 0.5% sodium hypochlorite solution for 5 min. to avoid contamination by microorganisms, and then rinsed with water. Afterwards the coffee berries were left to dry at room temperature for 24 hours. Then the coffee berries were stored in plastic boxes in complete darkness. The rearing was kept at a temperature of 25 ± 2°C, relative humidity of 70 ± 10%. Insects used in the tests were manually removed from the berries.

2.4 Mortality bioassay

To the *H. hampei* mortality test we used five treatments (0.2, 0.4, 0.6, 0.8 and 1.0 % *V. curassavica* EO concentration) and the control (distilled water and Tween® 80 (0.05%)). We applied 1.0 mL of the tested solutions in a filter paper disk placed in a Petri dish (9.0 cm diameter) and left then to dry at room temperature for five minutes. After the five minutes, we released five *H. hampei* females in the center of the Petri dish, that were then covered with plastic film and maintained at a temperature of 25 ± 2°C, relative humidity of 70 ± 10% in complete darkness for 48h. Females were differentiated of males by the size, as the males are visually smaller than the females (Reis et al., 2002). After this period, the number of dead insects were counted. Were considered dead all the insects that didn't show any movement when stimulated with a fine brush. The test was carried out in a completely randomized design with six treatments and 30 replicates, with each one consisting of a Petri dish.

2.5 Repellency Effect

To the EO repellency against *H. hampei* test we used one *V. curassavica* EO concentration (0.2 %) and the control (distilled water and Tween® 80 (0.05%)). Green coffee berries not infested with *H. hampei* were immersed in the tested solutions for one minute. Petri dishes (9.0 cm diameter) covered with filter paper disks were marked with a central line, and in one side of the plate was placed three coffee berries treated with the *V. curassavica* EO and in the other side three coffee berries of the control group (Fig. 1). Five *H. hampei* females were released in the center of each plate. Females were differentiated of males by size, as the males are visually smaller than the females (Reis et al., 2002). The Petri dishes were covered with PVC film and was evaluated the number of insects in treated and untreated halves after 15 min. of exposure. Repellency index (RI) was calculated as follows: $RI = [(C-T)/(C + T)] \times 100$, where C and T are the number of insects in the untreated and treated half, respectively (Villalobos & Robledo, 1998). The experimental design was completely randomized with 27 replicates, with each one consisting of a Petri dish with five *H. hampei* females.

2.6 Mobility bioassay

To the *H. hampei* mobility test we used one *V. curassavica* EO concentration (0.2 %) and the control (distilled water and Tween® 80 (0.05%)). We applied 1.0 mL of the tested solutions in a filter paper disk placed in a Petri dish (9.0 cm diameter) and left then to dry at room temperature for five minutes. After the five minutes, we released one *H. hampei* female in the center of the Petri dish, which were then covered with plastic film and maintained at a temperature of 25 ± 2°C, relative humidity of 70 ± 10% in complete darkness for 48h. We used 28 replicates per treatment, in which each replicate consisted of one *H. hampei* female in a Petri dish. After the 48h, each female was kept at room temperature for 1 h for acclimatization, transferred to a Petri dish (7.3 cm diameter), and recorded for 10 min using an automated video tracking system equipped with a charge-coupled device camera (ViewPoint Life Sciences, Montreal, Canada) for the calculation of the displacement (cm) and walking velocity (cm/s).

2.7 Data analyses

To the *H. hampei* mortality analysis we used a model simplification process by 'AICcmodavg' package (Mazerolle & Linden, 2019) and we determined the minimum adequate model(s) by comparing Akaike Information Criterion corrected (AICc) values. We used Generalized linear models (GLMs) with a negative binomial error distribution analyzed by χ^2 test of Analysis of Variance (ANOVA) and a contrast analysis using the lsmeans function from lsmeans package v.2.30-0 (Lenth, 2016) to identify the levels in which the differences occurred. For repellency and mobility, we used Analysis of Deviance and pairwise comparisons performed by emmeans. All analyses were performed using R 4.2.0 software (R Development Core Team, 2022).

3. RESULTS

3.1 Mortality bioassay

We found that the different concentrations of *V. curassavica* EO significantly affected the mortality rates of *H. hampei* ($\chi^2 = 272.42$, DF= 5, $p < 0.001$). The mortality proportion increased according to the increase in the *V. curassavica* EO concentrations, reaching 100% of mortality in the highest concentration (Fig. 2).

3.2 Repellency bioassay

There was no significant difference in the percentage of *H. hampei* females that moved to the area with coffee berries treated with *V. curassavica* EO and the area treated with control ($F = 0.755$, $p = 0.389$, DF = 1, fig. 3). The calculated repellence index (RI) was 7,56.

3.3 Mobility bioassay

The displacement was higher for *H. hampei* females treated with *V. curassavica* EO than in the control (F = 7.831, p = < 0.001, DF = 1, fig. 4). The walking velocity was similar for *H. hampei* females treated with *V. curassavica* EO and the control (F = 0.501, p = 0.482, DF = 1, fig. 5).

4. DISCUSSION

This study suggests that *V. currassavica* EO could be an alternative control of *H. hampei* in coffee crops. The *V. curassavica* EO was toxic to the coffee berry borer, in some level, in all the tested concentrations. The EOs can affect the insects in diverse forms: they can interact with the insect integument, affect the digestive and neurological enzymes, and cause physiological and/or behavioral effects (Koul et al., 2013; Campos et al., 2019). Their solubility in lipids allows them to penetrate in the lipoprotein matrix of the insect cell membrane (Bakkali et al., 2008; Satyan et al., 2009). The major chemical group of EOs compounds, the terpenoids, are directly connected with their modes of action (Isman, 2014). Terpenoids can act as growth inhibitors, reducing reproductive capacity, suppressing feeding and direct toxicity (Viegas, 2003; Tsukamoto et al., 2005). Monoterpenes are substances with high potential for toxic interference in basic biochemical processes (Ashour et al., 2018; Abdelgaleil et al., 2019). The major constituent compound of *V. curassavica* EO in this study, α-pinene, is a monoterpene that showed different rates of mortality for different insects at various stages of development (Viegas, 2003; Iori et al., 2005).

However, the synergy between all the compounds in an EO has an important function to the level of insecticidal efficacy (Pavela, 2015). Reyes et al. (2019) reported the importance of that synergetic effect to the mortality of the coffee berry borer working with the *Eucalyptus resinifera* (Myrtaceae) EO's major compounds, one of them the α-pinene, showing that the mortality rates to each compound was lower than the rate to them together. The synergy between constituents enhances the penetration through insects (Tak & Isman, 2015, 2017).

The *V. curassavica* EO in the tested concentration wasn't efficient to repel *H. hampei*. According to Villalobos & Robledo (1998), to be considered a repelling substance the RI should be higher than 50, what was not observed in this work. It's possible that at higher concentrations the results could be different. The EO of *V. curassavica* already was reported for its repellence effect in *Dorymyrmex thoracius* Gallardo (Formicidae: Dolichoderinae) (Oliveira et al., 2019). In addition to that, some of the *V. curassavica* compounds, such as β-caryophillene, is well-described as a repellent (Liu et al., 2010; Pavela, 2015; Ali et al., 2016; Benelli et al., 2018).

The displacement of *H. hampei* was affected by the *V. curassavica* EO, but the velocity was not affected. There are reports of natural compounds causing symptoms of neurotoxic activity in insects, such as hyperactivity and tremors (Gaire et al., 2019). The hyperactivity in insects is associated to compounds that inhibit the acetylcholinesterase (AChE), which leads to a higher displacement and irritability, because this enzyme is related to the general coordination in the neuromuscular system (Abdelgaleil et al., 2009; Cordeiro et al., 2010; Kang et al., 2013;). The α-pinene, as others terpenoids, has been reported to inhibit AChE activity (Viegas, 2003; Tsukamoto et al., 2005; Souza et al., 2012). That could explain the higher displacement observed in the treated insects of our study. Additionally, the contact of the insects with the EOs can alter their spatial perceptions through the olfactory or gustatory systems (Germinara et al., 2015; Martínez et al., 2018). Their spatial perceptions are responsible to modulate the functions of olfactory neurons in the sensilla and disrupt chemosensory receptors (Lee et al., 2010). Interference in this modulation can stimulate or reduce the mobility of the insects and affect their walking patterns (Plata-Rueda et al., 2018).

The EOs are potentially a sustainable and environmentally safe method of pest control. Their volatile characteristics and the natural origin of their compounds results in low persistence in the environment (Hincapié et al., 2008; Veronez et al., 2012). The risks of human toxicity are also decreased, because the EOs acts in the octopaminergic site, which inhibits or stimulates the octopamine, a neurotransmitter found only in invertebrates, representing low toxicity to mammals (Arun et al., 2009; Campos et al., 2019; Mossa et al., 2019). Therefore, our findings suggest that the EO of *V. curassavica* is a promising and safe strategy to be adopted by coffee farmers for the *H. hampei* management in coffee crops

References

Abdelgaleil, S. A. M., Mohamed, M. I. E., Badawy, M. E. I., & El-arami, S. A. A. (2009). Fumigant and contact toxicities of monoterpenes to *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) and their inhibitory effects on acetylcholinesterase activity. *Journal of Chemical Ecology*, 35(5), 518–525.

Abdelgaleil, S. A. M., Badawy, M. E. I., Mahmoud, N. F., & Marei, A. E. S. M. (2019). Acaricidal activity, biochemical effects and molecular docking of some monoterpenes against two-spotted spider mite (*Tetranychus urticae* Koch). *Pesticide Biochemistry and Physiology*, 156, 105–115. https://doi.org/10.1016/j.pestbp.2019.02.006

Ali, A., Tabanca, N., Amin, E., Demirci, B., & Khan, I. A. (2016). Chemical composition and biting deterrent activity of essential oil of *Tagetes patula* (marigold) against *Aedes aegypti*. *Natural Product Communications*, 11(10), 1535–1538. https://doi.org/10.1177/1934578X1601101028

Andrade, F. P., Venzon, M., das Dôres, R. G. R., Franzin, M. L., Martins, E. F., de Araújo, G. J., & Fonseca, M. C. M. (2021). Toxicity of *Varronia curassavica* Jacq. Essential Oil to Two Arthropod Pests and Their Natural Enemy. *Neotropical Entomology*, 50(5), 835–845. https://doi.org/10.1007/S13744-021-00906-X

Arun, K. T., Shikha, U., & Mantu, B. (2009). A review on prospects of essential oils as biopesticide in insect-pest management. *Journal of Pharmacognosy and Phytotherapy*, 1(5), 052-063.

Ashour, M., Wink, M., & Gershenzon, J. (2018). Biochemistry of Terpenoids: Monoterpenes, Sesquiterpenes and Diterpenes. *Annual plant reviews volume 40: biochemistry of plant secondary metabolism*, 258-303. https://doi.org/10.1002/9781119312994.apr0427

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M. (2008). Biological effects of essential oils - A review. *Food and Chemical Toxicology*, 46(2), 446-475. https://doi.org/10.1016/j.fct.2007.09.106

Benelli, G., Pavela, R., Petrelli, R., Cappellacci, L., Santini, G., Fiorini, D., Sut, S., Dall'Acqua, S., Canale, A., & Maggi, F. (2018). The essential oil from industrial hemp (*Cannabis sativa* L.) by-products as an effective tool for insect pest management in organic crops. *Industrial Crops and Products*, 122, 308–315. https://doi.org/10.1016/J.INDCROP.2018.05.032

Campos, E. V. R., Proença, P. L. F., Oliveira, J. L., Bakshi, M., Abhilash, P. C., & Fraceto, L. F. (2019). Use of botanical insecticides for sustainable agriculture: Future perspectives. *Ecological Indicators,* 105, 483-495. https://doi.org/10.1016/j.ecolind.2018.04.038

Carvalho Jr., P. M., Rodrigues, R. F. O., Sawaya, A. C. H. F., Marques, M. O. M., & Shimizu, M. T. (2004). Chemical composition and antimicrobial activity of the essential oil of *Cordia verbenacea* DC. *Journal of Ethnopharmacology,* 95(2-3), 297-301. https://doi.org/10.1016/j.jep.2004.07.028

Celestino, F. N., Pratissoli, D., Machado, L. C., Santos Junior, H. J. G. D., Queiroz, V. T. D., & Mardgan, L. (2016). Control of coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) with botanical insecticides and mineral oils. *Acta Scientiarum. Agronomy*, 38, 1-8.

Chen, M. S. (2008). Inducible direct plant defense against insect herbivores: a review. *Insect Science*, 15(2), 101-114.

Cordeiro, E. M. G., Correa, A. S., Venzon, M., & Guedes, R. N. C. (2010). Insecticide survival and behavioral avoidance in the lacewings *Chrysoperla externa* and *Ceraeochrysa cubana*. *Chemosphere*, 81, 1352–1357. https://doi.org/10.1016/j.chemosphere.2010.08.021

Cure, J. R., Rodríguez, D., Gutierrez, A. P., & Ponti, L. (2020). The coffee agroecosystem: bio-economic analysis of coffee berry borer control (*Hypothenemus hampei*). *Scientific Reports,* 10(1), 1-12.

Damon, A. (2000). A review of the biology and control of the coffee berry borer, Hypothenemus hampei (Coleoptera: Scolytidae). *Bulletin of entomological research*, 90(6), 453-465.

Feijó, E. V. R. S., Oliveira, R. A. D., & Costa, L. C. B. (2014). Light affects *Varronia curassavica* essential oil yield by increasing trichomes frequency. *Revista Brasileira de Farmacognosia*, 24(5), 516-523. https://doi.org/10.1016/j.bjp.2014.10.005

Gaire, S., Scharf, M. E., & Gondhalekar, A. D. (2019). Toxicity and neurophysiological impacts of plant essential oil components on bed bugs (Cimicidae: Hemiptera). *Scientific reports*, 9(1), 1-12.

Germinara, G. S., De Cristofaro, A., & Rotundo, G. (2015). Repellents effectively disrupt the olfactory orientation of *Sitophilus granarius* to wheat kernels. *Journal of Pest Science*, 88(4), 675–684.

Hincapié, C. A., López, G. E., & Torres, R. (2008). Comparison and Characterization of Garlic (*Allium sativum* L.) Bulbs Extracts and Their Effect on Mortality and Repellency of *Tetranychus urticae* Koch (Acari: Tetranychidae). *Chilean Journal of Agricultural Research*, 68(4), 317-327. https://doi.org/10.4067/s0718-58392008000400001

Infante, F. (2018). Pest Management Strategies Against the Coffee Berry Borer (Coleoptera: Curculionidae: Scolytinae). *Journal of Agricultural and Food Chemistry*, 66(21), 5275-5280. https://doi.org/10.1021/acs.jafc.7b04875

Iori, A., Grazioli, D., Gentile, E., Marano, G., Salvatore, G. (2005). Acaricidal properties of the essential oil of *Melaleuca alternifolia* Cheel (tea tree oil) against nymphs of *Ixodes ricinus*. *Veterinary Parasitology*, 129(1-2), 173-176. https://doi.org/10.1016/j.vetpar.2004.11.035

Isman, M. B. (2014). Botanical insecticides: A global perspective. In: *Biopesticides: State of the art and future opportunities,* 21-30. https://doi.org/10.1021/bk-2014-1172.ch002

Isman, M. B. (2020). Botanical insecticides in the twenty-first century - fulfilling their promise?. *Annual Review of Entomology*, 65, 233-249.

Jaramillo, J., Borgemeister, C., & Baker, P. (2006). Coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae): searching for sustainable control strategies. *Bulletin of Entomological Research,* 96(3), 223-233.https://doi.org/10.1079/BER2006434

Johnson, M. A., Ruiz-Diaz, C. P., Manoukis, N. C., & Verle Rodrigues, J. C. (2020). Coffee berry borer (*Hypothenemus hampei*), a global pest of coffee: perspectives from historical and recent invasions, and future priorities. *Insects*, 11(12), 882.

Kang, J. S., Kim, E., Lee, S. H., Park, I. K. (2013). Inhibition of acetylcholinesterases of the pinewood nematode, *Bursaphelenchus xylophilus*, by phytochemicals from plant essential oils. *Pesticide Biochemistry and Physiology,* 105(1), 50–56.

Koul, O., Singh, R., Kaur, B., Kanda D. (2013). Comparative study on the behavioral response and acute toxicity of some essential oil compounds and their binary mixtures to larvae of

Helicoverpa armigera, *Spodoptera litura* and *Chilo partellus*. *Industrial Crops and Products,* 49, 428-436. https://doi.org/10.1016/j.indcrop.2013.05.032

Lee, Y., Kim, S. H., & Montell, C. (2010): Avoiding DEET through insect gustatory receptors. *Neuron*, 67(4), 555–561.

Lenth, R.V. (2016). Least-Squares Means: The R Package lsmeans. *Journal of Statistical Software*, 69, 1-33.

Liu, Y., Xue, M., Zhang, Q., Zhou, F., & Wei, J. (2010). Toxicity of β-caryophyllene from *Vitex negundo* (Lamiales: Verbenaceae) to *Aphis gossypii* Glover (Homoptera: Aphididae) and its action mechanism. *Acta Entomologica Sinica*, *53*(4), 396–404.

Lorenzi, H., & Matos, F. J. A. (2008). Plantas medicinais no Brasil: nativas e exóticas, 2nd ed. Nova Odessa: Instituto Plantarum, 544p.

Marques, A. P. S., Bonfim, F. P. G., Dantas, W. F. C., Puppi, R. J., & Marques, M. O. M. (2019). Chemical composition of essential oil from *Varronia curassavica* Jacq. accessions in different seasons of the year. *Industrial Crops and Products*, 140, 111656. https://doi.org/10.1016/j.indcrop.2019.111656

Martínez, L. C., Plata-Rueda, A., Colares, H. C., Campos, J. M., Dos Santos, M. H., Fernandes, F. L., Serrão, J. E., & Zanuncio, J. C. (2018). Toxic effects of two essential oils and their constituents on the mealworm beetle, *Tenebrio molitor*. *Bulletin of Entomological Research*, 108(6), 716–725.

Matias, E. F. F., Santos, K. K. A., Falcão-Silva, V. S., Siqueira-Júnior, J. P., Costa, J. G. M., & Coutinho, H. D. M. (2013). Modulation of the norfloxacin resistance in *Staphylococcus aureus* by *Cordia verbenaceae* DC. *The Indian Journal of Medical Research*, 137(1), 178

Mawussi, G., Vilarem, G., Raynaud, C., Merlina, G., Gbongli, A. K., Wegbe, K., Sanda, K. (2009) Chemical composition and insecticidal activity of *Aeollanthus pubescens* essential oil against coffee berry borer (*Hypothenemus hampei* Ferrari) (Coleoptera: Scolytidae). *Journal of Essential Oil-Bearing Plants*, 12(3), 327–332. https:/doi.org/10.1080/0972060X.2009.10643727

Mawussi, G., Tounou, A. K., Ayisah, K. D., Vilarem, G., Raynaud, C., Merlina, G., Wegbe, K., & Sanda, K. (2012) Chemical composition and insecticidal activity of *Ocimum canum* essential oil against coffee berry borer (*Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae). *Journal of Essential Oil-Bearing Plants*, 15(6), 955–963. https://doi.org/10.1080/0972060X.2012.10662599

Mazerolle, M. J., & Linden, D. (2019). Model Selection and Multimodel Inference Based on (Q)AIC(c). R-CRAN. 233p

Mossa, A. T. H., Afia, S. I., Mohafrash, S. M. M., & Abou-Awad, B. A. (2019). Rosemary essential oil nanoemulsion, formulation, characterization and acaricidal activity against the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). *Journal of Plant Protection Research*, 59(1), 102-112. https://doi.org/10.24425/jppr.2019.126039_rfseq1

Oliveira, B. M. S., Melo, C. R., Santos, A. C. C., Nascimento, L. F. A., Nízio, D. A. C., Cristaldo, P. F., Blank, A. F., & Bacci, L. (2019). Essential oils from *Varronia curassavica* (Cordiaceae) accessions and their compounds (E)-caryophyllene and α-humulene as an alternative to control *Dorymyrmex thoracius* (Formicidae: Dolichoderinae). *Environmental Science and Pollution Research*, 26(7), 6602-6612. https://doi.org/10.1007/s11356-018-4044-1

Oliveira, C., Auad, A., Mendes, S. M., & Frizzas, M. R. (2013). Economic impact of exotic insect pests in Brazilian agriculture. *Journal of Applied Entomology*, *137*(1–2), 1–15. https://doi.org/10.1111/jen.12018

Pavela, R. (2015). Essential oils for the development of eco-friendly mosquito larvicides: A review. *Industrial Crops and Products*, 76, 174–187. https://doi.org/10.1016/J.INDCROP.2015.06.050

Pavela, R., & Sedlák, P. (2018). Post-application temperature as a factor influencing the insecticidal activity of essential oil from *Thymus vulgaris*. *Industrial Crops and Products*, 113, 46-49.

Plata-Rueda, A., Campos, J. M., Rolim, G. S., Martínez, L. C., Dos Santos, M. H., Fernandes, F. L., Serrão, J. E., & Zanuncio, J. C. (2018). Terpenoid constituents of cinnamon and clove essential oils cause toxic effects and behavior repellency response on granary weevil, *Sitophilus granarius*. *Ecotoxicology and Environmental Safety*, 156, 263–270.

R Development Core Team (2022). A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Regnault-Roger, C., Vincent, C., & Arnason, J. T. (2012). Essential oils in insect control: low-risk products in a high-stakes world. *Annual Review of Entomology*, 57, 405–424. https://doi.org/10.1146/annurev- ento-120710-100554

Reis, P. R., Souza, J. C., & Venzon, M. (2002). Manejo ecológico das principais pragas do cafeeiro. *Informe Agropecuário,* 23(214/215), 83–99.

Reyes, E. I. M., Farias, E. S., Silva, E. M. P., Filomeno, C. A., Plata, M. A. B., Picanco, M. C. P., & Barbosa, L. C. A. (2019). *Eucalyptus resinifera* essential oils have fumigant and repellent action against *Hypothenemus hampei*. *Crop Protection*, 116, 49–55. https://doi.org/10.1016/j.cropro.2018.09.018

Santos, A. A., Farder-Gomes, C. F., Ribeiro, A. V., Costa, T. L., França, J. C. O., Bacci, L., & Picanço, M. C. (2022). Lethal and sublethal effects of an emulsion based on *Pogostemon cablin* (Lamiaceae) essential oil on the coffee berry borer, *Hypothenemus hampei. Environmental Science and Pollution Research*, 1-11.

Santos, M. R. A., Lima, R. A., Silva, A. G., Lima, D. K. S., Sallet, L. A. P., Teixeira, C. A. D., & Facundo, V. A. (2013). Composição química e atividade inseticida do óleo essencial de *Schinus terebinthifolius* Raddi (Anacardiaceae) sobre a broca-do-café (*Hypothenemus hampei*) Ferrari. *Revista Brasileira de Plantas Medicinais*, 15, 757-762.

Satyan, R. S., Malarvannan, S., Eganathan, P., Rajalakshmi, S., & Parida, A. (2009). Growth Inhibitory Activity of Fatty Acid Methyl Esters in the Whole Seed Oil of Madagascar Periwinkle (Apocyanaceae) Against *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Journal of Economic Entomology,* 102(3), 1197-1202. https://doi.org/10.1603/029.102.0344

Souza, S. P., Valverde, S. S., da Silva, R. L., Lima, K. S., & Lima, A. L. (2012). Óleos essenciais como inibidores da acetilcolinesterase. *Revista Fitos*, 7(04), 259-266.

Städler, E., & Reifenrath, K. (2008). Glucosinolates on the leaf surface perceived by insect herbivores: review of ambiguous results and new investigations. *Phytochemistry Reviews* 8(1), 207–225. https://doi.org/10.1007/S11101-008-9108-2

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

Tak, J. H., & Isman, M.B. (2017). Penetration-enhancement underlies synergy of plant essential oil terpenoids as insecticides in the cabbage looper, *Trichoplusia ni*. *Scientific Reports*, 7(1), 1-11.

Tsukamoto, T., Ishikawa, Y., & Miyazawa, M. (2005). Larvicidal and adulticidal activity of alkylphthalide derivatives from rhizome of *Cnidium officinale* against *Drosophila melanogaster*. *Journal of Agricultural and Food Chemistry*, 53(14), 5549-5553. https://doi.org/10.1021/jf050110v

Vega, F. E., Infante, F., Castillo, A., & Jaramillo, J. (2009). The coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae): a short review, with recent findings and future research directions. *Terrestrial Arthropod Reviews,* 22, 129–147.

Vega, F. E., Infante, F., & Johnson, A. J. (2015). The Genus *Hypothenemus*, with emphasis on *H. hampei*, the Coffee Berry Borer, in: Vega, F.E., Hofstetter, R.W. (eds) *Bark Beetles*: Biology and Ecology of Native and Invasive Species, Academic Press: San Diego, CA, USA, pp. 427–494. http://dx.doi.org/10.1016/B978-0-12-417156-5.00011-3

Veronez, B., Sato, M. E., & Nicastro, R. L. (2012). Toxicidade de compostos sintéticos e naturais sobre *Tetranychus urticae* e o predador *Phytoseiulus macropilis*. *Pesquisa Agropecuária Brasileira*, 47(4), 511-518. https://doi.org/10.1590/S0100-204X2012000400006

Viegas, C. (2003). Terpenos com atividade inseticida: Uma alternativa para o controle químico de insetos. *Quim Nova*, 26(3), 390-400. https://doi.org/10.1590/s0100-40422003000300017

Villalobos, M. J. P. & Robledo, A. (1998). Screening for anti-insect activity in Mediterranean plants. *Industrial Crops and Products*, 8(3), 183-194.

Tables

Table 1. Chemical composition of the essential oil of *Varronia curassavica*. Information provided by the supplier Legeé Óleos Essenciais (Estiva Gerbi, São Paulo, Brazil).

No	Compound	Relative composition (%)
1	α-Tujene	0.57
2	α-Pinene	38.82
3	Campheno	0.15
4	Sabinene	0.56
5	β-Pinene	0.59
6	β-Myrcene	0.34
7	ρ-Cymene	0.13
8	β-Tujene	1.38
9	Eucalyptol	1.06
10	Bornyl Acetate	0.59
11	δ-Elemene	4.80
12	α-Cubebene	0.39
13	Copaene	0.46
14	β-Bourbonene	0.19
15	β-Elemene	2.26
16	7-epi-Sesquithujene	0.98
17	α-Bergamotene	0.86
18	β-Caryophyllene	21.84
19	γ-Elemene	1.29
20	Humulene	4.49
21	Alloaromadendrene	4.85
22	β-Cubebene	2.14
23	α-Zingibereneo	1.62
24	Bicyclogermacrene	1.10
25	β-Bisabolene	2.39
26	δ-Cadinene	2.14
27	γ-Bisabolene	0.55
28	Germacrene B	0.12
29	Caryophyllene oxide	0.42
30	Isospathulenol	0.82
31	α-Santalol	1.01

Figures

Figure 1. Arrangement used to the test of *Varronia curassavica* essential oil repellence against *Hypothenemus hampei* (Photo: Nancy Miranda).

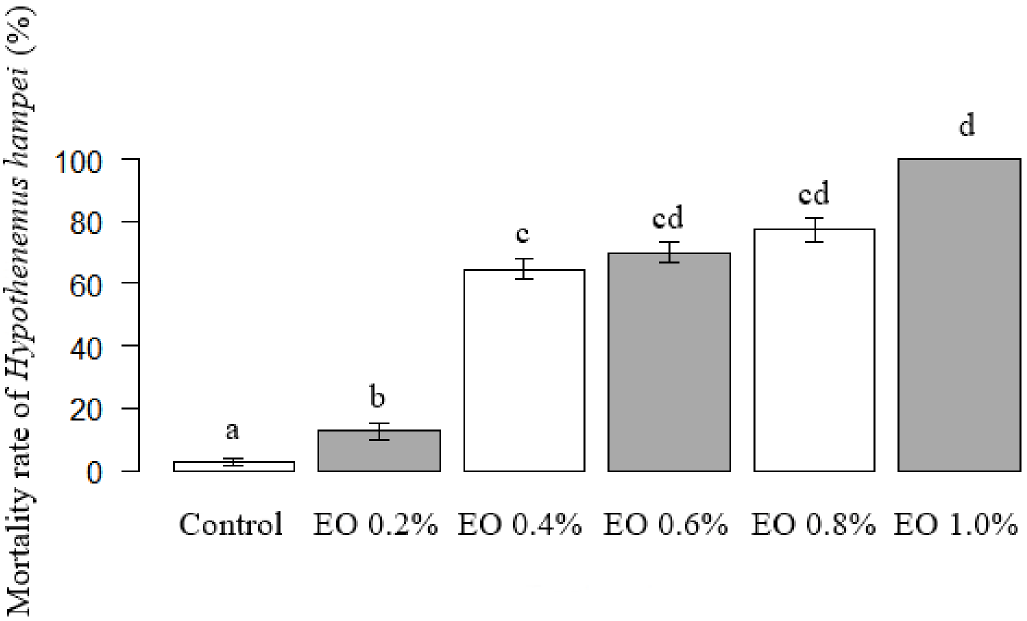

Figure 2. Mortality rate of *Hypothenemus hampei* exposed to different concentrations of *Varronia curassavica* essential oil (EO) and control (distilled water plus Tween® 80 (0.05%) (χ^2 = 272.42, DF= 5, p < 0.001). Different letters on the bars represent statistical differences.

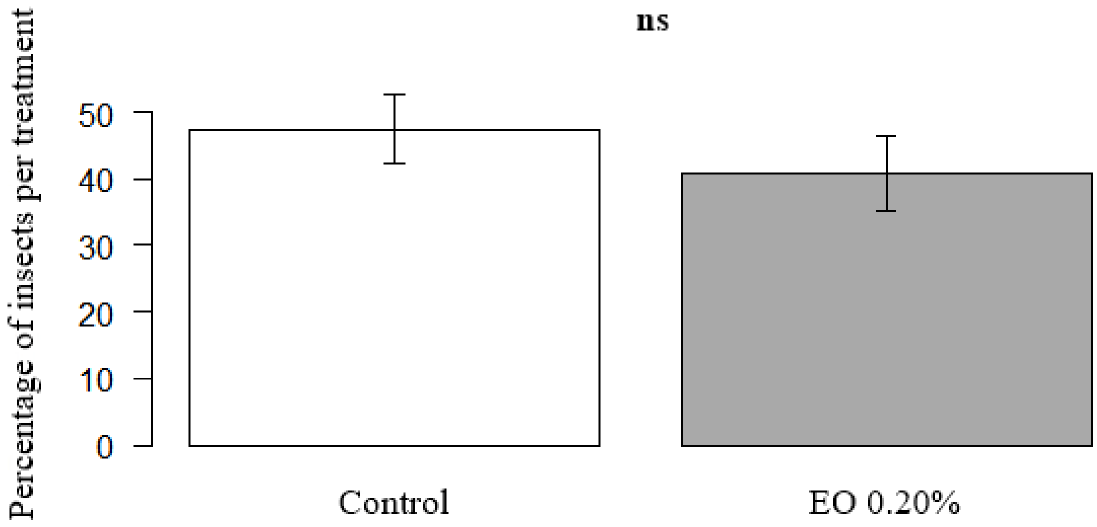

Figure 3. Percentage of *Hypothenemus hampei* per area containing coffee berries treated with *Varronia curassavica* essential oil (EO) and control (distilled water plus Tween® 80 (0.05%) ($F = 0.755$, $p = 0.389$, $DF = 1$). ns = No significant statistical difference.

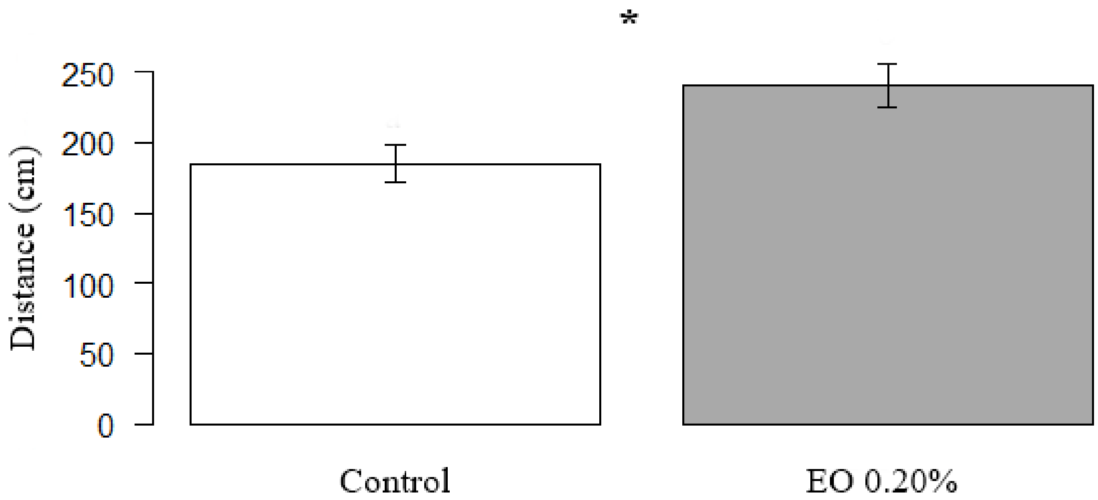

Figure 4. Displacement of *Hypothenemus hampei* 48h after exposure to *Varronia curassavica* essential oil (EO) and control (distilled water plus Tween® 80 (0.05%) ($F = 7.831$, $p = < 0.001$, $DF = 1$). Asterisks on the bars represent the statistical differences.

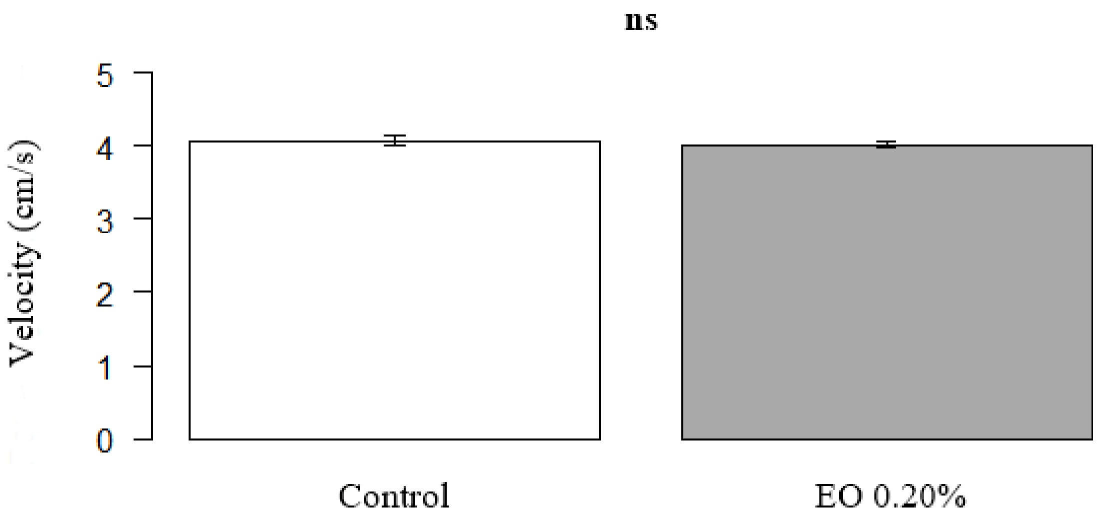

Figure 5. Walking velocity of *Hypothenemus hampei* 48h after exposure to *Varronia curassavica* essential oil (EO) and control (distilled water plus Tween® 80 (0.05%) (F = 0.501, p = 0.482, DF = 1). ns = No significant statistical difference.

GENERAL CONCLUSIONS

Varronia curassavica associated with coffee plants stimulate the oviposition of the green lacewing *Chrysoperla externa.*

The essential oil of *Varronia curassavica* inhibited the oviposition of *L. coffeella* but did not affect the development of its eggs and mines.

Varronia curassavica essential oil is toxic to *Hypothenemus hampei* and affects its mobility, but it wasn't repellent to the pest in the 0.2% EO concentration.

Therefore, this study can be used as a basis to new research's that involves prospection of new biopesticides and at the same time can be used by coffee farmers aiming the management of the key coffee pests.