

RODRIGO DONIZETI FARIA

ATIVIDADE PROTETORA DE GRÃOS DE ÓLEOS ESSENCIAIS EM *Zabrotes subfasciatus* E *Aspergillus nomius*

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ATIVIDADE PROTETORA DE GRÃOS DE ÓLEOS ESSENCIAIS EM *Zabrotes subfasciatus* E *Aspergillus nomius*

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Orientador: Carlos Gilberto Raetano

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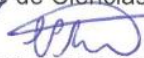
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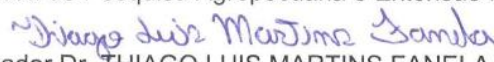
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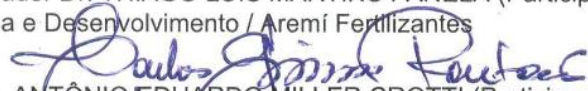
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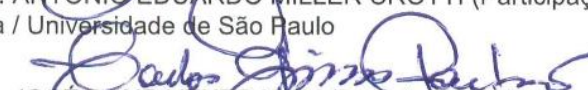
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Ao meu eterno professor e amigo,

Edson Luiz Lopes Baladin,

dedico

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“The scientific man does not aim at an immediate result. He does not expect that his advanced ideas will be readily taken up. His work is like that of the planter for the future. His duty is to lay the foundation for those who are to come, and point the way”.

TESLA, N. Radio power will revolutionize the world.
Modern Mechanix and Inventions, p. 261-266, 1934.

RESUMO

Após a colheita, o feijão-comum (*Phaseolus vulgaris* L.) necessita do armazenamento dos grãos secos em silos até o momento da comercialização. No entanto, medidas sanitárias são necessárias para evitar surtos de insetos-praga e microrganismos detritívoros. Entre as pragas do feijão armazenado destaca-se *Zabrotes subfasciatus* (Boh.) (Coleoptera: Crysomelidae: Bruchinae), responsável por danos diretos nas sementes e o favorecimento do desenvolvimento de fungos de armazenamento. Portanto, estudos com novas soluções ambientalmente amigáveis são imprescindíveis para o manejo integrado de pragas (MIP) de grãos armazenados. Alguns óleos essenciais (OEs) têm ação comprovada contra insetos-praga de diversas ordens e é compatível com as táticas abordadas pelo MIP. Neste sentido, o presente estudo, foi dividido em dois capítulos e teve por objetivos avaliar a bioatividade de cinco óleos essenciais [*Cordia verbanacea* DC. (Boraginaceae), *Elionurus latiflorus* (Nees Ex Steud.) Hack (Poaceae), *Dysphania ambrosioides* L. (Amaranthaceae), *Pelargonium graveolens* L'Hér (Geraniaceae) e *Piper callosum* Ruiz & Pav. (Piperaceae)] sobre *Z. subfasciatus* em diferentes métodos de exposição (fumigação, aplicação tópica e contato residual) e o comportamento de caminhamento do inseto (distância percorrida e velocidade). Com exceção dos óleos essenciais de *D. ambrosioides*, *P. graveolens* e *P. callosum* foi avaliada a bioatividade sobre *Aspergillus nomius* (Ascomycota) na produção de micotoxinas B₁, B₂, G₁ e G₂ e adicionalmente avaliada a mistura binária (1:1, v v⁻¹) dos mesmos. Todos os óleos essenciais apresentaram toxicidade para o caruncho-do-feijão nos diferentes métodos de exposição e foi possível determinar as concentrações e doses letais que ocasionaram a mortalidade 50% e 90% (CL₅₀, CL₉₀, DL₅₀ e DL₉₀) dos insetos. Quanto ao primeiro capítulo o OE de *E. latiflorus* apresentou maior toxicidade quando comparado com *C. verbanacea*. Para o comportamento de caminhamento o *E. latiflorus* apresentou efeito arrestante nas fêmeas. As análises de isoblograma indicaram efeito aditivo na mistura binária dos óleos essenciais para os métodos de exposição via contato residual, aplicação tópica e antagonista para fumigação. O OE de *E. latiflorus* inibiu o crescimento radial e a produção das aflotoxinas B₁, B₂, G₁ e G₂. No segundo capítulo, o OE de *D. ambrosioides* apresentou maior toxicidade aos adultos quando comparado com OE de *P. graveolens* e *P. callosum* em ambos os

métodos de exposição. Os OEs de *P. graveolens* e *P. callosum* influenciaram negativamente na oviposição e emergência dos adultos. Em adição, ambos os OEs influenciaram no comportamento de caminhada, destacando-se o OE de *D. ambrosioides* que apresentou potente efeito repelente para adultos de *Z. subfasciatus*. Os resultados encontrados neste estudo contribuem para elucidar os efeitos dos óleos essenciais nos organismos alvo, além da elaboração de novas soluções alternativas para a proteção de grãos e o manejo de insetos-praga em armazéns.

Palavras-chave: caruncho-do-feijão; controle alternativo; MIP; óleos essenciais.

ABSTRACT

After harvest, common beans (*Phaseolus vulgaris* L.) require dry grain storage in warehouses until they are sold. However, sanitary measures are necessary to prevent outbreaks of insect pests and detritivorous microorganisms. Among the pests of stored beans, *Zabrotes subfasciatus* (Boh.) (Coleoptera: Crysomelidae: Bruchinae) which is responsible for direct damage to the seeds and favoring the development of storage fungi. Therefore, studies with new eco-friendly solutions are essential for integrated pest management (IPM) of stored grains. Some essential oils (EOs) have proven action against insect pests of various orders and are compatible with the tactics addressed by IPM. In this sense, the present study was divided into two chapters and aimed to evaluate the bioactivity of five essential oils [*Cordia verbenacea* DC. (Boraginaceae), *Elionurus latiflorus* (Nees Ex Steud.) Hack (Poaceae), *Dysphania ambrosioides* L. (Amaranthaceae), *Pelargonium graveolens* L'Hér (Geraniaceae) and *Piper callosum* Ruiz & Pav. (Piperaceae)] on *Z. subfasciatus* in different exposure methods (fumigation, topical application and residual contact) and the walking behavior of the insect (distance traveled and speed). Except for the essential oils of *D. ambrosioides*, *P. graveolens* and *P. callosum*, the bioactivity on *Aspergillus nomius* (Ascomycota) in the production of mycotoxins B1, B2, G1 and G2 was evaluated and additionally the binary mixture (1:1, v v⁻¹) of these was evaluated. All essential oils showed toxicity to the Mexican bean weevil in the different exposure methods and it was possible to determine the lethal concentrations and doses that caused 50% and 90% mortality (LC₅₀, LC₉₀, LD₅₀ and LD₉₀) of the insects. Regarding the first chapter, the EO of *E. latiflorus* showed greater toxicity when compared to *C. verbanacea*. For walking behavior, *E. latiflorus* showed an arresting effect on females. The isobologram analyses indicated an additive effect in the binary mixture of essential oils for the exposure methods for residual contact, topical application and antagonist for fumigation. The EO of *E. latiflorus* inhibited radial growth and the production of aflatoxins B1, B2, G1 and G2. In the second chapter, the EO of *D. ambrosioides* showed greater toxicity to adults when compared to EO of *P. graveolens* and *P. callosum* in both exposure methods. The EOs of *P. graveolens* and *P. callosum* negatively influenced oviposition and adult emergence. In addition, both EOs influenced walking behavior, with *D. ambrosioides* standing out as an EO that showed a

potent repellent effect on *Z. subfasciatus* adults. The results found in this study contribute to elucidate the effects of essential oils on target organisms, in addition to developing new alternative solutions for grain protection and insect pest management in warehouses.

Keywords: alternative control; essential oils; IPM; Mexican bean weevil.

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INTRODUÇÃO GERAL

O feijão-comum (*Phaseolus vulgaris* L.) é um dos alimentos mais consumidos em todo o mundo por ser rico em proteínas. Os grãos podem ser consumidos na forma de vagens frescas ou na forma seca (FAO, 2024). Devido ao crescimento constante da população mundial, torna-se imprescindível aumentar a produção de alimentos, a fim de suprir toda a demanda no mercado. Desse modo, os cuidados com o período pós-colheita dos alimentos assumem grande importância, pois têm a finalidade de diminuir as perdas e manter características quantitativas e qualitativas desejáveis para o consumo final (Lorini *et al.*, 2015). Dentre os problemas fitossanitários comuns durante o armazenamento, destacam-se os insetos-praga, os quais têm o potencial de reduzir a qualidade e vigor dos grãos e inviabilizar o consumo ou para processamento industrial (Mingotte *et al.*, 2013).

No Brasil, o caruncho *Zabrotes subfasciatus* (Bohemann, 1833) (Coleoptera: Chrysomelidae: Bruchinae) tem grande relevância em feijão armazenado e é considerado praga primária. Essa espécie é originária das Américas Central e do Sul, e é hospedeiro primário de espécies ancestrais selvagens de *Phaseolus lunatus* L. e *P. vulgaris* (Dendy; Credland, 1991). Devido ao rápido estabelecimento e contínua reprodução, o inseto disseminou-se por meio do comércio de grãos e sementes nas regiões tropicais e subtropicais no mundo, caracterizando-se como praga cosmopolita (Aebi *et al.*, 2004).

As larvas de *Z. subfasciatus* são curculioniformes, de coloração branco-leitosa, dotadas de mandíbulas desenvolvidas, as quais são capazes de perfurar os cotilédones do grão de feijão já no primeiro instar, onde passam por quatro instares até a fase de pupa. As pupas apresentam de 2,5 a 3,5 mm de comprimento e 1,5 a 2,0 mm de largura e possuem coloração branco-leitosa. Nessa fase, é possível realizar a sexagem a partir do último segmento abdominal, retilíneo nas fêmeas e arqueado nos machos (Ferreira, 1960; Hill, 2002).

Os machos adultos de *Z. subfasciatus* medem em torno de 1,8 mm de comprimento, e pesam 1,7 mg; as fêmeas medem 2,5 mm, pesam 3 mg e têm coloração castanho-escura, com manchas claras no pronoto fortemente pubescente. É possível realizar a separação dos sexos, uma vez que essa espécie apresenta acentuado dimorfismo sexual (Ferreira, 1960). As fêmeas exibem uma mancha clara triangular na parte posterior da cabeça, outra da

mesma forma próxima ao escutelo e duas outras nos ângulos do pronoto, enquanto, nos machos ocorre apenas a mancha pré-escutelar. Os adultos emergem dentro do grão e são capazes de acasalar após 15 minutos, reiniciando a infestação (Southgate, 1978; Athié; De Paula, 2002).

Os ovos são arredondados, medindo de 0,46 a 0,60 mm de comprimento por 0,44 a 0,50 mm de largura. As fêmeas depositam os ovos na superfície dos grãos, de modo que ficam aderidos. Os ovos férteis são opacos e os inférteis são translúcidos (Ferreira, 1960; Hill, 2002). O ciclo total dessa espécie varia de 23 a 36 dias, dependendo das condições de temperatura e umidade em que o inseto está confinado. Segundo Ferreira (1960) o período total de desenvolvimento de *Z. subfasciatus* é de 36 dias, à temperatura de 27°C e umidade relativa de 75% (Ferreira, 1960). Outros autores reportaram o ciclo de 23 a 33 dias sob a temperatura de 32°C e umidade relativa de 70% (Carvalho; Rosseto, 1968).

Os danos diretos são caracterizados pela penetração das larvas logo após a eclosão e abertura de galerias levando a perda do peso dos grãos, redução da qualidade nutricional e do poder germinativo das sementes. Além disso, os danos indiretos associados são o favorecimento da entrada de organismos oportunistas (p.ex.: fungos e ácaros), conferindo aos grãos sabor indesejável e inviabilizando o consumo (Vieira, 1967; Pereira, 1993). As perdas ocasionadas por pragas de grãos armazenados podem chegar até 9% em países desenvolvidos e 20% ou mais em países subdesenvolvidos (Pimentel, 1991).

Os silos de armazenamento de grãos são ambientes ideais para o desenvolvimento de artrópodes-praga, principalmente quando combinados com a presença de grãos que são quebrados durante o processo de transporte (Phillips; Throne, 2010). Assim, é obrigatória a adoção de práticas de manejo que sigam normas de segurança alimentar, uma vez que produtos, em sua maioria, já estão prontos para o consumo final. As estratégias empregadas no manejo integrado de pragas (MIP) são técnicas já fundamentadas e desempenham um papel importante no controle de insetos-praga nas mais diversas culturas exploradas em todo o mundo (Kogan, 1998).

Os métodos físicos foram as primeiras técnicas empregadas no manejo de pragas de armazéns e consistem na manipulação de fatores físicos do ambiente com o intuito de deixar o nível populacional do inseto em um nível tolerável ou eliminá-lo (Brooker; Bakker-Arkema; Hall, 1992). Os métodos físicos

são: controle de temperatura, umidade relativa do ar, atmosfera controlada (CO₂, O₂, N₂), uso de pós inertes na dessecação (p. ex.: terra de diatomáceas), remoção física, radiação, luz e som. Esses métodos podem ser utilizados de forma isolada ou conjunta (Lorini *et al.*, 2015).

Os métodos de controle químico consistem no uso de inseticidas registrados para aplicação em armazéns e a adoção desta solução de manejo deve ser de forma para complementar outros métodos de controle, visando minimizar os possíveis resíduos nos grãos (Faroni; Silva, 2008). O método químico pode ser curativo ou preventivo e realizado após os grãos serem limpos, secos e expurgados. Quando o período de armazenamento for superior a 60 a 90 dias pode-se fazer o controle preventivo dos grãos ou sementes. Nesse caso, o controle é realizado por meio da aplicação de inseticidas na forma líquida sobre os grãos, onde passam por esteira transportadora e é realizado a aplicação, garantindo que todos os grãos recebam o tratamento com o inseticida de forma homogênea (Lorini *et al.*, 2015). Os ingredientes ativos mais utilizados em armazéns para aplicação direta nos grãos são do grupo químico dos piretroides (deltametrina e bifentrina) e organofosforados (pirimifós-metílico) (AGROFIT, 2024). Adicionalmente, quando os insetos já estão instalados no armazém é necessário o tratamento curativo por meio de expurgo ou fumigação, que consiste na eliminação dos agentes indesejáveis por meio do uso de gás (fluoreto de sulfúrio e fosfina). É necessário seguir as normas de segurança recomendadas pelo fabricante do produto e a concentração do gás em ambiente deve ser suficiente para controlar os carunchos (Upadhyay; Ahmad, 2011; Lorini *et al.*, 2013).

Embora o controle químico de pragas em armazéns seja eficiente e aceito pelas agências reguladoras de defensivos, o uso inadequado pode selecionar populações resistentes aos principais ingredientes ativos. Atualmente, a fosfina é o ingrediente ativo mais utilizado para fumigação de pragas de armazéns. Portanto, relatos de populações resistentes a esse ingrediente ativo são reportados na literatura desde os anos 70 (Champ; Dyte, 1976) e novos estudos evidenciaram aumento significativo na frequência da resistência em populações de pragas de armazéns no mundo (Pimentel *et al.*, 2009; Opit *et al.*, 2012; Daglish *et al.*, 2018). O aumento de populações de pragas resistentes está associado ao uso rotineiro dos mesmos ingredientes ativos ou ao mesmo grupo de inseticida

que age no mesmo sítio de ação nos insetos. Estudos sobre o monitoramento de populações de pragas em armazéns no Brasil constataram que em 74% das amostras coletadas havia insetos com acentuada resistência à fosfina (Lorini *et al.*, 2007). Em adição, outros trabalhos evidenciaram populações de pragas de armazéns resistentes a deltametrina (piretroide) (Khalid *et al.*, 2024). Além de problemas relacionados a populações resistentes, o uso indiscriminado de inseticidas químicos deixa resíduos prejudiciais à saúde humana e animal (Carvalho, 2017).

Nesse cenário, o desenvolvimento de novas soluções para o manejo de pragas em armazéns torna-se necessário. Tais medidas devem priorizar o baixo impacto ambiental e à saúde humana, contribuindo para a segurança alimentar global e viabilizar o fornecimento de produtos livres de resíduos químicos, além da exposição de aplicadores a esses ingredientes ativos. Assim, estudos com óleos essenciais para controle de pragas de grãos armazenados têm demonstrado resultados consistentes que os credenciam como uma alternativa valiosa no manejo de pragas e proteção dos grãos armazenados (Isman, 2020; Ribeiro *et al.*, 2023).

Os óleos essenciais (OEs), são misturas de constituintes químicos voláteis produzidos por plantas como parte de seu metabolismo secundário/especial com baixo peso molecular, apresentam alta volatilidade e variados compostos químicos (aleloquímicos) que possuem destacada ação atraente ou repelente sobre artrópodes (Regnault-Roger *et al.*, 2012), além de efeito inibidor de crescimento micelial de fungos produtores de micotoxinas prejudiciais à saúde humana (Zimmermann *et al.*, 2022). Esses compostos são produzidos pelo metabolismo secundário das plantas e a composição é influenciada por fatores genéticos, físicos, químicos, ambientais, sazonais, ocorrência de quimiotipo ou diferentes variedades de plantas, entre outros (Isman, 2016). A síntese e o acúmulo desses compostos ocorrem nas estruturas das plantas e pode variar conforme a família da planta. Por exemplo, dentre as plantas da família Lamiaceae existem algumas espécies que possuem estruturas secretoras complexas, como tricomas glandulares; já em espécies das famílias Myrtaceae e Rutaceae, as plantas apresentam cavidades secretoras e nas famílias Asteraceae e Apiaceae os óleos essenciais estão presentes em dutos de resina (Rodriguez *et al.*, 1984).

Embora a composição química dos óleos essenciais tenha influência na bioatividade sobre organismos, outros fatores podem estar envolvidos na toxicidade, entre eles, os modos de exposição. Rani *et al.* (2012) demonstraram que os óleos essenciais de *Pinus longifolia* Roxb. (Pinaceae), *Eucalyptus obliqua* L'hérit. (Myrtaceae) e *Coriandrum sativum* L. (Apiaceae) exibiram forte efeito fumigante nas espécies *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae) e *Corcyra cephalonica* Staint. (Lepidoptera: Pyralidae) quando comparados com o método de exposição por contato tópico. Outros trabalhos evidenciaram que o óleo essencial de *Citrus aurantium* L. (Rutaceae) aplicado via fumigação causa maior mortalidade de *Stomoxys calcitrans* L. (Diptera: Muscidae) em menores concentrações em relação à exposição tópica (Changbunjong *et al.*, 2022). Tais características estão relacionadas a alta volatilidade, principalmente dos compostos monoterpenos, e a penetração dos voláteis pelos sistemas respiratório, digestório ou pela cutícula do inseto (Kim; Ahn, 2001; Chaubey, 2018). Além disso, o método de fumigação permite que os compostos fiquem acumulados e confinados, resultando na rápida mortalidade nos insetos (Kiran; Prakash, 2015).

Além dos efeitos letais, são reportados na literatura efeitos subletais sobre insetos. Os efeitos subletais estão associados a interferência em aspectos biológicos dos insetos alvos, por exemplo diminuição da longevidade de adultos e/ou da progênie, redução da fecundidade e fertilidade, deterrentes de oviposição, além do efeito repelente, entre outros. Estudos com os óleos essenciais de *Eucalyptus camaldulensis* Dehn. (Myrtaceae) e *Heracleum persicum* L. (Apiaceae) evidenciaram os efeitos negativos na longevidade, fecundidade e fertilidade de fêmeas de *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae) (Izadmehr *et al.*, 2013). Pavela (2012) investigou o efeito subletal (CL₃₀) de 13 espécies de OEs sobre lagartas *Spodoptera littoralis* Bois. (Lepidoptera: Noctuidae). As lagartas sobreviventes foram monitoradas até a fase adulta, onde se avaliou a longevidade, fertilidade e natalidade da próxima geração. Os autores relataram redução significativa da porcentagem de natalidade nos tratamentos com CL₃₀ dos óleos essenciais de *Pimenta racemosa* L. (Myrtaceae), *Salvia sclarea* L. (Lamiaceae) e *Thymus vulgaris* L. (Lamiaceae) (Pavela, 2012).

Têm sido ainda reportados na literatura efeitos repelentes no comportamento de caminhada dos insetos. O óleo essencial de *Lippia sidoides*

Cham. (Verbanaceae) influenciou positivamente no caminharmento de *Cryptotermes brevis* Walk. (Isoptera: Kalotermitidae) (Santos *et al.*, 2017). Outros trabalhos demonstram efeito repelente do óleo essencial de *Origanum vulgare* L. (Lamiaceae) sobre adultos de *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (PLATA-RUEDA *et al.*, 2021). Entretanto, alguns óleos essenciais podem ter efeitos arrestantes em insetos. Rocha *et al.* (2018) constataram efeito residual do óleo essencial de *Pogostemon cablin* (Benth.) (Lamiaceae), que resultou na redução do caminharmento e velocidade de operárias de *Atta opaciceps* (Borg.) e *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae). Outros estudos reportaram redução da capacidade motora de *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) exposto ao óleo essencial de *Illicium verum* Hook. (Illiciaceae) (Peter *et al.*, 2022).

Além da bioatividade sobre artrópodes-praga, os óleos essenciais podem apresentar efeitos negativos em fungos saprofíticos produtores de micotoxinas prejudiciais à saúde humana. Fungos dos gêneros *Aspergillus* e *Penicillium* são comumente identificados em grãos armazenados e podem ocorrer em associação a infestações de insetos-praga em armazéns através de detritos gerados pela alimentação, aumento na umidade relativa e temperatura. As micotoxinas produzidas por esses microrganismos são conhecidas como aflotoxinas e afetam a segurança alimentar devido a atividade nefrotóxica, hepatóxica e potencialmente cancerígena para seres humanos (Reddy *et al.* 2004; Egal *et al.* 2005). Portanto, estudos com óleos essenciais que visam a proteção dos grãos de fungos saprofíticos, são relevantes para a prospecção de novas soluções de manejo. Trabalhos com os óleos essenciais de *Dysphania ambrosioides* (L.) Mosyakin & Clemants (Amaranthaceae) e *Conyza sumatrensis* Retz (Asteraceae) sobre o fungo *Aspergillus flavus* Link:Fr (Ascomycota) mostraram efeito inibidor de crescimento micelial e alta mortalidade dos adultos de *T. castaneum* (Azeem *et al.*, 2022). Outros estudos com *A. flavus* exposto ao óleo essencial de *Cymbopogon citratus* DC. (Gramineae) evidenciou forte efeito fungicida nas concentrações de 2,80 e 3,46 mg mL⁻¹ (Paranagama *et al.*, 2003).

Considerando a importância econômica de *Z. subfasciatus* para feijoeiro armazenado e os fungos saprofíticos associados, torna-se cada vez mais importante aprofundar o conhecimento de novas soluções ambientalmente amigáveis para a implementação do manejo de pragas, além de contribuir para

segurança alimentar. Neste sentido, o presente trabalho propõe a avaliação da bioatividade dos óleos essenciais em *Z. subfasciatus*, *A. nomius* e as aflotoxinas B1, B2, G1, G2.

CHAPTER 1

ESSENTIAL OILS FROM *Cordia verbenacea* AND *Elionurus latiflorus* AND THEIR BINARY MIXTURE: BIOACTIVITY AGAINST THE MEXICAN BEAN WEEVIL AND AN AFLATOXIN-PRODUCING FUNGAL SPECIES¹**ABSTRACT**

Chemically diversified essential oils (EOs) have a broad spectrum of biological activities, so they are promising sources of compounds with simultaneous action on stored-grain spoilers. This study has evaluated the insecticidal action and behavioral effects of the EOs obtained from *Cordia verbenacea* (Boraginaceae) and *Elionurus latiflorus* (Poaceae) leaves, tested in isolation or as binary mixture (1:1, v v⁻¹), against the Mexican bean weevil, *Zabrotes subfasciatus* (Coleoptera: Chrysomelidae: Bruchinae), a primary pest of stored beans in tropical regions. This study has also evaluated the antifungal and antiaflatoxigenic properties of these EOs against *Aspergillus nomius* (Ascomycota), which produces aflatoxins B₁, B₂, G₁, and G₂. GC-MS analyses indicated that sesquiterpene hydrocarbons (86.5%) and oxygenated monoterpenes (95.7%) predominated in the EOs obtained from *C. verbenacea* and *E. latiflorus* leaves, respectively. Both EOs and their binary mixture were toxic to *Z. subfasciatus* adults in fumigation, residual contact, and topical application trials; the EO obtained from *E. latiflorus* leaves was the most potent. In addition, computational behavioral tracking showed that the distance covered by females exposed to the EO obtained from *E. latiflorus* leaves at the previously estimated LC₅₀ was significantly reduced. Analysis of isobolograms indicated that the binary mixture of the EOs had an additive effect for both the residual contact and topical application exposure modes, while antagonism was verified for fumigation. The EO obtained from *E. latiflorus* leaves and the binary mixture also inhibited radial growth and production of aflatoxins B₁, B₂, G₁, and G₂ in a concentration-dependent manner. Thus, the tested EOs, especially from *E. latiflorus*, are promising grain-protectants with multiple actions against grain spoilers.

Keywords: “Baleeira herb”, “Brazilian lemongrass”, biological activities, binary mixture, walking behavior, grain spoilers.

¹Chapter published in Industrial Crops and Products

1.1 INTRODUCTION

Developing and implementing biorational approaches for pest management in stored grains is key to improving food security and safety (Adler et al., 2022; Berhe et al., 2022; Qin et al., 2023), mainly in undeveloped and developing countries (Kimiye, 2015; Zhang et al., 2016; Tadesse, 2020). Aflatoxin-producing fungal species and insects (especially Coleoptera and Lepidoptera species) directly and indirectly damage stored grains (Nayak and Daglish, 2018) and account for most of the losses verified in beans and cereals during post-harvest (Qin et al., 2023).

Among the fungi species of occurrence in warehouses, the genus *Aspergillus* includes saprophytic species, commonly found in stored grain debris that has been damaged mechanically or by primary insect pests (Nayak and Daglish, 2018). *Aspergillus* spp. can cause qualitative and quantitative damage to grains, including stimulating grain germination due of increasing temperature and humidity of stored grain mass, losses of protein and oil contents, grain deterioration and production of mycotoxins (Manu et al., 2019). Aflatoxins are mycotoxins produced mainly by species of *Aspergillus*, which are harmful to humans and animals when inhaled, ingested or absorbed through the skin (Shephard, 2008). The association of *Aspergillus* spp. with grain stored pests is related to the activities of insects in the grain mass, increasing the temperature, CO₂ levels and the amount of water in specific points of warehouse, making the environment favorable for the proliferation and development of these fungi species (Mohapatra et al., 2017).

In addition to the economic impact of grain spoilers, the search for environmentally friendly strategies and low-risk compounds that can be included in integrated pest management (IPM) of stored products has been encouraged by concerns about pest resistance to currently available insecticides (Boyer et al., 2012; López-Castilho et al., 2018; Nayak et al., 2020), environmental risks involved in the routine use of xenobiotics, and detection of agrochemicals residue in foodstuff (Teló et al., 2015; Han et al., 2016; Mebdoua and Ounane, 2019). Compounds originating from secondary plant metabolism (allelochemicals) are promising sources of new (bio)insecticides (Ribeiro et al., 2013; 2016; 2020) and have been widely studied to detect alternative tools that ensure effectiveness, safety, and selectivity standards (Viegar Jr, 2003; Ribeiro et al., 2016; Gonçalves

et al., 2017), which are essential precepts for IPM programs. Such compounds are also an interesting option for developing high-value products within the bioeconomy approach (Valli et al., 2018), particularly in megadiverse countries such as Brazil (Abranches, 2020).

Regarding stored-grain pest management, essential oils (EOs) are the most promising plant derivatives because they display a broad spectrum of activities against fungi and arthropod pests (Campolo et al., 2018), including repellency, feeding deterrence and fumigant and contact insecticidal actions (Ribeiro et al., 2020; Chaudhari et al., 2021) as well as inhibition of mycelial growth and toxin production of food-deteriorating fungi (Achar et al., 2020; Bomfim et al., 2020; Ribeiro et al., 2020). In the last decades, research on botanicals has increased considerably [from 1.43% in 1980 to 21.38% in 2012 of all the published papers on insecticides (Isman and Grienesen, 2014)], but some knowledge gaps need to be overcome to move botanical insecticides from basic laboratory studies and traditional uses (like homemade preparations) to commercialization (Isman, 2017; 2020). In this context, finding new and viable sources of compounds or their blends that can simultaneously act on stored-grain spoilers is crucial for the practical application of these natural products in warehouses. Neotropical plant species, which have diversified secondary metabolism, are an interesting source of volatile compounds (EOs) that could be applied in the phytosanitary management of row and horticultural crops, even during pre- and post-harvest practices (Fazolin et al., 2023).

Among Brazilian aromatic species, *Elionurus latiflorus* (Nees Ex Steud.) Hack (Poaceae), popularly known as “Brazilian lemongrass”, is a poorly studied medicinal plant species. It produces EO that is rich in citral (Braga et al., 2022), a compound that is extensively used in the food, cosmetic, and pharmaceutical industries (Buglia, 2021). Moreover, its antibacterial activity against phytopathogens (Braga et al., 2022) and bacteria that are important for human health (Bohm et al., 2012) has been reported. *Cordia verbenacea* DC (Boraginaceae), known as “Baleeira herb”, is a flowering shrub found along the Brazilian Atlantic Forest, the Brazilian coast, and low areas of the Amazon (Melo et al., 2021). This plant species produces an EO whose pharmacological properties, including its anti-inflammatory, anti-allergic, antibacterial, antiparasitic, antitumoral, and anti-ulcerogenic effects (Passos et al., 2007; Meccia et al., 2009;

Michielin et al., 2009; Souza et al., 2011; Matias et al., 2013; Vázquez-Sánchez et al., 2018; Martim et al., 2021; Melo et al., 2021; Pereira et al., 2021), have been investigated. Additionally, the EO obtained from this species acts against phytopathogens of agricultural relevance (Silva et al., 2009; 2012; 2014).

Although several studies have elucidated the promising bioactive effects of derivatives and/or isolated compounds obtained from these two species of aromatic plants, few studies have evaluated their action against arthropod pests. The EO obtained from *C. verbenacea* showed fumigant insecticidal activity, oviposition deterrence, and transgenerational effects against *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae), a pest species of stored cowpea [*Vigna unguiculata* (L.) Walp. (Fabaceae)] (Alves et al., 2015). However, the hexane and ethanolic extracts obtained from *C. verbenacea* did not cause lethal toxicity to *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae), *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae), or *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae) (Moreira et al., 2007). Furthermore, the cashew nut shell liquid associated with the EO obtained from *C. verbenacea* was effective against engorged females of *Rhipicephalus microplus* Canestrini (Acari: Ixodidae) (Castro et al., 2019). Nevertheless, the EO obtained from *E. latiflorus* has not been tested against arthropod pests, and the EO obtained from *C. verbenacea* has not been evaluated against *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae), a primary pest species of stored beans [*Phaseolus vulgaris* L. (Fabaceae)] in tropical regions (Barbosa et al., 2002; Guzzo et al., 2020).

This study has evaluated the insecticidal and behavioral actions of the EOs obtained from *C. verbenacea* and *E. latiflorus* leaves, tested in isolation or as binary mixture (1:1, v v⁻¹), against *Z. subfasciatus*. In addition, this study has evaluated the *in vitro* antifungal and antiaflatoxigenic effects of these EOs against an isolate of *Aspergillus nomius* Kurtzman, Horn & Hesseltine (Ascomycota), which produces aflatoxins B₁, B₂, G₁, and G₂.

1.2 MATERIAL AND METHODS

1.2.1 Essential oil: plant material obtaining, preparation, and chemical analysis

Seedlings of both species studied were obtained from “Banco Ativo de Germoplasma de Plantas Bioativas da Epagri” (BAG-BIO/EPAGRI, Accreditation N. 105/2013/SECEX/CGEN), located in Itajaí, SC, Brazil, and cultivated in the experimental area of Epagri/Cepaf (27°05'26.7"S; 52°38'8.75"W; elevation = 661 m), in Chapecó, SC, Brazil for at least one year until harvest. The leaves of the plant species were obtained in June 2022, between 8 and 9 h.

The EOs were extracted by hydrodistillation in a Clevenger apparatus at 100 °C for 2 h. Clean-cut fresh leaves (100 g) were used to optimize the extraction process by increasing their surface contact with the water vapor. The resulting water/oil mixture was allowed to separate, and water was removed by adding anhydrous sodium sulfate (Na₂SO₄). The extraction yield was determined on the basis of the fresh weight of the plant material (g of oil/g of fresh leaves). The obtained EOs were stored in a freezer at -10 °C until use.

For chemical analysis, the EOs were dissolved in ethyl ether and analyzed by gas chromatography-mass spectrometry (GC-MS) on a Shimadzu GCMS2010 Plus (Shimadzu Corporation, Kyoto, Japan) system. The column temperature was programmed to rise from 60 to 240 °C at 3 °C min⁻¹ and held at 240 °C for 5 min. The equipment was set to operate in the injection mode, and the injection volume was 0.1 µL (split ratio of 1:10). The injector and detector temperatures were 240 and 280 °C, respectively. Relative concentrations of the EO compounds were estimated from the relative peak areas (%) of the GC-MS chromatograms. The EO components were identified on the basis of their retention indices on an Rtx-5MS (30 m × 0.25 mm; 0.250 µm) capillary column relative to a homologous series of *n*-alkanes (C₈-C₂₀). The structures were computer matched with the Wiley 7, NIST 08, and FFNSC 1.2 spectral libraries, and their fragmentation patterns were compared with literature data (Adams, 2007; NIST Chemistry Weebook, 2022).

1.2.2 Tested insects and fungi species isolate

Insects originating from colonies maintained under controlled conditions (Temp.: 25±2 °C; R.U.: 60±10% and photoperiod of 14 L: 10 D h) were used in the bioassays. “Bolinha” common bean cv. “IAC Esperança” was used as a feeding and oviposition substrate for *Z. subfasciatus* rearing.

The *A. nomius* isolate used in the bioassays originated from Brazil nuts [*Bertholletia excelsa* Humb. & Bonpl. (Lecythidaceae)]. After isolation, pure colonies were multiplied in potato, dextrose, and agar (PDA) medium.

1.2.3 Bioassays

1.2.3.1 Evaluation of bioactivity on *Zabrotes subfasciatus*

All the bioassays were conducted under controlled laboratory conditions (Temp.: 25±2 °C; R.U.: 60±10% and photoperiod of 14 L: 10 D h) in a completely randomized experimental design. “Bolinha” common bean cv. IAC Esperança was used as the substrate for the tests; the beans had previously been disinfested and hygroscopically equilibrated (~ 13% humidity) following the procedure proposed by Ribeiro et al. (2013).

1.2.3.1.1 Fumigation bioassay

The fumigant insecticidal action of the EOs obtained from *C. verbenacea* and *E. latiflorus* leaves, tested in isolation or as binary mixture [1:1, v v⁻¹ (Tak and Isman, 2017; Brito et al., 2021; Santana et al., 2022)], was evaluated in 50-mL plastic bottles used as fumigation chambers. Ten *Z. subfasciatus* adults (5 males and 5 females) aged between 0 and 48 h were placed in each chamber. The EO was applied into filter paper (1 × 1 cm) which were placed separately [by a thin tissue (*voile*)] from the grains and insects to avoid direct contact among them. The tested EO concentrations, defined in previous tests according to the method described by Finney (1971), were 10, 15, 20, 25, 30, 35, 40, 45, 50, and 100 µL L⁻¹ of air for the EO obtained from *E. latiflorus* leaves; 10, 50, 100, 125, 150, 225, 250, and 275 µL L⁻¹ of air for the EO obtained from *C. verbenacea* leaves; and 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, and 80 µL L⁻¹ of air for the binary mixture of the EOs. A negative control (no application) was also employed. A phosphine-based commercial formulation (Phostoxin®; Bequisa Indústria Química do Brasil, São Vicente, SP, Brazil) was used as a positive control at the recommended rate (equivalent to 2 g of phosphine m⁻³) to control stored-common bean pests in Brazil (Agrofit, 2023). The number of dead insects was counted after exposure for 72 h. Five replicates were used for each concentration (n = 50). On the basis of the results of the bioassays performed with the EOs and their binary mixture, lethal concentrations (LC₅₀ and LC₉₀) were estimated.

1.2.3.1.2 Topical contact bioassay

To assess the insecticidal action of treatments via dorsal contact, 10 adults (5 females and 5 males) aged between 0 and 48 h were distributed on Petri® dishes (90-mm diameter × 15-mm height) covered with a thin tissue (*voile*). To adjust the concentrations, a pre-mixture of the EOs and their binary mixture in acetone (analytical grade) were prepared at a concentration of 10% (v v⁻¹). For each test, aliquots of the pre-mixed EOs were diluted in acetone according to the pre-established concentrations and considering the average weight (0.0022 g) of males and females used in the bioassay. Thus, the applied concentrations, defined according to the method described by Finney (1971), were 10, 15, 20, 25, 30, 35, 40, 50, 100, 300, and 600 mg g⁻¹ for the EO obtained from *E. latiflorus* leaves; 10, 30, 50, 60, 65, 70, 75, 80, 90, 100, 300, and 600 mg g⁻¹ for the EO obtained from *C. verbenacea* leaves; and 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, and 80 mg g⁻¹ for the binary mixture of the EOs. The negative control consisted of acetone only. Moreover, a deltamethrin-based commercial insecticide (K-Obiol® 25 EC, Bayer Corp., São Paulo, SP, Brazil) was used as positive control at the recommended rate (80 mL t⁻¹) to control weevils in stored beans in Brazil (Agrofit, 2023).

The tested EO or mixture was topically applied on the dorsal part of the adult insects (0.5 µL per insect) by using a microsyringe (Hamilton®). The number of dead insects was evaluated 72 h after the application. Each concentration was replicated five times (n = 50). On the basis of the results of the bioassays performed with the EOs and their binary mixture, Lethal Concentrations (LC₅₀ and LC₉₀) were estimated.

1.2.3.1.3 Residual contact bioassay

To evaluate the bioactivity of the EOs obtained from *C. verbenacea* and *E. latiflorus* leaves, tested in isolation or as binary mixture (1:1, v v⁻¹), through residual contact, they were solubilized in acetone and applied on grain samples at different concentrations [31.25, 62.50, 125, 250, and 500 µL kg⁻¹ of grain for each EO or the binary mixture, previously defined on the basis of preliminary tests (Ribeiro et al., 2013; 2020)]. The tested EO or binary mixture was applied by using a micropipette with spray volume equivalent to 30 L t⁻¹. The negative control consisted of the rearing substrate (grains) treated with pure acetone (analytical grade). Moreover, a deltamethrin-based commercial insecticide (K-Obiol® 25 EC, Bayer Corp., São

Paulo, SP, Brazil) was used as positive control at the recommended rate (80 mL t⁻¹). After that, the grains were manually mixed and shaken for 30 s. Subsequently, they were placed in a light-protected chamber for 30 min to allow acetone used to resuspend the EO or binary mixture to evaporate.

Each flask (experimental unit), covered with voile tissue, was infested with 5 pairs of *Z. subfasciatus* aged between 0 and 48 h; 5 repetitions were used per tested concentration (n = 50). Three days after infestation, the number of dead individuals was assessed, and Lethal Concentrations (LC₅₀ and LC₉₀) were estimated.

1.2.3.1.4 Walking behavior assessment (no-choice test)

The effect of the EOs on the walking behavior of *Z. subfasciatus* adults (aged between 0 and 48 h) was evaluated separately by sex in a no-choice test. For this purpose, arenas formed by Petri dishes (90-mm diameter × 15-mm height) containing discs of filter paper (Ø 90 mm) were used as experimental units. To prevent insects from escaping from the arenas, a solid layer of vaseline (paraffin) was applied on the edge of the Petri dishes (arenas).

The LC₂₅ and LC₅₀, previously estimated in the residual contact bioassay, of each EO and the binary mixture were applied on the filter paper. To dilute the EOs, acetone (analytical grade) was employed as solvent, and a volume of 200 µL of dilution was applied onto the filter paper by using a micropipette. The bioassay consisted of 20 arenas [repetitions (10 females and 10 males)] per treatment, while the negative control consisted of pure acetone.

After the solvent was evaporated (5 min), an individual *Z. subfasciatus* adult (male or female) was inserted in the center of the arena. The average walking speed and the total distance covered by each insect in the arena were recorded for 10 min through automated video tracking by the Ethovision® software (Noldus Information Technology Corp., Wageningen, Netherlands). In addition to the software, the computational system was composed of a camera BRIO 4K PRO WEBCMAX V-U0040 model (Logitech de México S.A., Ciudad de México, México).

1.2.3.2 Antifungal and antiaflatoxigenic activity assessments

The antifungal and antiaflatoxigenic activities of the tested EOs and their binary mixture on *A. nomius* isolate were evaluated by using the “poison food” method (Alvarez-Castellanos et al., 2001). Fungal mycelial growth was observed in yeast-extract sucrose (YES) culture medium (Himedia Co., Mumbai, MH, India)

containing different concentrations of each EO or binary mixture (312.5, 625, 1,250, 2,500, or 5,000 $\mu\text{L L}^{-1}$) solubilized with the aid of an emulsifier (Tween® 20) and incorporated by manual shaking into the non-solidified culture medium ($\sim 45^{\circ}\text{C}$). The medium + EO (10 mL) were placed in each Petri dish (60-mm diameter). The tests were conducted in triplicate. The negative control was the emulsifier. No positive control was legally available.

The fungus was inoculated on a sterilized paper disk (5 mm) and placed on the center of the surface of the medium after its solidification. A conidial solution (5 mL) was transferred with the aid of a micropipette. The conidial suspension was prepared by scraping the surface of a dish containing fungal colonies maintained in potato, dextrose, and agar (PDA) medium with a Drigalski loop for 7 days in potato, dextrose, and agar (PDA) medium with a Drigalski loop. The number of conidia in the solution was standardized to $2 \text{ to } 9 \times 10^6$ conidia mL^{-1} , measured in a hemocytometer-type Neubauer chamber. The inoculated dishes were incubated upside at $25 \pm 2^{\circ}\text{C}$ and 14 L: 10 D h photoperiod. Radial mycelial growth was assessed 144 h after fungal inoculation. The diameter of each colony was measured in two directions at right angles by using a caliper rule with a minimum measurement of 0.05 mm.

Aflatoxin (B_1 , B_2 , G_1 , and G_2) production by the *A. nomius* isolate growing in a culture medium containing an EO or the binary mixture was determined by thin-layer chromatography (TLC). The whole culture medium was removed from the dishes with stylets and spatulas and transferred to a Falcon tube (50 mL), and the aflatoxins were extracted. To extract the aflatoxins, 10 mL of chloroform was transferred to the Falcon tube, and the contents were shaken on a Vortex stirrer for 2 min.

The presence of aflatoxins in the extract was determined and quantified on an aluminum chromatoplate coated with silica gel. The chromatography plates were developed in vats containing 50 mL of the eluent system consisting of toluene/ethyl acetate/formic acid/chloroform (18.0: 14.5: 3.5: 14.0, v/v/v/v). The aflatoxins in the samples were identified by comparing the samples with the aflatoxin standards. The aflatoxin mix standard with B_1 , B_2 , G_1 , and G_2 , a certified reference material CRM 46303 (Sigma-Aldrich Corp., St. Louis, MO, USA), was used, and the concentration was determined by using the method 971.22 of the Association of Official Analytical Chemists (Association of Official Analytical

Chemists, 2005). For this analysis, the aflatoxin spot intensities present in the sample were compared with aliquots of 3, 4, and 6 μL of the standards. The contamination was calculated by using the following equation:

$$AF = [(YSV)/(XA)]$$

where AF = aflatoxin content in ng per square centimeter; Y = concentration of the standard in ng μL^{-1} ; S = μL of the toxin standard with fluorescence (emission at 365 nm) equivalent to the sample (same color appearance and retention factor – Rf); V = final volume of the extract (sample) in μL ; X = volume of the extract (sample) applied in μL ; and A = Area of the colony growth in the final evaluation day, in centimeters, in the final extract. An ultraviolet viewing cabinet model SL 204 (Solab Equipamentos para Laboratório Ltda., Piracicaba, SP, Brazil) with two 15-W tube lamps of 365-nm wavelength was used.

1.2.4 Data analysis

All the bioassays were conducted under a completely randomized design. The data obtained from walking behavior assessments were firstly analyzed for normality of residuals and homogeneity of variances; the Shapiro-Wilk test (Shapiro and Wilk, 1965) and the Bartlett test (Bartlett, 1964) were respectively employed. When these assumptions were not met, the non-parametric Kruskal-Wallis analysis of variance was performed, with the means compared by Dunn's test with Bonferroni correction at a 5% error probability. The statistical analyses were performed by using the R software version 4.2.1 (R Core Team, 2021).

To estimate the Lethal Concentrations or Lethal Doses (LC_{50} and LC_{90} or LD_{50} and LD_{90}), a binomial model with log-log complement function (Gompit model) was applied by using the Probit Procedure of SAS software version 9.2. (SAS Institute, 2011). To construct the isobolograms, the 'drc' package (Ritz et al., 2015) was adopted by using the additive concentration model proposed by Loewe (1928). The data from the concentration-response curves for the isolated EOs and their binary mixture (1:1, v v⁻¹) were adjusted by using Generalized Linear Models (GLM) with a binomial distribution (Nelder and Wedderburn, 1972). These statistical procedures and graphical representations were conducted by using the R software version 4.2.1 (R Core Team, 2021).

The data regarding the antifungal and antiaflatoxigenic actions were submitted to a linear model ($y = b_1 + b_2x$), where b_1 = intercept, b_2 = slope representing the rate of decay, and non-linear regression, adjusted by the Michaelis-Menten model ($y = b_1/(1+((1-0.05)/0.05) \times ((x/b_2)^{b_3}))$) with parameterization proposed by Zeviani (2013), in which: y = response variable; x = concentration tested; b_1 = maximum asymptote; b_2 = concentration that inhibited 99% of fungal growth and aflatoxin production (CI95%, with confidence intervals); and b_3 = parameter that controls the form of the function. Aflatoxin (B1, B2, G1, and G2) production was submitted to non-linear regression, by adjusting the exponential decay model ($y = b_1 \cdot \exp(b_2 \cdot x)$; b_1 represents the initial amount of B1, B2, G1, and G2 being measured, b_2 is a constant representing the rate of decay), and x the concentration tested. These regression analyses were also performed by using the R software version 4.2.1 (R Core Team, 2021).

1.3 RESULTS

1.3.1 EO extraction yield and chemical constituents

The extraction yields of the EOs from *C. verbenacea* and *E. latiflorus* leaves through hydrodistillation process were 0.36 and 0.78% (g of oil/g of fresh leaves), respectively. A total of 25 compounds were identified in the EO obtained from *C. verbenacea* leaves, and sesquiterpene hydrocarbons predominated (86.5%) (Table 1). The sesquiterpenes α -zingiberene (41.5%), β -sesquiphellandrene (20.9%), *trans*-caryophyllene (8.0%), and α -curcumene (6.6%) were the major compounds (Table 1). On the other hand, 15 compounds were identified in the EO obtained from *E. latiflorus* leaves, and oxygenated monoterpenes predominated (95.7%, Table 1). The major compounds in this EO were geranial (56.4%) and neral (35.1%) (Table 1).

1.3.2 Bioactivities of the EOs and their binary mixture on *Zabrotes subfasciatus*

Regardless of the exposure mode, the EOs obtained from *C. verbenacea* and *E. latiflorus* leaves showed lethal toxicity in a concentration/dose-dependent manner (Table 2). Nevertheless, the EO obtained from *E. latiflorus* leaves was more potent than the EO obtained from *C. verbenacea* leaves in all the evaluated

exposure modes, according to the confidence interval (CI95%) of adjusted values (Table 2). After 72 h of exposure, the phosphine-based commercial formulation (fumigation bioassay) and a deltamethrin-based commercial formulation (residual contact and topical application bioassays) resulted in 100% mortality of the exposed weevils in all exposure modes (data not shown).

The binary mixture (1:1, v v⁻¹) of the EOs demonstrated an additive effect in both the residual contact and topical application exposure modes (Figure 1). In the residual contact bioassay, the additive concentration method was accepted in the analysis of isobolograms [$F_{(1,73)} = 0.568$; $p = 0.453$] given that the model predicted by the additive concentration method intercepted the LC₅₀ value and its respective confidence interval for the binary mixture of the EOs (Figure 1D). Similar results were observed for the topical application exposure mode, where the additive concentration model was fitted for the binary mixture of the EOs [$F_{(1,78)} = 3.21$; $p = 0.077$] (Figure 1F). However, in the fumigation exposure, the additive method did not fit [$F_{(1,83)} = 9.91$; $p = 0.002$]: the LC₅₀ value and its respective confidence interval for the binary mixture were above the additivity line, suggesting an antagonistic effect of the binary mixture of these EOs (Figure 1B).

In addition to the observed lethal effects, the EO obtained from *E. latiflorus* leaves at its previously estimated LC₅₀ (residual contact bioassay) significantly reduced the distance covered by females compared to the control group (Figure 2) (kw = 26.039; df = 6; $p < 0.001$). Despite the differences between the treatments in terms of female walking speed (kw = 18.427; df = 6; $p = 0.005$), none of the treatments showed statistically significant differences in walking speed compared to the control (Figure 2). In general, males showed higher walking speed than females (Figure 2). The typical displacement behavior of *Z. subfasciatus* males and female in the treated arenas under the influence of the EOs or binary mixture are shown in Figure 3.

1.3.3 Fungicidal and antiaflatoxigenic effects of the EOs and their binary mixture on *A. flavus*

The EOs obtained from *E. latiflorus* and *C. verbenacea* leaves and their binary mixture inhibited the radial growth of the *A. nomius* isolate in a concentration-dependent manner (Figure 4A). A logistic model was fitted to describe the growth behavior of *A. nomius* as a function of the increasing concentration of the EO obtained from *E. latiflorus* leaves and the binary mixture,

while a linear model was fitted to describe the radial growth inhibition as a function of the concentration in culture medium (Figure 4A). The EO obtained from *E. latiflorus* leaves was more potent [CI99% = 687.70 $\mu\text{L L}^{-1}$ (486.20 – 889.19)] than the binary mixture [CI99% = 2918.75 $\mu\text{L L}^{-1}$ (1738.74 – 4098.75)] and the EO obtained from *C. verbenacea* leaves (CI99% $\geq 5,000 \mu\text{L L}^{-1}$). Isobologram analysis was employed to examine the interaction of both EOs in the binary mixture (CV/EL MIX) during the radial growth of *A. nomius*, but the fitted model was not significant [$F_{(1,41)} = 46.188$; $p < 0.001$].

Besides antifungal action, the EO obtained from *E. latiflorus* leaves and the binary mixture showed concentration-dependent antiaflatoxigenic action and considerably reduced the production of aflatoxins B₁, B₂, G₁, and G₂ (Figure 5). An exponential decay model was fitted to describe the inhibition of the production of aflatoxins B₁, B₂, G₁, and G₂ as a function of the concentration of the EO obtained from *E. latiflorus* leaves. The binary mixture in culture medium, while a linear model was used to describe the behavior of the EO obtained from *C. verbenacea* leaves (Figure 5).

1.4 DISCUSSION

EOs constitute an important source of insecticidal, fungicidal, and antiaflatoxigenic compounds that can be potentially used through different application forms in the IPM of stored-grain pests and aflatoxin-producing fungal species, especially in the organic grain market in which synthetic compounds are not allowed. Our results from different modalities of laboratory bioassays indicate that the EO obtained from *E. latiflorus* leaves is a promising source of grain protective compounds with simultaneous action on stored-grain spoilers. This is the first report on the bioactivity of *E. latiflorus* derivatives against a pest species of agricultural relevance. To a lesser extent, the EO obtained from *C. verbenacea* leaves also shows interesting insecticidal properties, and this is the first study that has reported on the lethal toxicity of this EO against *Z. subfasciatus*. EOs act differently on insects: they can inhibit the cholinergic system or P450 cytochromes, modulate the octopaminergic system, or interfere in GABA receptors (Pavela and Benelli, 2016; Li et al., 2020; Liu et al., 2022). Thus, these EOs can be used in the preventative management of stored-bean pests, including resistant insect populations.

Our GC-MS analyses indicate that oxygenated monoterpenes (95.7%) and sesquiterpene hydrocarbons (86.5%) predominate in the EOs obtained from *E. latiflorus* and *C. verbenacea* leaves, respectively. The compounds geranial (or (*E*)-citral; 56.4%) and neral (or (*Z*)-citral; 35.1%) are the major compounds in the EO obtained from *E. latiflorus* leaves. These data resemble the data reported by Braga et al. (2022), which indicated that citral (65.38%) predominates in the EO obtained from this plant species. By studying different populations of a related species, namely *Elionurus muticus* Humb. & Bompl Ex Willd (Poaceae), from the Pampa biome (Rio Grande do Sul State, Brazil), Füller (2008) verified allogamy predominance and high genetic variability, including variability in terms of EO yield and citral abundance. Citral is largely used in the perfumery, food, and cosmetic industries and is one of the main compounds in EOs commercialized worldwide. Thus, the *Elionurus* species is an interesting source of (*E/Z*) citral, and selection, domestication, and breeding procedures should be employed to improve its production and industrial applications (Dudai, 2011; Morshedloo et al., 2018; Navarro-Rocha et al., 2020).

Regarding the composition of the EO obtained from *C. verbenacea* leaves, we have detected the sesquiterpenes α -zingiberene (41.5%), β -sequiphellandrene (20.9%), *trans*-caryophyllene (8.0%), and α -curcumene (6.6%) as the major compounds. This result differs from previously published results showing considerable diversity in the major compounds in the EO obtained from *C. verbenacea* and their proportions. On this matter, α -pinene (Vila et al., 2009; Sciarrone et al., 2017; Pereira et al., 2021), α -humulene (Ribeiro et al., 2012), tricyclene (Meccia et al., 2009), α -santalene (Santos et al., 2015), sabinene (Matias et al., 2016), and methyl(2*E*,6*E*)-farnesoate and β -caryophyllene (Silva et al., 2012, 2014) were detected as the major compounds in the EO obtained from *C. verbenacea* of different origins. Environmental and developmental factors qualitatively and quantitatively influence secondary metabolites (allelochemicals) and, consequently, the biological properties of EOs (Li et al., 2020). Thus, further studies should be conducted to assess the genetic variability, including the incidence of chemotypes, as well as factors that affect EO production and composition in this Boraginaceae species.

Although further studies should be carried out with isolated compounds to offer clear evidence of the contribution of each of them to the overall bioactivity of

the EO, the insecticidal activity of the major compounds in the studied EOs has already been documented. For example, citral and geranial have been shown to have strong contact and fumigant toxicity against the cigarette beetle *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae), the booklouse *Liposcelis bostrychoplila* (Badonnel) (Psocoptera: Liposcelididae) (Yang et al., 2014), the rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), and the red flour beetle *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) (Elgizawy et al., 2019). Moreover, the major compounds in the studied EOs are important compounds of bioactive EOs obtained from different plant species, with spectra of action reported against stored-grain pest species (Fazolin et al., 2023).

Even though repellence is an outstanding characteristic of many EOs (Nerio et al., 2010; Benelli and Pavela, 2018), our findings from walking behavior assessments also indicate that the EOs obtained from *E. latiflorus* leaves have an arresting effect on *Z. subfasciatus* females. In general, odorant binding proteins present in the sensilla of insects respond to terpenoid substances (mainly volatile monoterpenes) and lead to irritations that alter the behavior and activate the taste receptor neurons present in the tarsi (Vosshall and Stocker, 2007; Deletre et al., 2015). Geranial, the major compound in the EO obtained from *E. latiflorus* leaves, changes the walking behavior of insects, as already reported for different species (Deletre et al., 2015; Larson et al., 2020; González-Morales et al., 2021). However, the reduced movement should also hypothetically be related to the action of active principles on the central nervous system because knock-down is a characteristic of these neurotoxins. Exposure to lemongrass EO (rich in citral and isomers) changes the behavior of both sexes of *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae), decreases the triacylglycerol content of virgin and mated females, and reduces the activities of acetylcholinesterase (females and males) and β -esterase (females) (Alves et al., 2019).

Combining EOs is an interesting approach in the framework of pest management given that a high diversity of compounds in the mixtures can increase their toxicity (Miresmailli et al. 2006; Jiang et al. 2009; Santana et al., 2022). For example, Haouel-Handi et al. (2021) showed that the binary mixture of EO from different species of *Mentha* (Lamiaceae) were more potent against *S. oryzae* than the same EO tested individually in fumigation and repellent bioassays. Additionally, synergistic mixtures of EOs can potentially reduce the costs associated with new

formulations of botanical insecticides because they require smaller quantities of active compound(s), while mitigating the selection of resistant insect populations (Akhtar and Isman 2013; Santana et al., 2022). Nevertheless, antagonistic interactions can occur due to, among other factors, competition for target-site receptors, especially among the enzymes of the insects' central nervous system (Savelev et al., 2003; Fazolin et al., 2023).

In this study, we employed isobologram analysis to elucidate the effects of the binary mixture (1:1, v v⁻¹) of the EOs derived from *E. latiflorus* and *C. verbenacea* leaves on *Z. subfasciatus* adults. Our results demonstrate an additive effect through topical and residual contact and antagonism through fumigation. These results are probably related to interactions of major and minor compounds present in the mixture, affecting the chemical characteristics and potency of blends in different exposure ways. The chemical structures and characteristics of each component in EO tested, the combination between major and minor compounds and the proportion of them in the final mixture are important factors to occurrence of additive or synergistic effects in pest control (Tak and Isman, 2015; Wangrawa et al., 2022; Fazolin et al., 2023). These findings underscore the need for further investigations to optimize the proportions of individual EOs in artificial blends and to explore the potential of more powerful combinations involving different EOs sourced from the diversified Neotropical flora. Such knowledge about interactions among chemical compounds will undoubtedly contribute to the development of optimized botanical insecticide formulations for the market (Gonçalves et al., 2015).

Stored-grain insect infestation increases the moisture of grain mass (Manu et al., 2019), creating conditions for fungal species to develop. Some of these species, such as *A. nomius*, can synthesize mycotoxins. In addition to its insecticidal properties, the EO obtained from *E. latiflorus* leaves and the tested binary mixture display promising fungicidal and antiaflatoxigenic effects on the *A. nomius* isolate, which produces the aflatoxins B₁, B₂, G₁, and G₂. Thus, products that act against mycotoxin-producing fungi, as well as protectants against insect pests are useful in the framework of pest management in warehouses, but they are currently unavailable in the market (Ribeiro et al., 2020; Zimmermann et al., 2022).

As the major components of EOs obtained from different plant species, geranial and neral display antifungal and antiaflatoxigenic actions against an

aflatoxin B₁ producer isolate of *Aspergillus flavus* (Shukla et al., 2009). Pandey et al. (2016) also verified that the EO obtained from *Lippia alba* (Mill.) N.E. Brow (Verbenaceae), which is rich in geranial (36.9%) and neral (29.3%), significantly inhibits fungal proliferation and aflatoxin production in green gram seeds during storage. Reduced aflatoxin production elicited by low concentrations of citral (an isomer of neral) may stem from down-regulated transcription of aflatoxin biosynthetic genes, as shown by Liang et al. (2015) and Moon et al. (2018). Furthermore, Tang et al. (2018) indicated that citral exhibits antifungal activity mainly by downregulating the sporulation- and growth-related genes of *Aspergillus ochraceus* Wilhelm and *A. flavus*. Although the binary mixture of the EOs tested here has significant insecticidal activity, only the EO obtained from *E. latiflorus* leaves presents promising fungicidal and antiaflatoxigenic activities, so further studies should be carried out with higher concentrations of the EO obtained from *C. verbenacea* leaves to elucidate its potency better. Zimmermann et al. (2023) demonstrated that EOs interact in combination, exhibiting an additive effect against *Fusarium graminearum* and *A. flavus*.

1.5 CONCLUSIONS

EOs constitute a compelling alternative strategy for controlling pests in stored grains, which makes these oils valuable sources of novel compounds that can serve as prototypes for the development of new grain protectants. The EOs tested here, particularly those derived from *E. latiflorus*, have proven promising grain protectants with multifaceted actions against grain spoilers. However, further investigations are warranted to elucidate the individual contributions of each component to the overall activity of the EOs, along with the assessment of their toxicological profiles and degradability characteristics under agricultural storage conditions. Such studies will contribute to obtaining more effective and environmentally sustainable pest management approaches in the context of grain storage.

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Table 1. Chemical composition of the essential oils (EOs) obtained from *Cordia verbenacea* and *Elionurus latiflorus* leaves as determined by GC-MS.

Compounds	<i>Cordia verbenacea</i>				<i>Elionurus latiflorus</i>			
	RT (min)	RI _{exp}	RI _{lit}	%RA	RT(min)	RI _{exp}	RI _{lit}	%RA
α -Thujene	6.17	925	931	0.8	6.18	930	931	0.1
α -Pinene	6.35	931	939	5.0	6.35	937	939	0.5
Sabinene	7.24	958	976	t	-	-	-	-
β -Myrcene	7.65	990	991	t	-	-	-	-
β -Phellandrene	8.77	1030	1031	t	-	-	-	-
Eucalyptol	8.86	1033	1033	t	-	-	-	-
Geranial	16.60	1268	1270	t	18.24	1268	1270	56.4
Citronellyl acetate	21.13	1352	1354	0.3	-	-	-	-
β -Elemene	22.64	1374	1375	0.5	-	-	-	-
<i>Trans</i> -caryophyllene	23.79	1428	1428	8.0	-	-	-	-
γ -Elemene	24.00	1431	1430	0.4	-	-	-	-
α - <i>Trans</i> -bergamotene	24.40	1440	1436	1.7	-	-	-	-
α -Humulene	25.08	1458	1455	1.5	-	-	-	-
(<i>E</i>)- β -Farnesene	25.25	1456	1458	0.7	-	-	-	-
Aromadendrene	25.35	1464	1464	0.3	-	-	-	-
α -Curcumene	26.34	1474	1474	6.6	-	-	-	-
α -Zingiberene	26.99	1498	1496	41.5	-	-	-	-
β -Bisabolene	27.35	1505	1509	4.0	-	-	-	-
γ -Cadinene	27.59	1511	1513	0.4	-	-	-	-
β -Sesquiphellandrene	28.02	1517	1515	20.9	-	-	-	-
(<i>E</i>)-Nerolidol	29.38	1562	1564	0.3	-	-	-	-
Sphatulenol	29.90	1574	1575	0.7	29.88	1574	1575	0.9
Caryophyllene oxide	30.09	1578	1581	0.2	28.82	1550	1581	-
Torreyol	32.42	1642	1645	0.9	-	-	-	-
<i>Z,E</i> -farnesol	34.03	1699	1697	2.9	-	-	-	-
Sulcatone	-	-	-	-	7.59	984	985	0.3
(<i>Z</i>)- β -Ocimene	-	-	-	-	9.02	1039	1040	0.1
(<i>E</i>)- β -Ocimene	-	-	-	-	9.35	1048	1050	0.1
Linalool	-	-	-	-	11.14	1095	1098	1.7
<i>Trans</i> -limonene oxide	-	-	-	-	13.56	1139	1139	0.6
<i>Cis</i> -verbenol	-	-	-	-	14.28	1141	1142	0.9
Decanal	-	-	-	-	15.16	1183	1185	0.2
Neral	-	-	-	-	16.89	1241	1240	35.1
Neryl acetate	-	-	-	-	22.43	1360	1362	1.0
Germacrene B	-	-	-	-	26.79	1559	1560	0.3
Globulol	-	-	-	-	30.81	1602	1604	0.1
Monoterpene hydrocarbons	5.3				0.8			
Oxygenated monoterpenes	0.3				95.7			
Sesquiterpene hydrocarbons	86.5				0.3			
Oxygenated sesquiterpenes	5.0				1.0			
Others	--				0.5			

Not identified	2.9	1.7
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RT: retention time (min) on a Rtx-5MS column; RI_{exp}: experimental retention index relative to a mixture of homolog *n*-alkanes (C₈-C₂₀) on a Rtx-5MS column. RI_{lit}: retention index value from the literature (Adams, 2007; NIST Chemistry Weebbook, 2022). %RA: relative area.

Table 2. Estimated lethal concentrations/doses (LC₅₀ and LC₉₀ or LD₅₀ and LD₉₀) of the essential oils obtained from *Cordia verbenacea* and *Elionurus latiflorus* leaves, in isolation or as binary mixture, to *Zabrotes subfasciatus* adults after exposure for 72 hours by means of different exposure modes.

Exposure mode	Treatments	n ¹	Slope ± SE (p-value)	LC ₅₀ or LD ₅₀ (CI) ²	LC ₉₀ or LD ₉₀ (CI) ²	χ ²⁽³⁾	df ⁴	h ⁵
Fumigation (LC, in µL L ⁻¹ of air)	<i>Cordia verbenacea</i> EO	300	2.63±0.30	68.92 (39.90 – 96.65)	211.39 (142.56 – 529.29)	9.97	4	2.49
	<i>Elionurus latiflorus</i> EO	300	8.12±0.87	13.81 (11.32 – 15.95)	19.86 (17.07 – 26.61)	9.60	4	2.40
	Binary mixture (CV:EL, 1:1)	300	4.60±1.36	34.26 (19.59 – 72.43)	143.56 (69.43 – 4303)	7.05	4	1.76
Residual contact (LC, in µL kg ⁻¹)	<i>Cordia verbenacea</i> EO	300	8.69±1.30	283.77 (259.06 – 305.27)	398.47 (365.58 – 454.68)	1.95	3	0.65
	<i>Elionurus latiflorus</i> EO	300	4.76±0.64	106.68 (92.12 – 121.89)	198.34 (168.32 – 252.95)	2.32	3	0.77
	Binary mixture (CV:EL, 1:1)	300	3.50±0.35	153.32 (133.72 – 171.52)	232.46 (203.55 – 292.94)	1.97	3	0.66
Topical application (LD, in mg g ⁻¹)	<i>Cordia verbenacea</i> EO	300	3.38±0.39	66.25 (47.55 – 95.33)	158.67 (106.15 – 514.48)	6.44	3	2.15
	<i>Elionurus latiflorus</i> EO	300	4.31±0.46	19.59 (17.39 – 22.03)	38.82 (33.82 – 48.42)	0.11	3	0.04
	Binary mixture (CV:EL, 1:1)	300	3.50±0.35	25.36 (18.20 – 32.97)	58.81 (43.09 – 114.39)	10.27	4	2.57

¹n: number of tested insects; ² CI: 95% confidence interval; ⁽³⁾χ²: Chi-square value; ⁴df: degrees of freedom; ⁵ h.: Heterogeneity factor.

Table 3. Estimated parameters of the regression models adjusted for the diameter of the colonies and production of aflatoxins B₁, B₂, G₁, and G₂ by *Aspergillus nomius* in culture medium containing different concentrations of the essential oils obtained from *Cordia verbenacea* (CV-EO) and *Elionurus latiflorus* (EL-EO) leaves, tested in isolation or as binary mixture (CV/EL MIX), after incubation for 144 h.

Treatment	Parameters			R ²
	b ₁	b ₂	b ₃	
Diameter of colonies				
CV-EO ^b	54.58 (54.22; 54.94)*	-0.0008 (-0.0009; -0.0006)		0.95
CV/EL MIX ^a	52.54 (48.80; 56.29)	1823.61 (1333.15; 2314.06)	3.51 (2.43; 4.58)	0.97
EL-EO ^a	54.77 (53.15; 56.38)	530.98 (426.34; 635.61)	6.38 (4.00; 8.76)	0.99
Production of aflatoxin B1 ^c				
CV-EO	836.70 (702.5; 970.84)	-0.0001 (-0.0002; -0.0000012)		0.37
CV/EL MIX	983.00 (835.86; 1130.06)	-0.0015 (-0.0021; -0.0010)		0.93
EL-EO	992.90 (851.74; 1134.11)	-0.0031 (-0.0041; -0.0021)		0.94
Production of aflatoxin B2 ^c				
CV-EO	591.50 (371.98; 811.04)	-0.00017 (-0.00042; 0.000081)		0.17
CV/EL MIX	954.10 (762.91; 1145.29)	-0.0071 (-0.0128; -0.0013)		0.88
EL-EO	955.10 (771.83; 1138.38)	-0.0109 (-0.0296; -0.0077)		0.90
Production of aflatoxin G1 ^c				
CV-EO	1786.00 (1642.20; 1929.69)	-0.00002 (-0.00005; 0.00001)		0.12
CV/EL MIX	2045.00 (1476.05; 2614.69)	-0.0009 (-0.0016; -0.00032)		0.77
EL-EO	1952.00 (1428.27; 2476.48)	-0.0021 (-0.0033; -0.0009)		0.81
Production of aflatoxin G2 ^c				
CV-EO	1612.00 (1336.85; 18887.80)	-0.00008 (-0.0002; 0.000004)		0.27
CV/EL MIX	2160.00 (1732.79; 2586.48)	-0.0013 (-0.0019; -0.00073)		0.87
EL-EO	2148.00 (1970.53; 2326.24)	-0.0048 (-0.0058; -0.0037)		0.98

^a Michaelis-Menten model ($y = b_1 / (1 + ((1 - 0.05) / 0.05) \times ((x / b_2)^{b_3}))$);

^b Linear model ($y = b_1 + b_2x$);

^c Exponential decay model ($y = b_1 \exp(b_2x)$);

* CI95% (LL, UL)

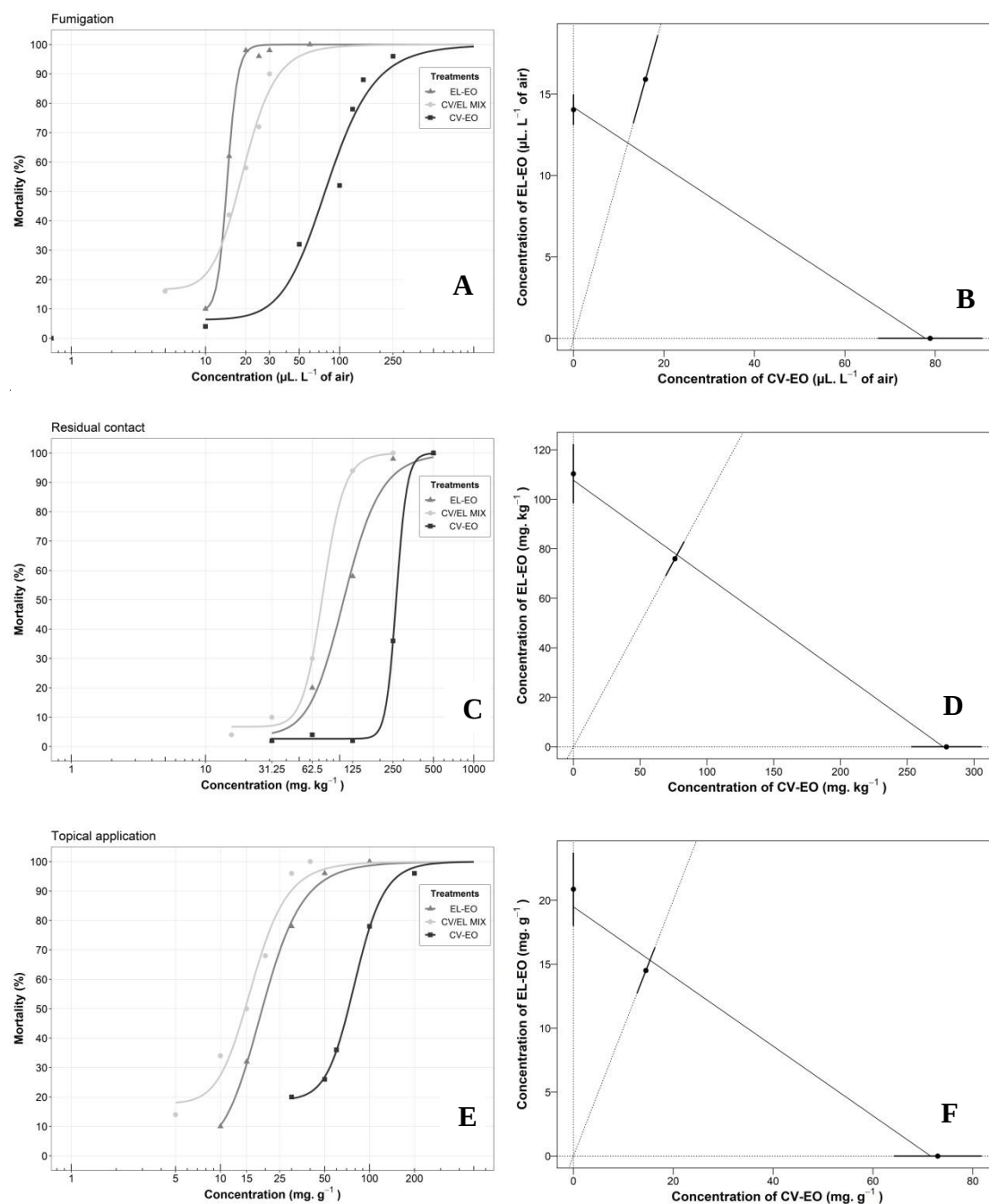


Figure 1. A, C, and E) Concentration/dose response curves and B, D, and F) isobolograms of the essential oils obtained from *Cordia verbenacea* (CV-EO) and *Elionurus latiflorus* (EL-EO) leaves, tested in isolation or as binary mixture (CV/EL MIX) on *Zabrotes subfasciatus* adults after exposure by different modes for 72 hours (fumigation, residual contact, or topical application).

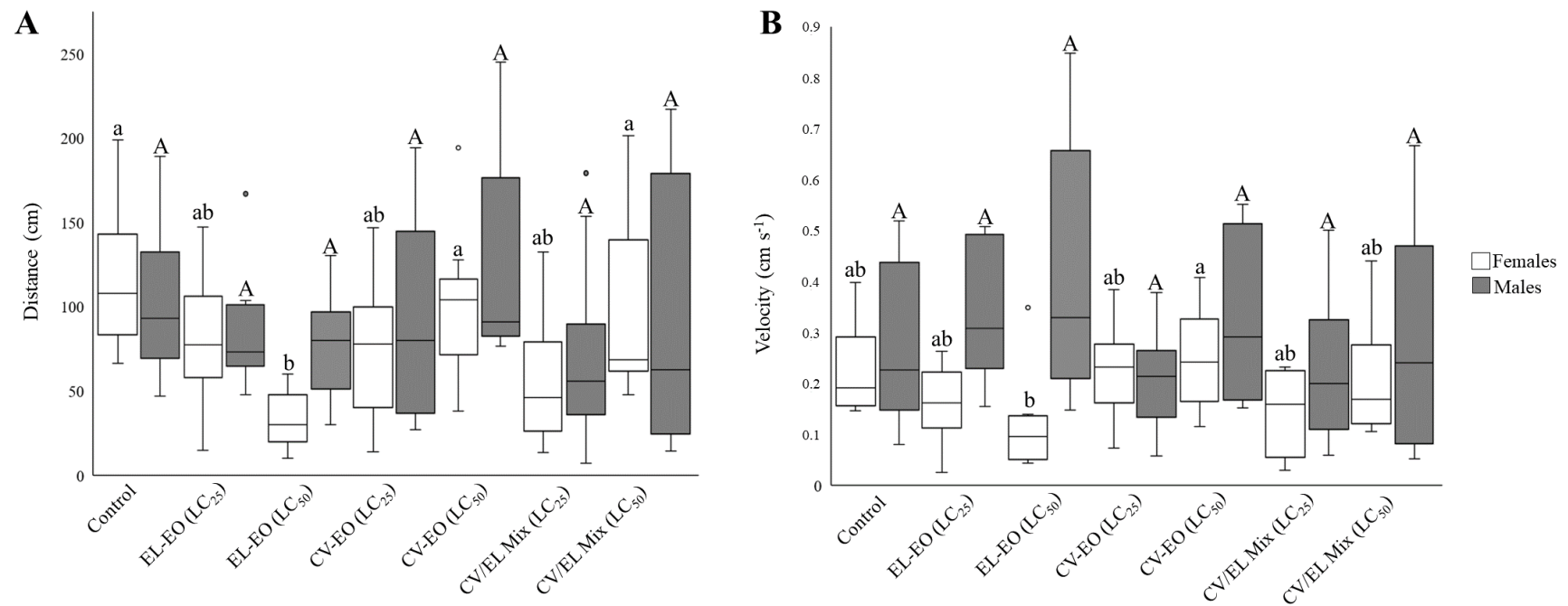


Figure 2. A) Mean distance covered and B) Mean speed of displacement of male and female *Zabrotes subfasciatus* adults exposed to surfaces treated with the essential oils (at previously estimated LC₂₅ and LC₅₀) obtained from *Cordia verbenacea* (CV-EO) and *Elionurus latiflorus* (EL-EO) leaves, tested in isolation or as binary mixture (CV/EL MIX, 1:1), in 10 min of computational monitoring (Ethovision® system). Data followed by the same letters, uppercase to compare males and lowercase to compare females in each assessed parameter, do not differ significantly by the Dunn's test with Bonferroni correction at 5% error probability.

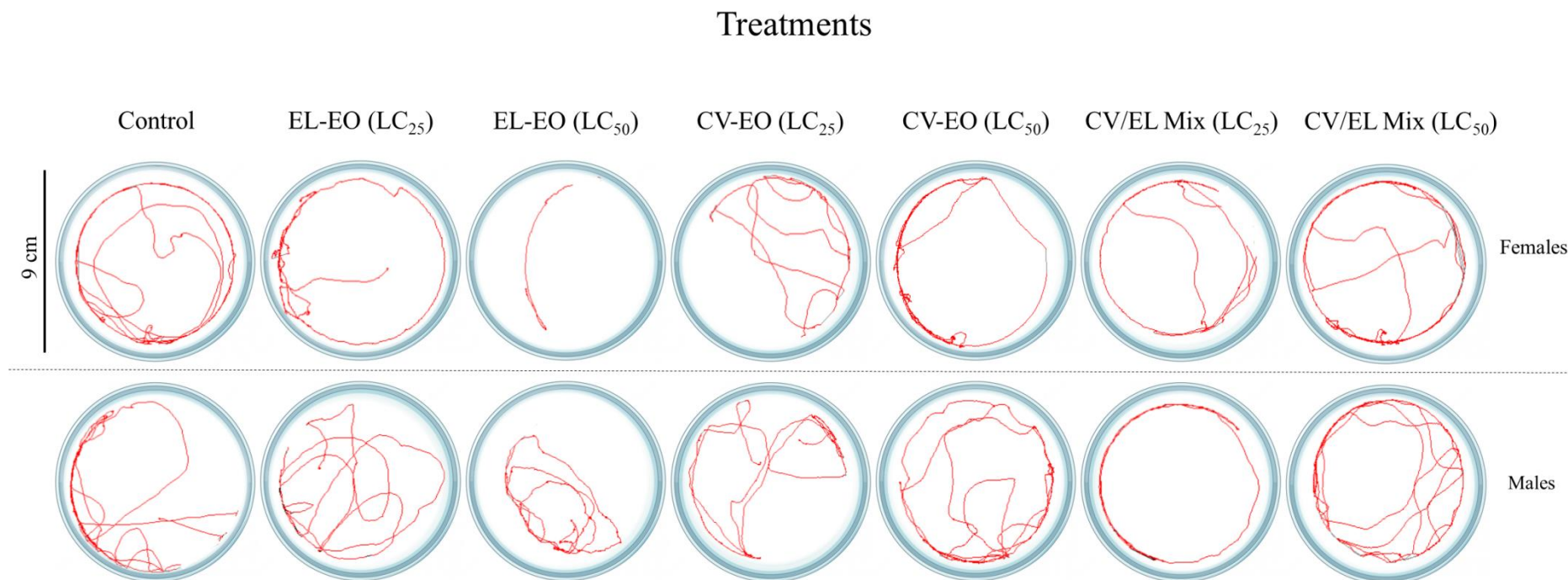


Figure 3. Lines showing the typical walking movement of *Zabrotes subfasciatus* male and female adults during 10 min of observation in Petri dishes (Ø 9 cm) superimposed with filter paper treated with the essential oils (at previously estimated LC₂₅ and LC₅₀) obtained from *Cordia verbenacea* (CV-EO) and *Elionurus latiflorus* (EL-EO) leaves, tested in isolation or as binary mixture (CV/EL MIX).

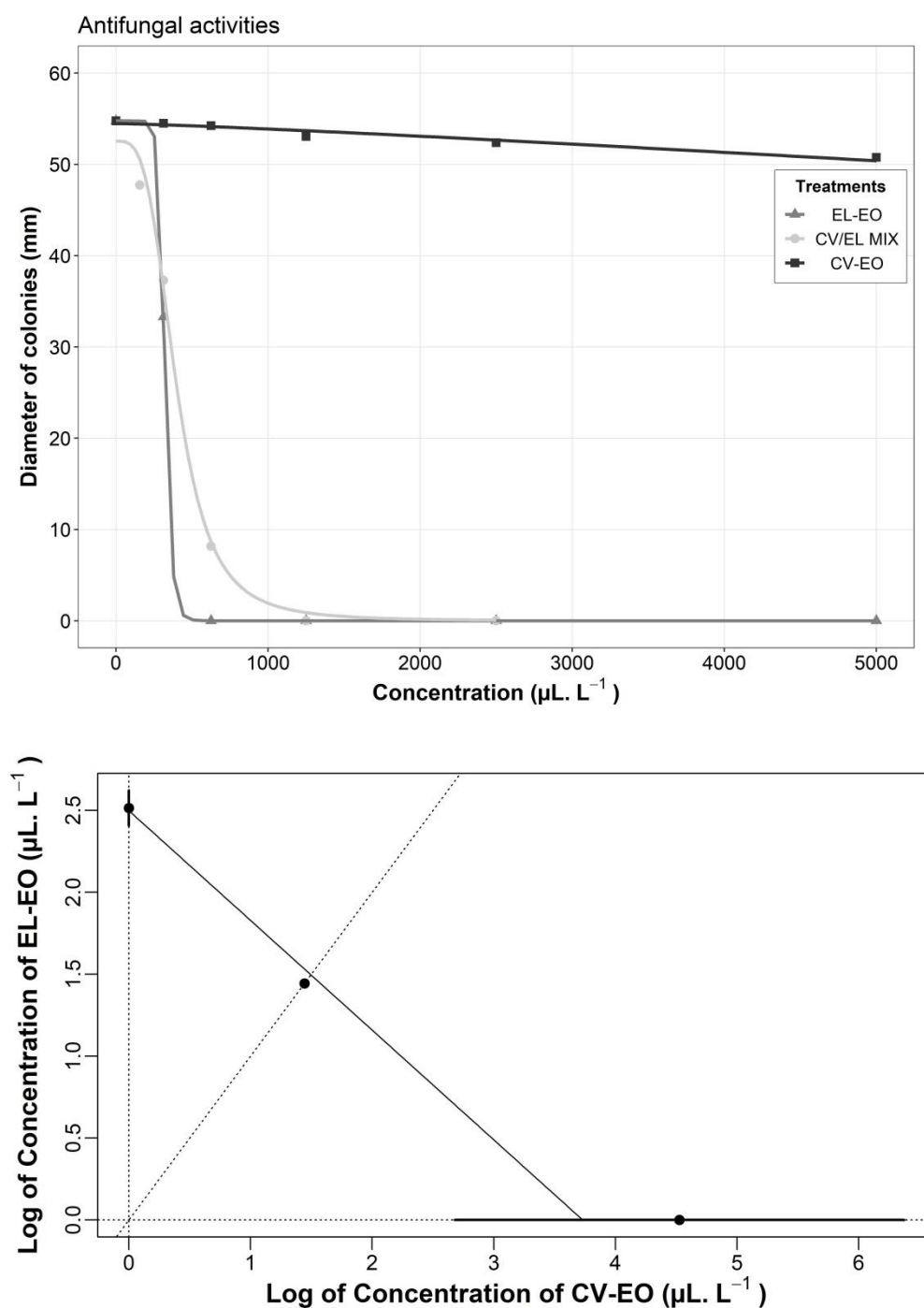


Figure 4. A) Radial growth of *Aspergillus nomius* colonies in culture medium containing different concentrations of the essential oils obtained from *Cordia verbenacea* (CV-EO) and *Elionurus latiflorus* (EL-EO) leaves, tested in isolation or as binary mixture (CV/EL MIX), after incubation for 144 h; B) isobologram showing the interaction of a binary mixture (CV/EL MIX) of both essential oils during the radial growth of *A. nomius*. Note: Details of the adjusted models are shown in Table 3.

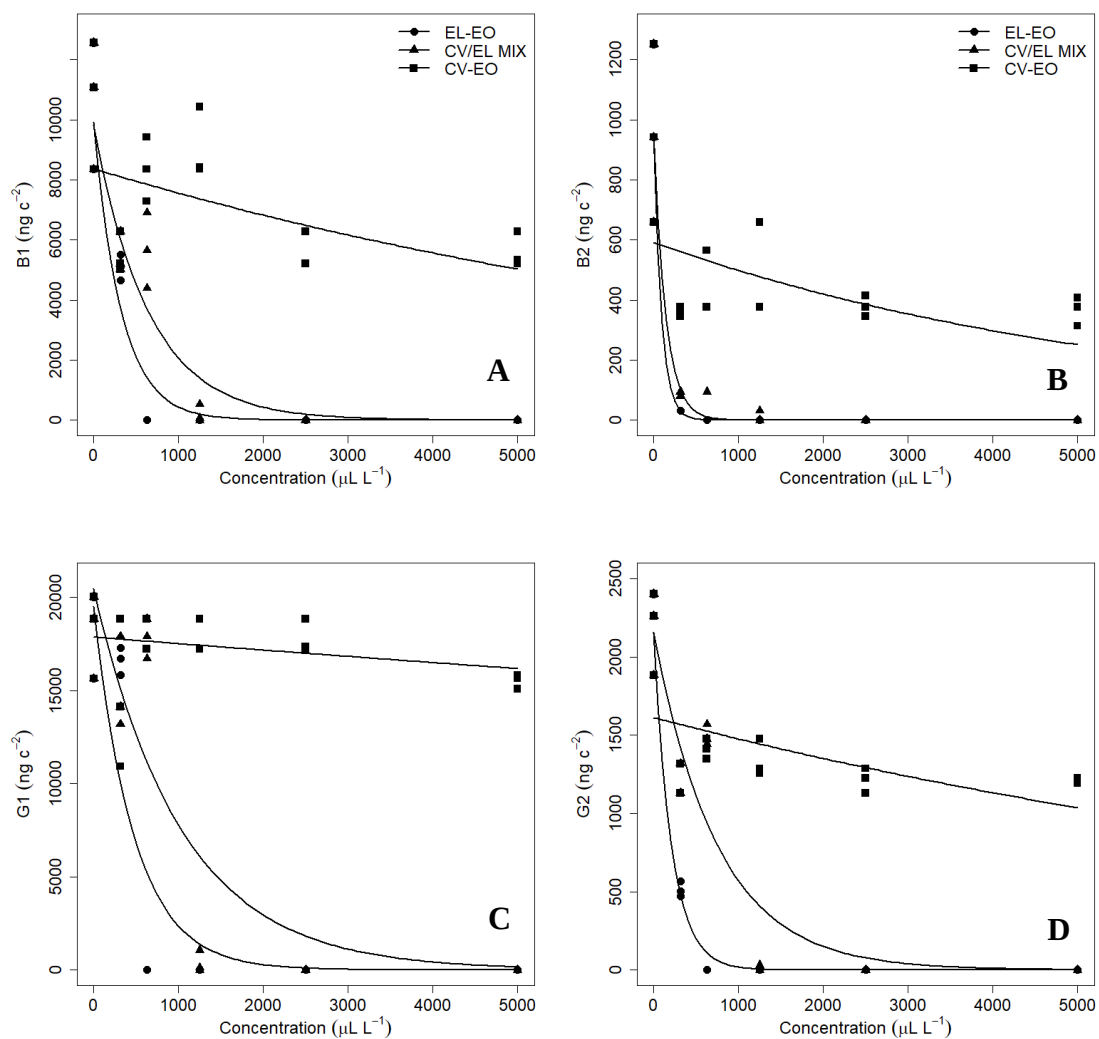


Figure 5. Production of aflatoxins B₁ (A), B₂ (B), G₁ (C), and G₂ (D) by *Aspergillus nomius* in culture medium containing different concentrations of the essential oils obtained from *Cordia verbenacea* (CV-EO) and *Elionurus latiflorus* (EL-EO) leaves, tested in isolation or as binary mixture (CV/EL MIX, 1:1), after incubation for 144 h. Note: Details of the adjusted models are shown in Table 3.

CHAPTER 2

GRAIN-PROTECTANT ACTION OF ESSENTIAL OILS AND WALKING
RESPONSES OF *Zabrotes subfasciatus* EXPOSED TO SELECTED
ESSENTIAL OILS¹**Abstract**

Mexican bean weevil, *Zabrotes subfasciatus* (Boh.) (Coleoptera: Chrysomelidae: Bruchinae), is one of the main pests of stored beans in Central and South America. The main control strategy is through synthetic insecticides. Considering this reality and the problems that the indiscriminate use of agrochemicals cause to the environment and human population, the development of eco-friendly solutions is essential to keep this pest population below control levels. Therefore, the use of essential oils (EOs) for pest management can be a promising solution. In this sense, we evaluated the chemical composition and bioactivity of three EOs from *Dysphania ambrosioides* (L.) Mosyakin & Clemants (Chenopodiaceae), *Pelargonium graveolens* L'Her (Geraniaceae) and *Piper callosum* Ruiz & Pav. (Piperaceae) against *Z. subfasciatus*. GC-MS showed that essential oil species are rich in monoterpenes, sesquiterpenes and phenylpropanoids. The EOs showed insecticidal activity in the fumigation, topical application and residual contact exposure methods in a concentration/dose dependent manner. The EOs of *P. callosum* and *P. graveolens* negatively influenced the oviposition and adult emergence of *Z. subfasciatus*. Furthermore, the EOs from species studied influenced the walking behavior of adults. The previously determined residual contact LC₅₀ of *D. ambrosioides* showed the strong repellent effect. Therefore, the results found in this study can contribute to the development of new solutions for pest control of stored grains and grain protection.

Keywords: Mexican bean weevil, *Dysphania ambrosioides*, *Pelargonium graveolens*, *Piper callosum*, stored grain IPM.

¹Chapter written in accordance with the journal's rules of the Journal of Pest Science

2.1 INTRODUCTION

After grain harvesting of common beans [*Phaseolus vulgaris* L. (Fabaceae)], storage techniques become necessary to maintain adequate sanitary requirements and preserve quality of produced grains as well as its adequability to human consumption. However, this process represents a challenge, since the bean grains are damage by arthropod pests and microorganisms that cause irreversible economic losses, in addition to being contaminated with substances (mycotoxins) that are harmful to human and animal health (Hill, 2002; Quintela et al., 2020). Among the coleopterans that occur in stored beans, the Mexican bean weevil, *Zabrotes subfasciatus* (Boh.) (Coleoptera: Chrysomelidae: Bruchinae), is considered the main insect pest of stored common beans. Its direct damage includes loss of mass, nutritional value reduction and losses of the germination power of seeds (Guzzo et al., 2018). On the other hand, indirect damages are related to changes in the storage environmental conditions, favoring the development of saprophytic fungi (e.g., *Aspergillus* and *Penicillium* species), which are potential producers of mycotoxins, in addition to reducing the level of quality and, consequently, consumption unfeasible or for the food industry (Mingotte et al., 2013; Guzzo et al., 2018; Quintela et al., 2020).

In ligh of this context, the search for new tools as alternative to synthetic pesticides is timely considering the growing concern of consumers about pesticide residues in foodstuff (Bajwa and Sandhu, 2014) and environmental aspects (Chawla et al., 2018). Moreover, efficient compounds are need to overlap the increasing selection of resistant populations to widespread active ingredients and reduce the economic impacts generated by the action of *Z. subfasciatus* in stored beans (Ribeiro et al., 2023).

Essential oils (EOs) from the secondary metabolism (allelochemicals) of plants have in their chemical composition substances with insecticidal and/or repellent effects, making them promising sources for the development of eco-friendly insecticides candidates compatible with precepts of integrated pest management (IPM) programs (Isman, 2020a; Ribeiro et al., 2023). Several research with EOs and different exposure methods (fumigation, topical application and residual contact) against insect pests has been reported as potential alternative control agents (Baldin et al. 2012, Ribeiro et al., 2013; Ortega-Nieblas et al., 2014; Fanela et al., 2016). In addition, the EOs promote behavioral changes

on target pests, including interference on insect walking behavior (Oliveira et al., 2018b; Antwi-Agyakwa et al., 2019; Faria et al., 2023).

The aromatic species *Dysphania ambrosioides* (L.) Mosyakin & Clemants (Amarantaceae) is a plant native of South and Central America and has been implemented in tropical and subtropical regions around the world (Blanckaert et al., 2012). This plant is commonly used in tea preparations to relieve gastrointestinal pain and has antioxidant, anti-inflammatory and antimicrobial properties (De Feo and Senatore, 1993). In addition to the benefits for human health, the EO of *D. ambrosioides* has in its chemical composition oxygenated monoterpenes and monoterpene hydrocarbons that are associated with promising insecticidal effects (Koba et al., 2009; Bernardes et al., 2018). *Pelargonium graveolens* L'Her (Geraniaceae) is an aromatic herb endemic to the South of the African continent (Rana et al., 2002). The derivatives from this Geraniaceae species are rich in geraniol, linalool, and citronellol (Ghedira and Goetz, 2015). These compounds can be used by pharmaceutical, food and cosmetic industries to create fragrances, lotions, ointments, medicines and flavor constituents (Southwell and Stiff, 1995; Lis-Balchin, 2006). In addition, some previous studies demonstrated that EO from *P. graveolens* had fumigant effect and oviposition deterrent against *Bemisia tabaci* MEAM 1 (Hemiptera: Aleyrodidae) (Baldin et al., 2015). Abouellata et al. (2016) showed that the EO of *P. graveolens* had a strong fumigant and oviposition-inhibiting effect on adults of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae). On the other hand, *Piper callosum* Ruiz & Pav. (Piperaceae) is an aromatic plant originating from the Amazon rainforest and is used by the local population as an aphrodisiac, astringent, treatment of gastrointestinal diseases and mosquito bites (Andrade et al., 2009). The EO of *P. callosum* contains safrole, α -pinene and β -pinene substances with potential insecticidal effects (Santos et al., 2021). Previous studies reported that *P. callosum* EO has an insecticidal effect against *Z. subfasciatus* and *B. tabaci* (Fanela et al., 2016; Ayres et al., 2021). Thus, studies on the bioactivity of EOs in different exposure methods, as well as the effect on the walking behavior of insects, assist in the development of assertive management strategies and in decision-making for the application of EOs in warehouses. In this sense, we evaluate the bioactivity and walking behavior responses of Mexican bean weevil exposed to EOs from *D. ambrosioides*, *P. graveolens* and *P. callosum* in different modalities of bioassays.

2.2 MATERIAL AND METHODS

2.2.1 Tested insects

The adults of *Z. subfasciatus* used in the bioassays were collected from stock colony maintained in the Plant Resistance to Insects and Insecticide Plants Lab (LARESPI). The insects were maintained in 0.5 L jars with 300 g of “bolinha” common bean cv. IAC “Esperança”. The bottles were covered with thin tissue (*voile*) and closed with rubber bands and kept in climatic chambers with controlled conditions (26 ± 2 °C temperature, $60 \pm 10\%$ RH and 12 h photophase).

2.2.2 Essential oils: plants collection, extraction procedures and chemical analysis

Plants of *D. ambrosioides* and *P. graveolens* were collected in the urban perimeter of Patrocínio municipality, located in western portion of the Minas Gerais state, Brazil and in physiographic zone of the Triângulo Mineiro and Alto Paranaíba ($18^{\circ}56'35''\text{S}$; $46^{\circ}59'31''\text{W}$). The identification of *D. ambrosioides* was performed by Dr. Rosângela de Oliveira Araújo and a voucher specimen was deposited in the Herbarium Uberlandense (HUFU) of the Federal University of Uberlândia (UFU) under number 61.679. On the other hand, *P. graveolens* was identified by Dr. Wagner Antônio Bernardes and a voucher specimen was deposited at the Centro Universitário do Cerrado Patrocínio (UNICERP), under number SPFR12023. Finally, *P. callosum* was collected at the Instituto de Ciências Exatas e Tecnologia of Itacoatiara ($58^{\circ}26'54.3''\text{S}$; $3^{\circ}8'28.8''\text{W}$), Itacoatiara, AM, Brazil., and identified by Dr. Renata Takeara. The voucher specimen was deposited in the Herbarium of the Federal University of Amazonas (HUAM/UFAM), under number 11.650.

The plant parts used in the extraction process were fresh leaves and branches (samples of 150 g) of *D. ambrosioides* (DA-EO), only fresh leaves (samples of 300 g) of *P. graveolens* (PG-EO) and fresh leaves, branches, and inflorescences (samples of 450 g) of *P. callosum* (PC-EO). The extraction of EO was performed using the steam drag hydrodistillation method in a Clevenger apparatus. For the extraction process, 500 mL of distilled water was added in round bottom flask. Then, the samples were subjected to an extraction temperature of 100°C for a period of 1.5 h for DA-EO, 4 h for PG-EO and 6 h for PC-EO. After the extraction process, the EOs were stored in amber bottles and kept in a freezer at -

10°C until their use in the bioassays. Determination of extraction yield was calculated based on fresh mass of plant material submitted to extraction (g of fresh plant material/g of EO).

The chemical constitutions of the essential oils were determined by gas chromatography coupled to mass spectrometry (GC-MS), in a Shimadzu QP2010 Plus device (Shimadzu Corp., Kyoto, Japan), equipped with an AOC-20i automatic injector, electron ionization source (IE-EM) operating at 70 eV and quadrupole mass filter analyzer. Spectroscopic grade n-hexane (Malinkrodt brand) to dissolve the sample was used.

Chromatographic separation was performed on an Rtx5-MS capillary column (Restek) of fused silica (30 m × 0.25 mm i.d. × 0.25 µm film), composed of 5% diphenylpolysiloxane and 95% dimethylpolysiloxane. Helium (99.999%) was used as carrier gas, at a constant flow of 1.0 mL min⁻¹. The injector temperature was 240°C and the injection volume was 0.1 µL. The oven temperature was programmed from 60°C to 240°C at a speed of 3°C min⁻¹. To calculate retention index (RI), a mixture of saturated aliphatic hydrocarbons (C8 to C40) was added to the essential oil samples. Retention rates were calculated according to equation 1 (E1):

$$RI = 100n + 100 \frac{(tx - tn)}{tn+1 - tn} \quad (E1)$$

Where n is the number of carbons of the first hydrocarbon of the standard mixture whose retention time tn is immediately less than the retention time tx of the essential oil constituent, and $tn+1$ is the retention time of the hydrocarbon of the standard mixture immediately larger. The RI values obtained were compared to those present in the literature.

The sample eluted from the chromatographic column was directed by the flow divider to the ionization source, at a ratio of 1:20. The source temperature was set at 280°C and the electron beam energy was 70 eV. The analyzer was controlled to separate ions of m/z between 40 and 600. The mass spectra obtained were compared to those from the Wiley 7, NIST 08 and FFNSC 1.2 Libraries using the GC-MS Solution software (Shimadzu), which provided the similarity index (SI), expressed as a percentage. The identification of each chemical constituent present in the EO was made based on the combination of the RI and SI values. Compounds

whose peaks presented SI below 85% and experimental RI with a difference greater than 20 in relation to the literature were considered as "not identified". The percentages of each chemical constituent in essential oils were estimated from the peak areas, obtained by normalizing the area, in relation to the total area of the chromatogram.

2.2.3 Bioassays

The bioassays were conducted under controlled conditions ($25 \pm 2^\circ\text{C}$ temperature; $60 \pm 10\%$ RH and 12 h photophase). All bioassays followed the completely randomized experimental design. "Bolinha" common bean cv. IAC "Esperança" was used as substrate of tests, which was previously disinfested and the relative humidity standardized at 13% following the procedure proposed by Ribeiro et al. (2013).

2.2.3.1 Fumigation bioassay

To assess the fumigant insecticidal action of DA-EO, PG-EO and PC-EO, five couples of *Z. subfasciatus* (age 0-48 h) were placed into 50 mL bottles used as fumigation chambers. The bottle lids were internally covered with aluminum foil. The EOs were applied into 1×1 cm filter paper placed in the center of the lids and were covered with a thin tissue (*voile*) to prevent insects from contacting the treated filter paper. Finally, the fumigation chambers were wrapped with plastic film to prevent the volatiles from the EO from escaping. Previous tests were carried out to define the rates (Finney, 1971). Based on this procedure, the concentrations defined were: 1, 2, 3, 4, 5, 15, 20, 25, 30, 35, 40 $\mu\text{L L}^{-1}$ of air for DA-EO; 2.5, 5, 7.5, 15, 20, 25, 30, 35, 40 $\mu\text{L L}^{-1}$ of air for PG-EO and 20, 30, 40, 100, 150, 200, 250, 300 $\mu\text{L L}^{-1}$ of air for PC-EO. The positive control was composed by a phosphine-based insecticide (Phostoxin®; Besquita Indústria Química do Brasil, São Vicente, SP, Brazil) applied to the recommended rate (2 g m^{-3}) for management of stored grain pests in Brazil (Agrofit, 2024). On the other hand, the negative control was composed by no treated experimental units. For each concentration, five replications were performed ($n=50$). The number of dead insects was assessed after 72 h of exposure to EOs. The data collected in the experiment were analyzed to estimate the lethal concentrations (LC_{50} and LC_{90}) of EOs.

2.2.3.2 Topical application bioassay

The insecticidal performance of EO applied via dorsal contact was evaluated in 5 adult couples aged between 0 and 48 h. The adults were confined in Petri dishes (90 mm diameter × 15 mm height), which were covered with a thin tissue (*voile*) aiming at gas exchange with the environment. The doses were diluted in acetone considering the average weight of males and females (0.0024 g). The EOs were applied on the dorsal part of the adult insect (0.5 µL per insect) using a Hamilton® microsyringe.

Preliminary tests were carried out with rates of 10, 100, 200, 600 and 1000 mg g⁻¹ to define the doses of EO. Based on this procedure, doses of 2, 4, 5, 6, 7, 8, 9, 10, 40, 50, 70, 80, 90 mg g⁻¹ were established for DA-EO; 10, 15, 20, 30, 40, 50, 60, 100, 200, 600 mg g⁻¹ for PG-EO; 10, 20, 30, 40, 60, 70, 80, 100, 200 mg g⁻¹ for PC-EO. The negative control consisted of the application of pure acetone. On the other hand, the positive control was composed by the recommended dose (80 mL t⁻¹) of a synthetic insecticide based on deltamethrin (K-Obiol® 25 EC, Bayer Corp., São Paulo, SP, Brazil) for stored pest management in Brazil (Agrofit, 2024). The evaluations were carried out after 72 h of exposure to EOs and the dead insects were counted using a brush (diameter 3 mm). Each dose consisted of five replications (n=50). The results were subjected to analysis of the lethal doses (LD₅₀ and LD₉₀) of the EOs.

2.2.3.3 Residual contact bioassay

The bioactivity of EOs from DA-EO, PG-EO and PC-EO was evaluated for residual contact. The concentrations were previously defined as proposed by Ribeiro et al. (2013, 2020) and were diluted in acetone. For the negative control, pure acetone was applied, while the positive control was composed by the recommended dose (80 mL t⁻¹) of the synthetic insecticide based on deltamethrin (K-Obiol® 25 EC). The bioassay consisted of 5 replications with 5 adult couples (age 0-48 h) for each treatment (n=50). Each repetition was individualized in 250 mL bottles with 10 g of “bolinha” common beans cv. IAC “Esperança” and were covered with a thin tissue (*voile*). The concentrations (31.25; 62.50; 125; 250 and 500 µL kg⁻¹) of EO were applied using a micropipette on the grains and the volume of solution was equivalent to 30 L t⁻¹. After application, the bottles were stirred for

30 s to improve distribution of treatments on the beans. Subsequently, the samples were kept in light-free chambers for 30 min until the acetone evaporates. After this stage, adult couples were released into the bottles. The assessment consisted of counting the number of adults killed after 72 h of exposure and the data were used to estimate lethal concentrations (LC₅₀ and LC₉₀).

2.2.3.4 Oviposition and transgenerational bioassay

For the oviposition bioassay, was adopted the same method and concentrations of the residual contact test with DA-EO, PG-EO and PC-EO was used. For this purpose, 250 mL jars containing 10 g of “bolinha” common beans cv. IAC “Esperança” were used. After applying the treatments, five couples (age 0-48 h) were released, and the bottles were covered with a thin tissue (*voile*). Five replications were performed for each concentration (n=50). The adults were removed after 7 days of exposure to the treated beans. After 14 days, the number of viable and inviable eggs per grain was counted. The number of emerged adults and holes in the grains was counted 56 days after infestation as proposed by Ribeiro et al. (2020).

2.2.3.5 Walking behavior assay (no-choice test)

The effect of DA-EO, PG-EO and PC-EO was evaluated on walking behavior (no choice) using males and females of *Z. subfasciatus*. The arenas consisted of Petri dishes with (60 mm diameter × 20 mm height) containing filter paper. To prevent insects from escaping, fluon was applied to the edges of each arena.

The LC₂₅ and LC₅₀, previously estimated in the residual contact bioassay to each tested EO, were applied to the filter paper. Therefore, the EO were diluted in acetone and a volume of 200 µL was applied to the filter paper with the aid of a micropipette. For each treatment, 30 arenas (repetitions) were made, 15 for males and 15 for females of *Z. subfasciatus*. The negative control consisted of the application of pure acetone. After evaporation of the solvent, *Z. subfasciatus* adults were released into the center of the arenas. The recorded parameters were the mean distance and walking speed over a period of 10 min recorded by video with the aid of the BRIO 4K PRO WEBCMAx V-U0040 camera (Longitech México S.A.,

Ciudad de México, México) and the Ethovision® software (Noldus Information Technology Corp., Wageningen, Netherlands).

2.2.4 Data analyses

The effective EOs dose-response data were determined using the two-parameter log-logistic curve of the drc package of the R statistical software (Nguwen et al., 2014; Arena et al., 2017). A two-parameter model is usually reserved to binomial/quantal responses data, while the three or four parameter log-logistic model is useful to describe continuous dose-response data (Oliveira et al., 2018a). The model is showed by equation 2 (E2).

$$Y = 1/\{1+\exp [b(\log(x) - \log(e))]\} \quad (E2)$$

In this model, Y is the proportion of dead insects of the EO [dead insects/total of population insects in each EO dose/concentration (n=50)] and represents the LD₅₀ value. The parameter b is the relative slope and x is the EO dose. LL.2 or LL2.2 functions provide the two-parameter log-logistic function where the lower limit is fixed at 0 and the upper limit is fixed at 1 (Ritz et al., 2015). A chi-square goodness-of-fit test (χ^2) was used to evaluate how well the selected model fits the data (p-values ≥ 0.05 indicate that the model fits the data well).

For oviposition analyses, continuous variables of the parameters number of total eggs oviposited, viable eggs, inviable eggs, adults and considering the treatments: three EOs in different concentrations ($\mu\text{L/kg}$), negative control and positive control, was applied a multivariate analysis. We used a hierarchical clustering on principal components (HCPC) approach, which allowed combined three standard methods: principal component analysis, hierarchical clustering and K-means algorithm, to aim to identify groups (clusters) of similar objects within our data set (Maugeri et al., 2021). The description of each cluster by quantitative variables was analyzed by t-statistics. Each treatment had 5 repetitions The mean value of the repetitions was used in the analyses. The HCPC analyses was performed on the R software (R version 4.3.2), with FactoMineR and factoextra packages (R Development core Team 2024).

Data on the distance and walking speed interations and (isolated evaluations) were analyzed using Generalized Linear Models (GLM) with Gaussian

distribution. Statistically significant were calculated using the function “anova” in the package “car” with pairwise comparison from LS means with Tukey adjustment ($p \leq 0.05$), using “LS means” package, all analyses were performed using R software (R version 4.3.2) (R Development core Team 2024).

2.3 RESULTS

2.3.1 Essential oils: extraction yield and chemical composition

The extraction yield of DA-EO, PG-EO and PC-EO through the hydrodistillation process were 1.12%, 1% and 0.99% (g of oil/g of fresh plant material), respectively. The DA-EO presented a total of 12 compounds, and oxygenated monoterpenes and monoterpene hydrocarbons were predominant (96.7%) (Table 1). The major compounds were cis-ascaridol (29.9%), trans-ascaridole (23.50%), p-cymene (22.2%) and α -terpinene (15.2%). The PG-EO exhibited a total of 41 compounds, of which oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and monoterpene hydrocarbons represented 90% of the total composition (Table 1). The major compounds were citronellol (22.3%), geraniol (13.6%), β -linalool (7.1%), citronellyl formate (6.8%) and geranyl acetate (4.8%) (Table 1). Finally, 35 compounds were identified in the PC-EO and the phenylpropanoids, monoterpene hydrocarbon, oxygenated monoterpene, oxygenated sesquiterpene and sesquiterpene hydrocarbon compounds represented 95.8% of the total composition (Table 1). The majority compounds identified in this EO were (Z)-Isosafrole (46.9%), β -pinene (9.6%), methyleugenol (7.1%), eucalyptol (6.9%) and α -pinene (4.7%) (Table 1).

2.3.2 Toxicity tests

Adult mortality varied depending on the plant species studied, concentration and exposure methods. The DA-EO had a greater effect on mortality when compared to PG-EO and PC-EO in both exposure methods, according to the results of the binomial model log-log (Table 2). As for the positive control, 100% mortality was observed in adults exposed to phosphine (fumigation test) and deltamethrin (topical application, residual contact and oviposition test) after 72 h of exposure (data not shown due to null variance).

2.3.2.1 Lethal concentration fumigation bioassays

The fumigant effect of EO showed promising results on Mexican bean weevil mortality and showed results similar to phosphine (100% adult mortality) at the highest concentrations. It was the most effective exposure method when compared to the other exposure methods studied in relation to the amount of EO displayed in LC_{50} and LC_{90} (Table 2). LC_{50} and LC_{90} differed significantly. The LC_{50} value of DA-EO was $3.75 \mu\text{L L}^{-1}$ of air and the LC_{90} was $11.94 \mu\text{L L}^{-1}$ of air, for PG-EO the LC_{50} was $14.75 \mu\text{L L}^{-1}$ of air and the LC_{90} was $27.74 \mu\text{L L}^{-1}$ of air and the LC_{50} value of the PC-EO was $LC_{50} 24.41 \mu\text{L L}^{-1}$ of air and the LC_{90} $62.04 \mu\text{L L}^{-1}$ of air (Table 2).

2.3.2.2 Lethal concentration topical application

The EOs showed toxicity via topical application on the back of Mexican bean weevil adults (Table 2). Our results showed a 100% effect on adult mortality at the highest dosages, as observed in the positive control (deltamethrin). The LD_{50} ($6.81 \mu\text{L mg g}^{-1}$) and LD_{90} ($14.33 \mu\text{L mg g}^{-1}$) of DA-EO differed significantly from those of PC-EO and PG-EO. However, the PC-EO ($LD_{50} 23.58 \mu\text{L mg g}^{-1}$; $LD_{90} 72.73 \mu\text{L mg g}^{-1}$) and PG-EO ($LD_{50} 30.02 \mu\text{L mg g}^{-1}$; $LD_{90} 89.80 \mu\text{L mg g}^{-1}$) did not differ from each other (Table 2).

2.3.2.3 Lethal concentration residual contact

Exposure of EOs residual contact demonstrated toxicity to adults. PC-EO ($LC_{50} 154.21 \mu\text{L L}^{-1}$ and $LC_{90} 325.80 \mu\text{L L}^{-1}$) and PG-EO ($LC_{50} 187.52 \mu\text{L L}^{-1}$ and $LC_{90} 371.12 \mu\text{L L}^{-1}$) did not differ significantly. The DA-EO ($LC_{50} 48.35 \mu\text{L L}^{-1}$; $LC_{90} 97.94 \mu\text{L L}^{-1}$) differed significantly from the EO PC-EO and PG-EO (Table 2).

2.3.2.4 Oviposition and transgenerational bioassay

In the hierarchical tree with graphical output, which showed the relative proximity of the treatments to each other by loss of inertia was possible to observe loss of inertia in the two firsts bars, especially in the first one, while there was barely any loss of inertia from the third bar. The gain of inertia from one class to two classes (first bar) was 3.64. From two to three was 0.80 and from three for four (third bar) the values have become very low, below at 0.22 following a linearity. Therefore, it was retained three classes/clusters in our study (Figure 1).

The five quantitative variables were linked with to clusters variables with high statistically significance. The mean number of inviable eggs was that most linked to the cluster variables ($p = 0.00000$) followed by total of eggs, adults, viable eggs and holes with respective correlations ratios of 0.96, 0.91, 0.88, 0.86 and 0.83 (Table 3).

Regarding the summary of each cluster by quantitative variables, in the first cluster (group of treatments with null/ lowest means in category), the linked variables (mean category/t-value), in descending order, were total eggs (9.24/-3.77), viable eggs (4.44/-3.56), adults (3.28/-3.51), inviable eggs (4.80/-3.09) and holes (4.45/-3.07) (Table 3). The means values of all quantitative variables in first cluster (means in category) were lower than overall means, resulting in these negative t-values. For the second cluster, only the variable inviable eggs were linked (Table 3). The mean in category (82.5) was higher than overall mean (25.38) with positive t-value (3.33). As for the third cluster (group of treatments with highest means in category), the linked variables, in descending order, were number of holes (82.96/+3.63), adults (55.97/+3.57), viable eggs (66.68/+3.46) and total eggs (99.25/+2.65) (Table 3).

Considering the cluster description by PCA dimensions (Dim1 = 82.73% and Dim2 = 16.48%), the first cluster had significant smaller coordinate values than the other clusters (t-value = -3.73) in the first dimension. The treatments in cluster two had significant higher values than the other clusters in second dimension (t-value = 3.80). Treatments in the third cluster had significant larger coordinate values in the first dimension (t-value = 3.23) (Table 4).

Regarding the representative treatments of each cluster, the best five closest treatments to the barycenter for cluster 1 were *P. graveolens* (Pgrave 4) (0.121), *P. callosum* (Pcalo 4) (0.270), *D. ambrosioides* (Damb 5) (0.282), *P. graveolens* (Pgra 5) (0.335) and *P. callosum* (Pcal 5) (0.342). For cluster 3 were, *D. ambrosioides* (Damb 4) (0.317), *D. ambrosioides* (Damb 3) (0.364), negative control (NegControl) (0.559), *D. ambrosioides* (Damb 2) (0.562) and *D. ambrosioides* (Damb 1) (0.955). For the cluster 2, *P. callosum* (P cal 1) and *P. callosum* (P cal 2) had the same distance (0.591) (Figure 2).

2.3.2.5 Walking behavior assessment

EOs influenced walking behavior in adult male and female Mexican bean weevils. Significant differences were observed specially in the LC_{50} of the residual contact of the EOs when compared to the control (acetone) on the average of *Z. subfasciatus* adults stimulating walking (distance covered cm^{-1}) and speed ($cm\ s^{-1}$). A significant interaction was evidenced in the LC_{50} of the residual contact of the PC-EO in the distance covered by the males of *Z. subfasciatus* ($1,299.56 \pm 232.63$ cm) and in the mean speed ($1.81 \pm 0.22\ cm\ s^{-1}$) when compared with control (Tables 5 and 6). Except for PG-EO LC_{25} , the other EOs did not show significant results either in isolated factors or interactions (Table 5 and 6). The isolated analysis of the treatments revealed a significant difference in distance and speed (Tables 7 and 8). Adults exposed to LC_{50} from residual contact of DA-EO and PG-EO stimulated walking behavior (Table 7). The LC_{25} of the PG-EO ($1,066.24 \pm 106.35$ cm; $1.83 \pm 0.18\ cm\ s^{-1}$) differed significantly from the LC_{25} of the PC-EO (625.35 ± 95.21 cm) (Table 8), demonstrating the arresting effect of the PC-EO and stimulant for PG-EO (Tables 7 and 8).

2.4 DISCUSSION

EOs on insect pests have shown promising results in the control and they are potential candidates in the formulation of eco-friendly pesticides (Isman, 2020b; Brito et al., 2021; Ribeiro et al., 2023). Our results indicated the insecticidal effect of the DA-EO, PG-EO and PC-EO in different exposure methods on the Mexican bean weevil. EO have different action modes in insects, which mainly related to neurotoxic effects, including interference with the octopamine neuromodulator, or gamma-aminobutyric acid (GABA) receptors, inhibition of ATPases enzymes, acetylcholinesterase (AChE) and cytochromes P450 (CYPs) (Kim et al., 2013; Oyedele et al., 2020; Liu et al., 2021; Liu et al., 2022).

Our results from GC-MS analysis of DA-EO indicated the major compounds cis-ascaridol (29.9%), followed by trans-ascaridol (23.5%), p-cymene (22.2%) and α -terpinene (15.2%). Some studies on the chemical composition of *D. ambrosioides* EO indicate that the composition is a complex mixture of oxygenated monoterpenes and monoterpene hydrocarbons (Koba et al., 2009; Singh; Pandey, 2022). Our results showed the presence of 58.8% of oxygenated monoterpene compounds and 37.9% of monoterpene hydrocarbons. The major compound ascaridol (cis and trans isomers), p-cyneme and α -terpinene are reported in other

studies as the main constituents of the *D. ambrosioides* EO, but with different percentages of them (Nenaah; Ibrahim, 2011; Almadiy, 2020; Zriouli et al., 2023; Subaitha et al., 2024).

The chemical analyzes of the *P. graveolens* EO showed 41 compounds, of which oxygenated monoterpenes represented 72.6% of the total composition. Our results highlighted the main compounds citronellol (22.3%), geraniol (13.6%), citronellyl formate (6.8%), β -linalool (7.1%) and geranyl acetate (4.8%). Abouelatta et al. (2020) reported that the main compounds of *P. graveolens* EO are citronellol (29.76%), geraniol (12.53%) and citronellyl formate (7.10%), similar to those found in our study. Other studies reported the presence of the same major compounds found in our study associated with the *P. graveolens* EO in different percentages (Džamić et al., 2014; Baldin et al., 2015; Abouellata et al., 2016; Ibrahim et al., 2022). Additionally, phenylpropanoids, monoterpene hydrocarbon, and oxygenated monoterpene compounds represented 87.4% of the 35 compounds detected in our analysis of *P. callosum* EO. The compounds (Z)-isosafrole (46.9%), β -pinene (9.6%), methyleugenol (7.1%), eucalyptol (6.9%) and α -pinene (4.7%) were the main constituents of the total EO composition. However, previous studies have shown that safrole is the predominant major constituent of *P. callosum* EO (Maia et al., 1987; Fanela et al., 2016; Almeida et al., 2018; Ayres et al., 2021), different from found in our study that did not detect the presence of the safrole compound. On the other hand, the components β -pinene, α -pinene and methyleugenol are present in other studies in different percentages in the *P. callosum* EO (Fanela et al., 2016; Ayres et al., 2021).

The EOs compounds have a complex variation in composition (quantitative/qualitative) due to genetic, physical, chemical, environmental, seasonal factors, chemotypes or different varieties of the plant, among others and should be considered as a limiting factor in bioactivity studies against arthropods pest. In addition, they can directly influence the response of organisms exposed to these volatiles (Isman, 2016; Ribeiro et al., 2023). New studies on genetic improvement of aromatic plants must be modified to standardize these constituents present in secondary metabolism, in addition to standardizing cultivation.

Our analysis results showed that the EOs studied are rich in monoterpenes, sesquiterpenes and phenylpropanoids. These compounds can penetrate through the cuticle, respiratory and digestive systems, severely affecting several neuronal

pathways, such as GABA, AChE and the cholinergic and octopaminergic system (Yeom et al., 2012; Gaire et al., 2019). Previous study showed a strong fumigant effect of the major compound ascaridole against maize weevil, *Sitophilus zeamais* (Mot.) (Coleoptera: Curculionidae), followed by α -terpinene and *p*-cymene (Chu et al., 2011). In the same study, the compound ascaridole showed a strong toxic effect for topical application against maize weevil. It was reported in the literature that the major compounds citronellol and geraniol at a concentration of $0.2 \mu\text{L L}^{-1}$ had a fumigation effect on the stored grain pest *Sitophilus oryzae* L. (Coleoptera: Curculionidae) with mortality rates of 64% and 70%, respectively, and for *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) with 58% and 68%, respectively after 72 h of exposure (Brari; Kumar, 2020). Studies with pure *P. graveolens* EO and the isolated major compounds (geraniol, citronellol and citronellyl formate) demonstrated an insecticidal effect against *Stephanitis pyrioides* (Scott) (Hemiptera: Tingidae) and the major compound geranyl acetate showed a larvicidal effect against *Aedes aegypti* L. (Diptera: Culicidae) (Ali et al., 2013). Furthermore, other studies showed that the compound isosafrole extracted from the plant species *Piper betle* L. (Piperaceae) showed high mortality in *Musca domestica* (L.) (Diptera: Muscidae) (LC_{50} 2.3 mg dm^{-3}) after 24 h of exposure (Mohottalage et al., 2007). A recently study on the fumigant bioactivity of the major compounds in the EO of *Pimenta pseudocaryophyllus* (Gomes) (Myrtaceae) against *Z. subfasciatus* reported that a concentration of $25.50 \mu\text{L L}^{-1}$ of the compound methyleugenol caused 100% mortality in adults (Ribeiro et al., 2024). Other studies with major compounds showed a toxic effect of α -pinene on stored grain pests *Sitophilus granaries* (L.), *S. zeamais* (L.) (Coleoptera: Curculionidae), *Tribolium confusum* (du Val) (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) (Atay et al., 2023). Additionally, the major compound eucalyptol showed an insecticidal effect against *T. castaneum* by both topical application and fumigation (Tine et al., 2023).

In relation to our results from the fumigation and topical application bioassays, the EO showed a high mortality rate, especially *D. ambrosioides*, followed by *P. graveolens* and *P. callosum*. Previous study on the fumigant effect of *D. ambrosioides* EO reported LC_{50} $0.5 \mu\text{L L}^{-1}$ on *Z. subfasciatus* adults exposed for 24 h (Bernardes et al., 2018). Our results showed an LC_{50} of $3.75 \mu\text{L L}^{-1}$ on Mexican bean weevil adults exposed for 72 h. The variation in our results may be

related to the part of the plants from which the essential oils of *D. ambrosioides* were extracted. The parts of the plants may influence the quantity and quality of the major and minor compounds present in the EO (Isman, 2017). Other studies reported the fumigant effect of *P. callosum* EO against *Z. subfasciatus* (LC₅₀ 0.56 $\mu\text{L L}^{-1}$, after 72 h) (Ayres et al., 2021), our results were 24.41 $\mu\text{L L}^{-1}$ for LC₅₀. However, the majority compound reported by Ayres et al. (2021) was safrole (29.3%), unlike what was found in our study, where the analyzes showed (Z)-isosafole (46.9%). Future works should be carried out with these isolated compounds to investigate differences related to bioactivity on Mexican bean weevil. Additionally, the bioactivity of *P. graveolens* EO against *Z. subfasciatus* has not been reported in the literature to date, but insecticidal and repellent activity is known for other species of stored grain pests (Bouzenna; Krichen, 2013; Abouelatta et al., 2020; Santana et al., 2022).

Regarding the residual contact bioassay, our results suggest that the EOs studied may be promising for protecting grains from adult infestation. Furthermore, our results from the oviposition test showed a reduction in the number of viable eggs, this effect may be related to the high mortality of adults and the repellent characteristics of the EOs, specially in treatments with higher rates. However, this positive effect on insect control may also have been verified, through HCPC analysis, in EOs with low and intermediate rates such as, in PC-EO (31.25 and 62.5 $\mu\text{L Kg}^{-1}$) there was the highest number of inviable eggs “cluster 2” for PC-EO and PG-EO (125 $\mu\text{L Kg}^{-1}$) in terms of lower oviposition, viable eggs and emerged adults, resulting in less direct damage to beans “cluster 1”. Previous studies showed that the EO of *Thymus vulgaris* (L.) (Lamiaceae) has a deterrent effect on oviposition and a reduction in the emergence of adults of *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae) (Lazarević et al., 2020). Santana et al. (2021) showed a strong effect on reducing oviposition of *Z. subfasciatus* females and the ovicidal effect of EO from *Acorus calamus* (L.) (Acoraceae) and *Betula lenta* (L.) (Betulaceae). Other studies showed no preference for oviposition of *Z. subfasciatus* females with bean grains treated with *Vitis vinifera* (L.) (Vitaceae) EO and reduction in the number of eggs per grain for the *Brassica napus* (L.) (Brassicaceae) EO. The authors suggest that the EO of *B. napus* have the protective function of the grains, hindering the insect development in the initial stages of the life cycle (Silva et al., 2023). Such sublethal effects found in our study

can be strategic in the practical application of EO for the protection of bean grains, contributing to the reduction of progeny. New studies must be carried out regarding the influence of the *P. callosum* and *P. graveolens* EOs, as well as the major compounds on the eggs of *Z. subfasciatus*.

Our results from the walking behavior assay showed that LC₅₀ DA-EO, PG-EO and PC-EO repelled *Z. subfasciatus* adults, influencing walking behavior (distance and speed) regardless of sex. In analyzes between males and females, the LC₅₀ of the residual contact of *P. callosum* EO showed a repellent effect on males. Some species of EOs present repellent characteristics in different groups of insects (Bougherra et al., 2015; Andrade et al., 2016; Pang et al., 2020; Cantó-Tejero et al., 2022; Pinto et al., 2022). The *Lippia sidoides* (Cham.) (Verbenaceae) EO and the compounds thymol and p-cymene at a concentration of 1.0% influenced the walking behavior (distance and speed) of *S. zeamais* (Oliveira et al., 2018b). Although EO and the main compounds may have repellent characteristics on insects, some oils may have a deterrent effect on walking behavior, due to neurotoxic properties (e.g. GABA and acetylcholine receptor) (Araújo et al., 2017). In addition, the sensilla present in the antennae can detect the chemical compounds present in the EO, leading to a reinforcement of the insect walking (Antwi-Agyakwa et al., 2019). Our results demonstrated that the LC₂₅ of the residual contact of the *P. callosum* EO had arresting effect on adults of *Z. subfasciatus* adults when compared with LC₂₅ of *P. graveolens*. Previous studies had similar results on the *Elionurus latiflorus* EO showed arresting effect on the walking behavior of females of *Z. subfasciatus* (Faria et al., 2023).

2.5 CONCLUSIONS

EOs from *D. ambrosioides*, *P. graveolens* and *P. callosum* show stronger toxicity against *Z. subfasciatus* adults in different exposure methods and sublethal effects on walking behavior. The EO of *D. ambrosioides* show high effect by fumigation, topical application, residual contact exposure methods and repellent compared to the others EOs on Mexican bean weevil. The EOs of *P. callosum* and *P. graveolens* shown negative effects on oviposition and adult emergence. The EOs from the species studied are promising alternative sources for the protection of stored grains. Future studies with the main isolated compounds are essential to elucidate the bioactivity on *Z. subfasciatus*.

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Table 1. Chemical composition of essential oils from *Pelargonium graveolens*, *Piper callosum*, *Dysphania ambrosioides*.

Compounds	<i>Pelargonium graveolens</i>				<i>Piper callosum</i>				<i>Dysphania ambrosioides</i>			
	¹ RT (min)	² %RA	RI _{exp}	RI _{lit}	RT (min)	%RA	RI _{exp}	RI _{lit}	RT (min)	%RA	RI _{exp}	RI _{lit}
α-pinene	7.29	1.5	938	939	7.30	4.7	935	939				
β-pinene	8.61	0.5	981	980	8.63	9.6	975	980				
β-myrcene	9.09	0.2	992	991								
2-(2-ethoxyethoxy)-ethanol	9.65	2.5	1003	1006								
Limonene	10.39	2.8	1027	1031								
<i>trans</i> -linalool oxide	12.02	0.2	1071	1074								
<i>cis</i> -linalool oxide	12.61	0.2	1085	1088								
β-linalool	13.12	7.1	1093	1098								
<i>cis</i> -rose oxide	13.44	0.8	1107	1111								
phenylethanol	13.61	2.7	1111	1110								
<i>trans</i> -rose oxide	14.04	0.3	1119	1115								
<i>cis</i> -β-terpineol	14.69	0.4	1145	1144								
menthone	14.98	2.0	1154	1154								
Isomenthone	15.38	2.9	1160	1164								
phenethyl formate	15.87	0.3	1178	1174								
α-terpineol	16.38	2.5	1188	1189					14.83	0.3	1187	1189
γ-terpineol	16.62	1.2	1192	1193								
citronellol	18.02	22.3	1237	1233								
(<i>Z</i>)-neral	18.26	0.4	1234	1240								
geraniol	18.92	13.6	1238	1235								
citronellyl formate	19.45	6.8	1272	1275								
geranyl formate	20.32	3.2	1302	1300								
α-cubebene	21.79	0.1	1349	1351								
citronelly acetate	21.96	1.0	1355	1354								

Table 1. Continuation...

neryl acetate	22.35	2.4	1364	1365						
α -copaene	22.63	0.5	1372	1376	22.65	0.7	1378	1376		
geranyl acetate	22.99	4.8	1381	1383						
α -gurjunene	24.02	1.2	1407	1409						
<i>trans</i> -caryophyllene	24.32	0.1	1415	1418	24.03	0.7	1422	1418		
α -guaiene	24.62	0.1	1435	1439						
aromadendrene	24.78	1.2	1441	1439						
α -muurolene	25.75	0.7	1457	1455						
γ -muurolene	25.92	0.7	1480	1476						
germadrene D	26.36	0.4	1482	1480						
β -selinene	26.51	0.1	1484	1485						
α -amorphene	27.19	0.8	1488	1485	25.94	1.4	1482	1482		
citronelly isobutyrate	27.32	0.5	1512	1514						
α -cadinene	28.27	0.8	1537	1538						
spathulenol	29.99	3.1	1578	1575						
guaiol	31.28	1.5	1593	1595						
γ -eudesmol	32.17	1.1	1616	1617						
α -pinene										
camphene					7.74	0.4	951	953		
β -pinene										
myrcene					9.11	0.6	992	994		
α -phellandrene					9.56	0.1	1003	1005		
α -terpinene					9.97	1.5	1014	1018	10.01	15.2
<i>para</i> -cymene					10.27	0.7	1024	1026		1015
eucalyptol					10.49	6.9	1031	1033		1018
β - <i>trans</i> -ocimene					11.14	0.1	1049	1050		

Table 1. Continuation...

γ -terpinene	11.50	3.9	1065	1062	11.50	0.5	1061	1062
<i>cis</i> -sabinene hydrate	11.81	0.2	1069	1068				
α -terpinolene	12.58	1.1	1084	1088				
linalool	13.04	0.9	1097	1098				
camphor	14.63	0.1	1141	1143				
isoborneol	15.09	0.3	1152	1156				
terpinen-4-ol (4-terpineol)	15.856	1.2	1182	1182				
alpha-terpineol	16.36	1.1	1193	1189				
(Z)-Isosafrole	20.21	46.9	1317	1315				
α -copaene								
methyleugenol	23.74	7.1	1403	1401				
<i>trans</i> -caryophyllene								
α -humulene	25.09	0.2	1457	1455				
germacrene D	25.81	0.4	1479	1480				
α -amorphene								
curzerene	26.44	0.3	1495	1496				
δ -cadinene	26.96	0.3	1528	1524				
viridiflorol	27.20	0.6	1591	1590				
β -elemenone	27.95	0.5	1595	1594				
<i>cis</i> -asarone	29.25	1.6	1620	1622				
cubenol	28.71	0.2	1643	1641				
torreyol	30.59	0.2	1647	1645				
α -cadinol	30.71	0.3	1658	1653				
τ -muurolol	30.81	0.5	1665	1660				
bulnesol	30.91	0.4	1669	1666				
germacrone	32.05	0.1	1697	1693				

Table 1. Continuation...

α -terpinene						
p-cymene			10.35	22.2	1024	1026
γ -terpinene						
<i>cis</i> -p-Mentha-2.8-dien-1-ol			13.79	0.7	1134	1138
<i>trans</i> -caren-4-ol			14.33	0.8	1175	1178
α -terpineol						
<i>trans</i> -carveol			15.94	1.0	1213	1217
<i>trans</i> -chrysanthenyl acetate			16.38	1.5	1234	1235
<i>cis</i> -ascaridol			18.29	29.9	1237	1237
<i>cis</i> -piperitone oxide			18.68	0.1	1251	1254
<i>trans</i> -piperitone oxide			19.55	1.0	1257	1258
<i>trans</i> -ascaridole			20.64	23.5	1299	1300
Monoterpene hydrocarbons	5.0	21.5		37.9		
Oxygenated monoterpenes	72.6	11.9		58.8		
Sesquiterpene hydrocarbons	6.7	4.0				
Oxygenated sesquiterpenes	5.7	4.4				
Phenylpropanoids	-	54.0				
Others	5.5	-		-		
Not identified	4.5	4.2		3.3		

¹RT: retention time (min) on the Rtx-5MS column; R_{exp}: experimental retention index relative to a mixture of homologous n-alkanes (C8-C20) on the Rtx-5MS column. Retention index value was compared in the Wiley 7, NIST 08 and FFNSC 1.2 libraries using the GC-MS Solution software (Shimadzu, Japan). ²%RA: relative area.

Table 2. Estimation of concentrations/doses (LC₅₀ and LC₉₀ or LD₅₀ and LD₉₀) of essential oils from *Dysphania ambrosioides*, *Piper callosum* and *Pelargonium graveolens* on adults of *Z. subfasciatus* after 72 hours of exposure in different exposure mode.

Exposure mode	Treatments	N ^a	Slope (± EP)	LC ₅₀ or LD ₅₀ (CI) ^b	CL ₉₀ or DL ₉₀ (CI)	DF ^d	χ ² ^c	P-value
Fumigation (LC, in µ.L ⁻¹ ar)	<i>D. ambrosioides</i> EO	300	-1.90 (0.19)	3.75 (3.13-4.48)	11.94 (9.18-15.5)	4	9.129	0.058
	<i>P. callosum</i> EO	250	-2.36 (0.31)	24.41 (21.35-27.91)	62.04 (47.75-80.60)	3	6.954	0.073
	<i>P. graveolens</i> EO	350	-3.48 (0.37)	14.75 (13.46-16.16)	27.74 (24.28-31.69)	5	10.685	0.058
Topical application (LD, in mg.g ⁻¹)	<i>D. ambrosioides</i> EO	400	-2.95 (0.38)	6.81 (6.26-7.40)	14.33 (11.47-17.89)	6	11.458	0.075
	<i>P. callosum</i> EO	350	-1.95 (0.21)	23.58 (19.93-27.91)	72.73 (57.89-91.38)	5	3.123	0.681
	<i>P. graveolens</i> EO	400	-2.01 (0.22)	30.02 (26.28-34.29)	89.80 (69.78-115.56)	6	12.569	0.050
Residual contact (LC, in µ.kg ⁻¹)	<i>D. ambrosioides</i> EO	250	-3.11 (0.44)	48.35 (42.11-55.52)	97.94 (78.62-122.01)	3	5.094	0.165
	<i>P. callosum</i> EO	250	-2.94 (0.35)	154.21 (134.66-176.60)	325.80 (260.19-407.96)	3	6.980	0.072
	<i>P. graveolens</i> EO	250	-3.29 (0.22)	187.52 (164.70-213.51)	371.12 (299.67-459.61)	3	2.736	0.434

^a N: number of tested insects.

^b CI: 95% confidence interval.

^c χ²: Chi-square value.

^d DF: degrees of freedom.

Table 3. Description of each cluster by quantitative variables (Cluster characterizations in terms of variables).

Variables that describe the partition				
Variables	Eta2^a		p.value	
Inviab. eggs	0.96		0.00000	
Total eggs	0.91		0.00000	
Adults	0.88		0.00000	
Viable eggs	0.86		0.00000	
Holes	0.83		0.00000	
Summary of each cluster				
Cluster 1	v.test	Mean in Category	Overall Mean	p. value
Total eggs	-3.77	9.24	59.51	0.00016
Viable eggs	-3.56	4.44	34.14	0.00038
Adults	-3.51	3.28	27.84	0.00045
Inviab. eggs	-3.09	4.80	25.38	0.00199
Holes	-3.07	4.45	38.11	0.00211
Cluster 2	v.test	Mean in Category	Overall Mean	p. value
Inviab. eggs	3.33	82.50	25.38	0.00089
Cluster 3	v.test	Mean in Category	Overall Mean	p. value
Holes	3.63	82.96	38.11	0.00028
Adults	3.57	55.97	27.84	0.00036
Viable eggs	3.46	66.68	34.14	0.00055
Total eggs	2.65	99.25	59.51	0.00817

^a Correlation ratio (all of them were significantly different to zero).

Table 4. Description of each cluster by quantitative variables (Dimensions of PCA as quantitative variables).

Cluster 1	v.test	Mean in Category	Overall Mean	p. value
Dim1	-3.73	-2.02	0.0000	0.00018
Cluster 2	v.test	Mean in Category	Overall Mean	p. value
Dim2	3.80	2.36	0.0000	0.000147
Cluster 3	v.test	Mean in Category	Overall Mean	p. value
Dim1	3.23	1.96	0.0000	0.001255

Table 5. Analysis of variance of the effects of insect (male/female), “oil and negative control” treatments and interaction between them regarding the distance traveled (cm).

<i>Dysphania ambrosioides</i> LC ₂₅				<i>Dysphania ambrosioides</i> LC ₅₀		
	DF	F	p-value	DF	F	p-value
Sex	1	0,8740	0,3529	1	1,7713	0,1874
Treatments	1	1,3641	0,2466	1	14,861	0,0003 *
Sex×Treatments	1	1,4038	0,2399	1	0,4667	0,4967
Residual	74	-----	-----	72	-----	-----
Significant result for the “Treatments” effect						
Oil		-----			1.263,16 ¹ ± 112,56 a	
Control		-----			762,86 ± 69,60 b	
<i>Piper callosum</i> LC ₂₅				<i>Piper callosum</i> LC ₅₀		
	DF	F	p-value	DF	F	p-value
Sex	1	0,9421	0,3356	1	0,0000	0,9992
Treatments	1	1,5000	0,2254	1	7,0943	0,0095 *
Sex×Treatments	1	2,9241	0,0924	1	4,4900	0,0376 *
Residual	61	-----	-----	70	-----	-----
Significant Results for Interaction						
		-----			Male	Female
Oil		-----			1.299,56 ± 232,63 Aa	963,65 ± 129,00 Aa
Control		-----			632,96 ± 83,80 Ab	875,81 ± 103,71 Aa
<i>Pelargonium graveolens</i> LC ₂₅				<i>Pelargonium graveolens</i> LC ₅₀		
	DF	F	p-value	DF	F	p-value
Sex	1	0,2995	0,5860	1	0,8615	0,3563
Treatments	1	6,4980	0,0130 *	1	10,5541	0,0018 *
Sex×Treatments	1	3,0694	0,0842	1	0,6673	0,4166
Residual	70	-----	-----	74	-----	-----
Significant results for the “Treatments” effect						
Oil		1.066,24 ± 106,35 a			1.235,32 ± 137,28 a	
Control		762,86 ± 69,60 b			762,86 ± 69,60 b	

¹Distance in centimeters (cm)

* Significant for the variance analysis tukey 5%

Table 6. Analysis of variance of the effects of insect sex, “oil and negative control” treatments and interaction between them regarding speed.

<i>Dysphania ambrosioides</i> LC ₂₅				<i>Dysphania ambrosioides</i> LC ₅₀		
	DF	F	p-value	DF	F	p-value
Sex	1	0,5307	0,4686	1	1,4357	0,2348
Treatments	1	1,3074	0,2566	1	17,5657	0,0000*
Sex×Treatments	1	1,7041	0,1958	1	0,6525	0,4219
Residual	74	-----	-----	72	-----	-----
Significant result for the “Treatments” effect						
Oil		-----			2,22 ¹ ± 0,18 a	
Control		-----			1,31 ± 0,12 b	
<i>Piper callosum</i> LC ₂₅				<i>Piper callosum</i> LC ₅₀		
	DF	F	p-value	DF	F	p-value
Sex	1	0,7710	0,3834	1	0,0650	0,7995
Treatments	1	1,5937	0,2116	1	4,7016	0,0335*
Sex×Treatments	1	2,8513	0,0964	1	3,1198	0,0817
Residual	61	-----	-----	70	-----	-----
Significant result for the “Treatments” effect						
Oil		-----			1,81 ± 0,22 a	
Control		-----			1,31 ± 0,12 b	
<i>Pelargonium graveolens</i> LC ₂₅				<i>Pelargonium graveolens</i> LC ₅₀		
	DF	F	p-value	DF	F	p-value
Sex	1	0,1373	0,7121	1	0,6174	0,4345
Treatments	1	6,3109	0,0143*	1	10,5932	0,0017*
Sex×Treatments	1	3,4915	0,0659*	1	0,8581	0,3573
Residual	70	-----	-----	74	-----	-----
Significant results for Interaction and “Treatments” effect						
	Male		Female		-----	
Oil	2,00 ± 0,22 Aa		1,62 ± 0,30 Aa		2,11 ± 0,23 a	
Control	1,09 ± 0,12 Ab		1,49 ± 0,18 Aa		1,31 ± 0,12 b	

¹Speed in centimeters per second (cm⁻¹)

* Significant for the variance analysis Tukey 5%

Table 7. Isolated statistical analysis to differentiate treatments in relation to distance and speed – LC₅₀

Treatments	Distance	DF	F	p-value	Speed	DF	F	p-value
<i>D. ambrosioides</i>	1.263,16 ± 112,56 a	3	4,7986	0,003*	2,22 ± 0,18 a	3	5,2909	0,002*
<i>P. graveolens</i>	1.235,32 ± 137,28 a				2,11 ± 0,23 a			
<i>P. callosum</i>	1.126,19 ± 132,03 ab				1,81 ± 0,22 ab			
Control	762,86 ± 69,60 b				1,31 ± 0,12 b			

¹Means followed by the same letters do not differ significantly from the Tukey 5% test

Table 8. Isolated statistical analysis to differentiate treatments in relation to distance and speed – LC₂₅

Treatments	Distance	DF	F	p-value	Speed	DF	F	p-value
<i>P. graveolens</i>	1.066,24 ± 106,35 a	3	3,4782	0,0180	1,83 ± 0,18 a	3	3,4813	0,0179*
<i>D. ambrosioides</i>	905,73 ± 104,51 ab				1,55 ± 0,18 ab			
Control	762,86 ± 69,60 ab				1,31 ± 0,12 ab			
<i>P. callosum</i>	625,35 ± 95,21 b				1,06 ± 0,16 b			

¹Means followed by the same letters do not differ significantly from the tukey 5% test

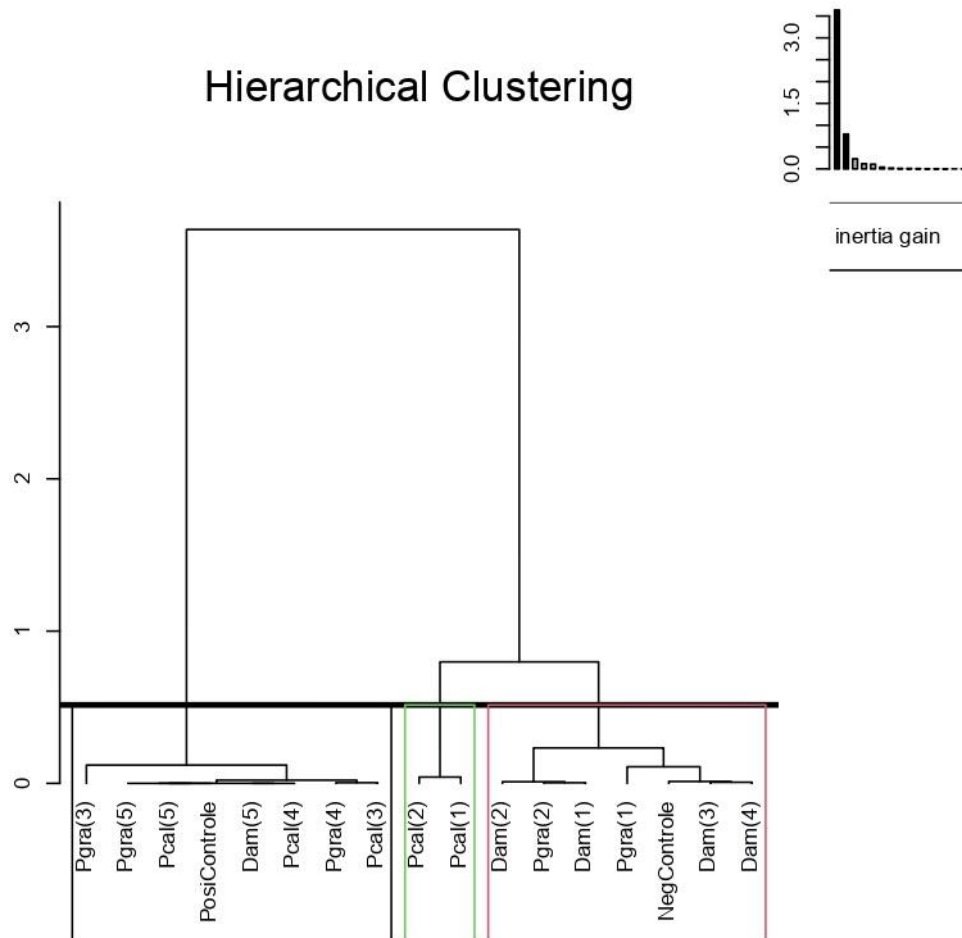


Figure 1 Clustering on Principal Components (PCs). Treatments: Control (1) (NegControl); *D. ambrosioides* 31,25 $\mu\text{L/kg}$ (Dam 1); *D. ambrosioides* 62,5 $\mu\text{L/kg}$ (Dam 2); *D. ambrosioides* 125 $\mu\text{L/kg}$ (Dam 3); *D. ambrosioides* 250 $\mu\text{L/kg}$ (Dam 4); *D. ambrosioides* 500 $\mu\text{L/kg}$ (Dam 5); *P. graveolens* 31,25 $\mu\text{L/kg}$ (Pgra 1); *P. graveolens* 62,5 $\mu\text{L/kg}$ (Pgra 2); *P. graveolens* 125 $\mu\text{L/kg}$ (Pgra 3); *P. graveolens* 250 $\mu\text{L/kg}$ (Pgra 4); *P. graveolens* 500 $\mu\text{L/kg}$ (Pgra 5); *P. callosum* 31,25 $\mu\text{L/kg}$ (Pcal 1); *P. callosum* 62,5 $\mu\text{L/kg}$ (Pcal 2); *P. callosum* 125 $\mu\text{L/kg}$ (Pcal 3); *P. callosum* 250 $\mu\text{L/kg}$ (Pcal 4); *P. callosum* 500 $\mu\text{L/kg}$ (Pcal 5); Positive control (Deltamethrin).

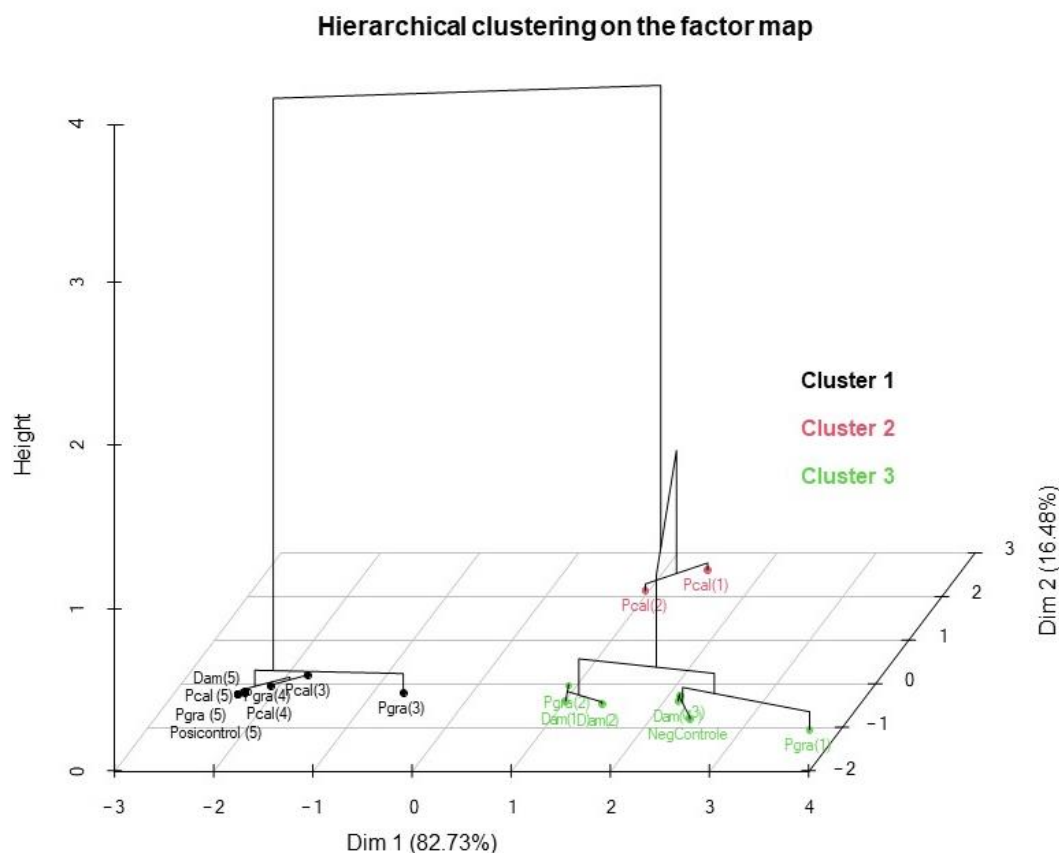


Figure 2. Dendrogram of Hierarchical Clustering based on the Ward's criterion. The height of the branches indicates the dissimilarity between clusters. Cluster solution is indicated by tree-colored panels. (B) Tree-dimensional Score plot illustrating how clusters were distributed on PCs. The number of clusters to be retained was set according to the dendrogram and cluster solution was consolidated by K-means. Treatments: Control (1) (NegControl); *D. ambrosioides* 31,25 $\mu\text{L/kg}$ (Dam 1); *D. ambrosioides* 62,5 $\mu\text{L/kg}$ (Dam 2); *D. ambrosioides* 125 $\mu\text{L/kg}$ (Dam 3); *D. ambrosioides* 250 $\mu\text{L/kg}$ (Dam 4); *D. ambrosioides* 500 $\mu\text{L/kg}$ (Dam 5); *P. graveolens* 31,25 $\mu\text{L/kg}$ (Pgra 1); *P. graveolens* 62,5 $\mu\text{L/kg}$ (Pgra 2); *P. graveolens* 125 $\mu\text{L/kg}$ (Pgra 3); *P. graveolens* 250 $\mu\text{L/kg}$ (Pgra 4); *P. graveolens* 500 $\mu\text{L/kg}$ (Pgra 5); *P. callosum* 31,25 $\mu\text{L/kg}$ (Pcal 1); *P. callosum* 62,5 $\mu\text{L/kg}$ (Pcal 2); *P. callosum* 125 $\mu\text{L/kg}$ (Pcal 3); *P. callosum* 250 $\mu\text{L/kg}$ (Pcal 4); *P. callosum* 500 $\mu\text{L/kg}$ (Pcal 5); Positive control (Deltamethrin).

CONSIDERAÇÕES FINAIS

Os resultados encontrados nessa pesquisa auxiliam no desenvolvimento de novas soluções ambientalmente amigáveis de forma alternativa ao controle químico. Assim, os óleos essenciais (OE) podem ser estratégicos para controle de pragas de grãos armazenados e para a proteção de grãos. Os nossos resultados evidenciaram a toxicidade dos OEs de *Cordia verbenacea*, *Elionurus latiflorus*, *Dysphania ambrosioides*, *Pelargonium graveolens* e *Piper callosum* nos métodos de exposição via fumigação, aplicação tópica e contato residual sobre adultos de *Z. subfasciatus*. O OE de *E. latiflorus* apresentou inibição do crescimento do fungo *Aspergillus nomius*, e a produção das aflotoxinas B1, B2, G1 e G2, além do efeito arrestante no comportamento de caminhamento das fêmeas. O OE *D. ambrosioides* apresentou maior toxicidade quando comparado com os de OE *P. graveolens* e *P. callosum* e, adicionalmente, maior efeito repelente sobre os adultos. O OE de *P. graveolens* e de *P. callosum* mostraram efeitos negativos na oviposição das fêmeas e na emergência de adultos, sugerindo que esses OE possam ter propriedades ovicidas e/ou efeitos na biologia das larvas de *Z. subfasciatus*. Os resultados encontrados nesse estudo contribuem na busca de novas fontes de soluções de manejo de pragas de armazéns de baixo impacto ambiental e na saúde humana e ambiental. Novos estudos devem ser elaborados com os compostos majoritários isolados dos OE e investigar os efeitos nos insetos e ovos.

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