GABRIEL TADEU DE PAIVA SILVA

SURVIVAL AND DEMOGRAPHY OF THE TOMATO BORER (*Tuta absoluta*) EXPOSED TO CITRUS ESSENTIAL OILS AND MAJOR COMPOUNDS

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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em entomologia, para a obtenção do título de Mestre.

Prof. Dr. Geraldo Andrade Carvalho
Orientador
Profa. Dra. Dejane Santos Alves
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SOBREVIVÊNCIA E HISTÓRIA DE VIDA DA TRAÇA-DO-TOMATEIRO (*Tuta absoluta*) EXPOSTA A ÓLEOS ESSENCIAIS DE *Citrus* spp. E A SEUS COMPONENTES MAJORITÁRIOS

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À minha mãe Clarice, pelo amor incondicional e apoio.

Dedico

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RESUMO

A traça-do-tomateiro *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) é uma praga global de rápida dispersão e de difícil controle, devido aos inúmeros casos de resistência aos ingredientes ativos empregados para o seu controle. Com isso a avaliação de óleos essenciais para desenvolvimento de novos produtos com potencial inseticida é importante. O objetivo desse trabalho foi avaliar os efeitos letal e subletais de óleos essenciais (OEs) de *Citrus aurantifolia* (lima), *Citrus aurantium* (petitgrain) e *Citrus aurantium bergamia* (bergamota) e de seus compostos majoritários sobre *T. absoluta*. Para tanto, primeiramente os OEs foram solubilizados em acetona na concentração de 100 µg µL^{-1} e alíquotas (1 µL) foram aplicadas no dorso de lagartas de 2º ínstar com microseringa (Hamilton 50 µL). A avaliação da sobrevivência foi feita a cada 12 h até o total de 72 h; em todos os experimentos desse estudo o tratamento controle consistiu de lagartas expostas apenas a 1 µL de acetona. O tempo letal mediano (TL_{50}) das lagartas expostas aos óleos de petitgrain e lime foi de 13,57 h enquanto as tratadas com bergamota tiveram TL_{50} de 19,34 h. Os OEs foram submetidos à análise de cromatografia gasosa acoplada a espectrometria de massas (CG-EM) e identificados os compostos majoritários. No OE de lima foi encontrado 44,74% de alfa-terpinol; 55,45% e 58,12% de acetato de linalila para petitgrain e bergamota, respectivamente. Para determinação da dose letal mediana (DL_{50}) utilizaram-se seis doses dos OEs baseadas em progressão aritmética e em experimentos prévios, sendo de 100; 75; 55; 41,3 e 31,6 e 17,3 µg µL^{-1}. As DL_{50} dos OEs de lima, petitgrain e bergamota foram 33,75, 38,78 e 35,05 µg µL^{-1}, respectivamente. Para determinação da toxicidade dos componentes majoritários usaram-se soluções em concentração equivalente à DL_{50} dos OEs; sendo 16,2 µg µL^{-1} (alfa-terpineol) e 25,8 µg µL^{-1} (acetato de linalila). Foi aplicado uma alíquota de 1 µL da solução no dorso de lagartas de 2º ínstar e a sobrevivência foi avaliada durante período de 72 h. Os compostos majoritários apresentaram toxicidade inferior à encontrada dos OEs, não sendo capazes de eliminar metade da população testada. Para construção da tabela de vida, primeiramente as lagartas de 2º ínstar foram expostas a 1 µL da DL_{50} dos OEs e avaliadas diariamente. Os tratamentos reduziram a duração dos ínstares larvais, duração do período de pupa, fecundidade, oviposição e viabilidade de ovos. Com isso houve redução dos parâmetros de crescimento populacional da praga. Sendo assim, nesse estudo comprovou-se que os OEs de *C. aurantifolia* (lima), *C. aurantium* (petitgrain) e *C. aurantium bergamia* (bergamota) causam efeitos letais em *T. absoluta.* Quando OEs foram empregados em doses subletais prejudicaram negativamente parâmetros demográficos dessa praga.

Palavras-chave: Solanaceae. Lepidóptero-praga. Produtos botânicos. Controle.

ABSTRACT

The tomato borer, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) is a global pest that have dispersed quickly over the countries. That pest shows high level of resistance to insecticides commonly used. Therewith the evaluation of essential oils toxicity it's important for development of new insecticide molecules. In this study we evaluated the lethal and sublethal effects of *Citrus aurantifolia* (lime), *Citrus aurantium* (petitgrain) *and Citrus aurantium bergamia* (bergamot) essential oils and its major compounds against *T. absoluta*. Therefore, the EOs were solubilized in acetone at 100 µg μL^{-1} and 1 µL of that solution were applied to the mid-dorsal abdominal region of 2º instar larvae using a microsyringe (Hamilton 50 µL). The survival was assessed every 12 h up to a total of 72 h; in all experiments in this study, the control treatments consisted of 2º instar larvae exposed to only 1 µL of acetone. The lethal time 50 (LT_{50}) of larvae exposed to petitgrain and lime EOs was 13.57 h while those treated with bergamot had LT_{50} of 19.34 h. For chemical characterization, EOs were submitted to gas chromatography analysis coupled to mass spectrometry (GC-ME) and the major compounds were identified. Results show lime EO had 44.74% of alpha-terpinol; petitgrain and bergamot have 55.45% and 58.12% of linalyl acetate respectively. To determine the median lethal dose (LD_{50}), six doses of EOs were used based on arithmetic progression and previous experiments, being them, 100; 75; 55; 41.3 and 31.6 and 17.3 µg μL^{-1}. The LD_{50} of the EOs for lime, petitgrain and bergamot were 33.75, 38.78 and 35.05 µg μL^{-1}, respectively. To determine the toxicity of the major components, solutions were used in the concentration equivalent to LD50 in the OEs; 16.2 µg μL^{-1} (alpha-terpineol) and 25.8 µg μL^{-1} (linalyl acetate). After applying a 1 µL aliquot on the dorsal abdominal region of 2º instar larvae, survival was assessed for a period of 72 h. The majority compounds showed toxicity lower than that found in EOs, not being able to eliminate half of the tested population. To life table analysis, the 2º instar larvae were exposed to 1 µL of the LD_{50} of the EOs and evaluated daily. The treatments reduced the duration of larval instars, duration of the pupal period, fertility, oviposition and egg viability. As a result, there was a reduction in the population growth parameters of *T. absoluta*. Thus, in this study it was proved that the EOs of *C. aurantifolia* (lime), *C. aurantium* (petitgrain) and *C. aurantium bergamia* (Bergamot) cause lethal effects in *T. absoluta*. When EOs were used in sublethal doses, they affected reproductive and demographic parameters of this pest.

Keywords: Solanaceae. IPM. Botanical products. Control.

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Parte I

1. INTRODUÇÃO GERAL

Dentre as pragas do tomateiro, a espécie *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) se destaca como uma praga severa dessa cultura, sendo que em altas infestações pode causar até 100% de perda na produção se não controlada. Esse micro lepidóptero popularmente conhecido como traça-do-tomateiro, em sua fase jovem, se alimenta de todas as partes aéreas da planta, como ramos, folhas, flores e frutos (IMENES et al., 1990; DESNEUX et al., 2010). A lagarta penetra na folha de tomateiro após 20 a 45 minutos de sua eclosão, e em seu último estádio mede cerca de 7 mm de comprimento. Possuem uma mancha marrom escuro em seu dorso, podendo variar de verde a amarelo. A mariposa tem um padrão de cor acinzentada e tem cerca de 5 mm de comprimento. O ciclo de vida de *T. absoluta* é de aproximadamente 30 dias sob temperatura média de 23ºC (IMENES et al., 1990; COCCO et al., 2015).

A traça-do-tomateiro é uma praga sul americana, nativa do Peru e sua entrada no Brasil ocorreu na década de 60, se tornando um grande problema em cultivos de tomate desde então (IMENES et al., 1990; GONTIJO et al., 2013; BIONDI et al., 2018; HAN et al., 2019; ZHANG et al., 2020). Em 2006 esse inseto tornou-se um problema ainda maior no mundo quando foi registrada na Espanha, de onde rapidamente se dispersou para outros países europeus, africanos e asiáticos (BIONDI et al., 2018). Recentemente sua presença foi registrada em cultivos da China, o maior produtor de tomates do mundo (ZHANG et al., 2020).

O controle da *T. absoluta* é feito utilizando inseticidas sintéticos de amplo espectro (BUENO et al., 2013). Porém, a taxa de controle vem diminuindo devido ao uso indiscriminado dos ingredientes ativos, de forma que já existem populações com resistência a múltiplos inseticidas. Exemplo disso são os sucessivos relatos da ineficiência de alguns produtos dos groups químicos dos piretroides, espinosinas, organofosforados, avermectinas, cartap, indoxicarb, oxadiazinas, diamidas e benzoilureias (GUEDES, 2012; GUEDES; PICANÇO, 2012; CAMPOS et al., 2014; BIONDI et al., 2018). Isso demonstra que o uso exclusivo do controle químico sem um manejo racional é ineficaz a longo prazo. Desta forma, a racionalização e o desenvolvimento de novos ingredientes ativos menos

tóxicos e mais seletivos para o controle da traça do tomateiro é essencial para o sucesso de seu controle (GONTIJO et al., 2013).

Nesse contexto, a busca por novos ingredientes ativos menos tóxicos ao homem e ambiente e mais seletivos aos inimigos naturais, utilizando-se de moléculas presentes nos óleos essenciais (OEs) e extratos vegetais, tem sido cada vez mais incentivada (ISMAN, 2006; BAKKALI et al., 2008; WALIA et al., 2017; SOARES et al., 2019). Os OEs possuem aceitação maior do mercado à medida que possuem diversos usos medicinais e alimentícios (KERDCHOECHUEN et al., 2010; MAIA; MOORE, 2011; LOIZZO et al., 2012; DE SOUSA et al., 2015; ZARRAD et al., 2017). No controle de insetos pragas, moléculas botânicas de extratos de plantas são utilizadas desde o começo da agricultura (BAKKALI et al., 2008).

Dentre os gêneros de plantas dos quais são extraídos OEs, destaca-se o *Citrus* que contém plantas com grande potencial no controle de pragas. Vários trabalhos evidenciaram a bioatividade de óleos de plantas de *Citrus sinensis, Citrus lemon, Citrus aurantifolia, Citrus aurantium, Citrus bergamia* para diferentes artrópodes pragas como *Bemisia tabaci* (Gennadius) (Hempitera: Aleyrodidae), *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Rhizopertha dominica* (Fabricius) (Coleoptera: Bostrichidae), *Pseudococcus calceolariae* (Maskell) (Hemiptera: Pseudococcidae), *Pseudococcus longispinus* (Targioni Tozzetti) (Hemiptera: Pseudococcidae), *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), *Callosobrunchus maculatus* (Fabricius) (Coleoptera: Bruchidae) e *Sitophilus zeamais* (Motshulsdy) (Coleoptera: Curculionidae) (ZARRAD et al., 2015, 2017; CAMPOLO et al., 2017; TACOLI et al., 2018; OYEDEJI et al., 2020).

Para o controle de *T. absoluta* com OEs de plantas, os trabalhos são escassos, sendo que Campolo et al. (2017) e Zarrad et al. (2017) avaliaram a toxicidade de óleos essenciais de *C. aurantium* (laranja amarga), *C. limon* (limão), *Citrus reticulata* (laranja mandarim) e *Citrus sinensis* (laranja doce) e encontraram resultados promissores. Dentre as espécies do gênero *Citrus*, destaca-se *Citrus aurantifolia* (lima), *Citrus aurantium* (petitgrain) e *Citrus aurantium bergamia* (bergamota) que demonstram potencial de controle para *T. absoluta* e outros artrópodes pragas (KERDCHOECHUEN et al., 2010; MAIA; MOORE, 2011; LOIZZO et al., 2012; ZARRAD et al., 2017; TCHAMENI et al., 2018).

A toxicidade de OEs está relacionada com a sua composição química, geralmente formada de uma mistura complexa de hidrocarbonetos, terpenoides e outras moléculas como aldeídos, álcoois, ésteres e ácidos orgânicos (BORA et al., 2020). Essa mistura pode atuar em diversos pontos na fisiologia do inseto, sendo que em alguns trabalhos, os autores sugeriram que a toxicidade dos OEs de *Citrus* spp. está associada ao sistema nervoso central dos insetos, porém o seu verdadeiro mecanismo de ação ainda não está claro, necessitando de novos estudos para este propósito (KOSTYUKOVSKY et al., 2002; ZARRAD et al., 2015; OBOH et al., 2017). Além disso, a toxicidade ainda pode estar relacionada com o componente majoritário da mistura, a outros componentes em menor quantidade ou ao sinergismo entre as moléculas (BAKKALI et al., 2008). Os componentes ou constituintes majoritários são moléculas que estão em maior proporção no OE, e por vezes são responsáveis pela toxicidade ao organismo alvo. Contudo o sinergismo é um fator determinante à medida que alguns componentes em menor quantidade podem agir como facilitadores para que a molécula bioativa encontre o sítio de ação (KARPOUHTSIS et al., 1998; CAL, 2006; TCHAMENI et al., 2018).

Além do estudo do efeito letal dos óleos essenciais sobre *T. absoluta*, avaliar seus impactos na história de vida, por meio de tabelas de vida, e os efeitos subletais é de grande valia para o desenvolvimento de programas de manejo dessa praga (BREVIK et al., 2018; SZABÓ; SERES; BAKONYI, 2020). Tradicionalmente as tabelas de vida desconsideravam o efeito dos machos na história de vida dos insetos, levando a interpretações errôneas dos parâmetros estudados (CHI, 1988; CHI; HUANG; CHI, 2011). Contudo, esse problema tem sido contornado por meio do método desenvolvido por Chi & Liu (1985), denominado de tabela de vida para dois sexos por idade e estágio de desenvolvimento (*Age-stage, two-sex, life table*). Por esse método considera-se a sobrevivência e o desenvolvimento tanto de machos quanto de fêmeas. Dessa forma, essa metodologia permite avaliar os efeitos das moléculas químicas em parâmetros demográficos que indicam o crescimento ou não da população do inseto e parâmetros reprodutivos como fecundidade e oviposição, demonstrando os prováveis efeitos na população como um todo (STARK; SUGAYAMA; KOVALESKI, 2007; TUAN; LEE; CHI, 2014; JIANG et al., 2020).

Assim, o presente trabalho teve como objetivo avaliar a toxicidade aguda dos OEs de *C. aurantifolia, C. aurantium* e *C. aurantium bergamia* e seus componentes majoritários

para *T. absoluta*. Adicionalmente o efeito de doses subletais dos OEs foi avaliado sobre parâmetros demográficos desse inseto.

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Part II

SURVIVAL AND DEMOGRAPHY OF THE TOMATO BORER (*Tuta absoluta*) EXPOSED TO CITRUS ESSENTIAL OILS AND MAJOR COMPOUNDS

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ABSTRACT

The tomato borer (*Tuta absoluta*) is a pest of great economic importance, quick to disperse and difficult to control due to the countless cases of resistance to different insecticide active ingredients. Thus, studies with essential oils (EOs) to search for new molecules for control should be intensified. The objective of the present study was to evaluate the toxicity of EOs from *Citrus aurantifolia* (lime), *Citrus aurantium* (petitgrain) and *Citrus aurantium bergamia* (bergamot) and its major compounds against *T. absoluta*, in a topical application test. Additionally, the demographic parameters of *T. absoluta* were studied after the application of EOs. The median lethal time (LT_{50}) required to cause mortality in 50% of the population was 13.57 h (petitgrain and lime) and 19.34 h for bergamot. The median lethal dose (LD_{50}) was 33.75; 38.78 and 35.05 µg μL^{-1} for lime, petitgrain and bergamot, respectively. By gas chromatography coupled to mass spectrometry (GC-MS) quantification, the EO of lime contains 44.74% of α-terpineol, while the essential oil of petitgrain and bergamot contain 55.45% and 58.12 % linalyl acetate. All EOs negatively affected the demographic parameters of *T. absoluta*, with a decrease in the duration of larval instars, duration of the pupal period, fecundity, oviposition, and viability of eggs, implying a reduction in the population growth parameters of this pest. The EOs of lime, petitgrain and bergamot are toxic to *T. absoluta* and subdoses cause deleterious effects on the reproductive and population parameters of *T. absoluta*.

Keywords: Solanaceae. Sublethal. Botanical insecticides. Rutaceae. life table.

Introduction

The tomato borer, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), is a pest commonly found in plants of the Solanaceae family and can cause great economic losses when feeding on plant tissues. Its life cycle is short, which increases its destructive potential, and in approximately 30 days under an average temperature of 23ºC a new generation of insects already occurs (Imenes et al. 1990; Tropea Garzia et al. 2012). This pest is native to South America, but it took on a more prominent role in global terms from 2006 when it was detected in Spain and then spread quickly to other countries in Europe, Africa and Asia, reaching China, which is the world's largest tomato producer (Desneux et al. 2010; Guedes and Picanço 2012; Biondi et al. 2018; Han et al. 2019; Zhang et al. 2020). According to Santana et al. (2019) *T. absoluta* are a major threat to other regions in Oceania and North America since they have favorable climatic conditions for their survival and multiplication.

The use of synthetic insecticides is commonly the most used method for the control of *T. absoluta*. However, the efficiency of insecticides has been decreasing due to indiscriminate use, which has led to the selection of resistant populations of this pest (Guedes et al. 2019). There are numerous reports of the inefficiency of active ingredients belonging to the chemical groups of pyrethroids, spinosyns, organophosphates, avermectins, cartap, indoxacarb, oxadiazines, diamides and benzoylureas (Guedes and Picanço 2012; Guedes 2012; Campos et al. 2014; Biondi et al. 2018; Guedes et al. 2019) to this insect. Thus, studies looking for new molecules to control *T. absoluta* should be encouraged.

In this context, essential oils (EOs) have been studied for the control of *T. absoluta* (Moreno et al. 2012; Campolo et al. 2017; Zarrad et al. 2017; Chegini et al. 2018; Sammour et al. 2018; Yarou et al. 2018; Piri et al. 2020). Secondary metabolites present in EOs can be used as model molecules for the synthesis of new insecticides, such as pyrethroids that were synthesized from pyrethrins of plants of the genus *Tanacetum*. In addition, studies with EOs show that their residual effect is quite low and that they have selectivity for non-target individuals, such as aquatic fauna organisms, pollinators, natural enemies and soil organisms (Pavela and Benelli 2016; Walia et al. 2017; Campolo et al. 2020).

EOs extracted from plants of the genus *Citrus* present bioactivity against several species of arthropod pests such as *T. absoluta*, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Rhizopertha dominica* (Fabricius) (Coleoptera: Bostrichidae), *Pseudococcus calceolariae* (Maskell) (Hemiptera: Pseudococcidae), *Pseudococcus longispinus* (Targioni Tozzetti) (Hemiptera: Pseudococcidae), *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), *Callosobrunchus maculatus* (Fabricius, 1775) (Coleóptera: Bruchidae) and *Sitophilus zeamais* (Motshulsdy) (Coleoptera: Curculionidae) (Zarrad et al. 2015, 2017a; Campolo et al. 2017; Tacoli et al. 2018; Oyedeji et al. 2020). Among the species of the genus *Citrus*, *Citrus aurantifolia* (lime), *Citrus aurantium* (petitgrain) and *Citrus aurantium bergamia* (bergamot) EOs have several findings reported on their bioinsecticidal action (Kerdchoechuen et al. 2010; Campolo et al. 2014, 2020; Zarrad et al. 2015, 2017; Tacoli et al. 2018).

It is known that there is a natural variation in the concentration and components of EOs (Isman e Grieneisen 2014); these differences in products extracted from a species occur due to genetic and environmental factors, which act on the plant's physiology (Kesterson et al. 1971; Hili et al. 1997; Tadeo et al. 2008; Bora et al. 2020). Thus, the use of gas chromatography analysis coupled to mass spectrometry (GC-MS) to quantify and identify the components is important (Koul et al. 2008; Isman and Grieneisen 2014).

In addition, the bioactivity of EOs on pest arthropods may be related to the major component of the oil or to the synergism between the chemical molecules of the mixture, since components in lesser amounts can improve the physical-chemical characteristics of the oil, helping for example in mobility of toxic molecules present in the insect's body (Karpouhtsis et al. 1998; Cal 2006; Bakkali et al. 2008; Tchameni et al. 2018). This justifies the importance of assessing the toxicity of major compounds.

Few studies have been done on the lethal and sublethal effects of the EOs of *C. aurantifolia*, *C. aurantium* and *C. aurantium bergamia* on *T. absoluta* (Zarrad et al. 2017). The study of acute toxicity of OEs is traditionally the most used method for studying the lethal effect on insect pests (Biondi et al. 2013), However, the sublethal effects of these chemicals are also important for toxicological studies, since part of the population in the field is exposed only to subdoses of the products (Desneux et al. 2007). The sublethal

effects can increase the mortality of future generations and reduce reproductive parameters such as fertility and the period of oviposition, causing the reduction of future populations of the target insect (Desneux et al. 2007; He et al. 2013). Thus, evaluating the impact of subdoses of EOs on the life history of *T. absoluta* is important for understanding the toxicity in this insect (Desneux et al. 2007; Brevik et al. 2018).

For the study of sublethal effects and the life history of insects, fertility life tables are traditionally used. However, these methods disregard the effect of males and the different stages of development in the life history of insects, leading to erroneous interpretations of the studied parameters (Chi 1988; Chi; Huang; Chi 2011). To solve this problem, Chi and Liu (1985), proposed a method called Age-stage two-sex life table, where the survival and development of both males and females at all stages are considered. Thus, this methodology allows to evaluate the effects of chemical molecules on demographic parameters that indicate the growth or not of the insect population and reproductive parameters such as fertility and pre-oviposition and oviposition period, demonstrating effects on the population as a whole (Stark et al. 2007; Tuan et al. 2014).

Considering the hypothesis that EOs negatively affect the biological characteristics of *T. absoluta* and that this toxic effect is caused by the major compounds of the mixture, the present study aimed to i) evaluate the acute toxicity of EOs and the major compounds of *C. aurantifolia*, *C. aurantium* and *C. aurantium bergamia* against *T. absoluta*; ii) study the effects of sublethal doses of EOs of *C. aurantifolia*, *C. aurantium* and *C. aurantium bergamia* on demographic parameters of *T. absoluta*.

Material and Methods

All bioassays were conducted at a temperature of 24 ± 2 °C, relative humidity of 70 ± 10% and a 12-h photophase, with laboratory rearing insects from the second to sixth generation.

Biological Material

Tomato seedlings, *Solanum lycopersicum* cv. Santa Clara were transplanted in 5-liter pots containing a mixture of 3 parts of ravine soil and 1 of vermiculite and kept in a greenhouse, free from contamination by insects, pathogens, or chemicals until the cutting period (60 days after planting).

Tomato leaves containing *T. absoluta* larvae were collected in crops of this Solanaceae at the Campus of the Universidade Federal de Lavras (UFLA) and at the company Agroteste LTDA (21º12’ S, 45º03’ W). After collection, the larvae were placed in acrylic cages (60 x 30 x 30 cm), along with other specimens from the already existing rearing at the Ecotoxicology and MIP Laboratory (LEMIP). After the emergence of adults, males and females of the same age were placed together in a new cage containing tomato twigs as substrates for oviposition and cotton moistened with a solution of 1 part of water and 1 part of honey that for feeding. The twigs with the eggs were removed and used to maintain the laboratory rearing. After six generations, the second instar larvae of the F6 and F7 were used in the studies.

Essential oils

The EOs of *C. aurantifolia*, *C. aurantium* and *C. aurantium bergamia* were acquired from Empresa Ferquima Indústria e Comércio Ltda., Vargem Grande Paulista, São Paulo - Brazil. The extraction of EOs was made by steam distillation and cold pressing of the fruits (lime), leaves (petitgrain) and fruit peels (bergamot).

Chemical composition

The analyzes were conducted using a gas chromatograph coupled to a mass spectrometer (model QP2010, Shimadzu, Japan), with RTX-5MS capillary column (30 m ×

0,25 mm ID × 0,25 µm film thickness; Restek). The EOs were diluted in acetone at a concentration of 10 mg mL^{-1}, with 1 µL of the solution injected into the gas chromatograph, in which helium was used at 1.0 mL min^{-1} as the carrier. The conditions followed the one proposed by Adams (2007), being: Split/splitless temperature: 220 ºC; Split injection ratio: 1:20; initial column temperature: 60 ºC; rate of elevation in column temperature: 2 ºC min^{-1} to 200 ºC, after 200 ºC the rate of elevation changed to 5 ºC min^{-1}; final column temperature: 250 ºC; interface temperature between the chromatograph and the mass spectrophotometer: 220 ºC; ionization of spectrophotometer molecules: electron impact at 70 eV; mass/load range (m/z) analyzed on the mass spectrophotometer: 45–400; Mass spectrum acquisition time: 0.5 s. The components were identified based on comparisons with the relative retention index using data from a series of n-alkanes (C9-C20). All spectra were compared with NIST 05 Mass Spectral Library 2005; peaks with similarity less than 90% were discarded.

Screening of EOs against *T. absoluta*

The EOs of *C. aurantifolia, C. aurantium* and *C. aurantium bergamia* were diluted in acetone at a concentration of 100 µg $µL^{-1}$. From this solution, aliquots (1 µL) were applied to the dorsal abdominal region of each second instar larva using a Hamilton® 50 µL microsyringe. In the control treatment, the larvae were exposed only to acetone. After application, the larvae were individualized in wells of plastic rearing tray containing a piece of tomato leaf (3 cm x 3 cm) and a piece of filter paper (2 cm x 2 cm) that was previously moistened with 500 µL distilled water to maintain leaf tissue moisture and turgidity.

The bioassay was carried out in a completely randomized design, with four treatments and 60 repetitions, each formed by a second instar larva, maintained individually in each well of the tray. The evaluation of the mortality of the larvae was made every 12 h up to 72 h after the application of the EOs. Larvae that did not show movements at the touch of a fine-tipped and soft bristles brush were considered dead.

EOs doses-time-mortality response against *T. absoluta*

To determine the values of median lethal doses (LD_{50}), lethal dose to 90% of the population (LD_{90}) and median lethal time (LT_{50}), six doses of EOs diluted in acetone were

used, which were determined using arithmetic progression and previous tests, being of 100; 75; 55; 41.3; 31.6 and 17.3 µg µL^{-1}. *Tuta absoluta* 2nd instar larvae were treated with 1 µL of the solution using microsyringe. In the control treatment, only the acetone solvent was used.

The application of EOs was carried out in a manner similar to that previously described. Then, the larvae were individualized in wells of rearing trays containing a piece of tomato leaf (3 cm x 3 cm) and a piece of filter paper moistened with water to maintain the turgidity of the leaf tissue. The bioassay was carried out in a completely randomized design, with four treatments and 50 replicates, each one formed by a larva kept individualized in each well of the plate. The evaluation of the mortality of the larvae was done every 12 h up to 72 h of their treatment, to determine the response-time-mortality. The accumulated survival of insects after 72 h after the application of the treatments was used to calculate the dose-mortality-response.

Acute toxicity of the major compounds of the EOs against *T. absoluta*

The major compounds of the EOs of *C. aurantifolia, C. aurantium* and *C. aurantium bergamia* (α-terpineol, 93% pure and linalyl acetate, 95% pure) were obtained from the Chemistry Department of UFLA. To calculate the equivalence of the concentrations of major compounds, the percentage of α-terpineol and linalyl acetate found in the gas chromatography analysis coupled to a mass spectrophotometer (CG-EM) was used, using the following formula:

$$CBT = CEO \times CMC(\%)$$

Where: CBT is the concentration to be tested; CEO: concentration of essential oil and CMC: content of the major compound present in the oil in percentage.

The treatments consisted of pure substances in concentrations equivalent to the LD_{50} of the EOs, that is, 16.2 µg µL^{-1} (terpineol) and 25.8 µg µL^{-1} (linalyl acetate). Aliquots (1 µL) were applied to second instars of *T. absoluta* using microsyringe. The design was completely randomized, with four treatments and 60 repetitions per treatment, each of which consisted of a second instar treated larva.

The evaluation of insect mortality was carried out at 12, 24, 36, 48, 60 and 72 h after application of the compounds, to calculate the median lethal time (LT_{50}). Insects that did not move at the touch of a brush with a fine, soft tip were considered dead.

Life tables of *T. absoluta* treated with LD_{50} of *Citrus* spp. EOs

About 200 adults of the same age (72h) of *T. absoluta* were kept in an acrylic cage (60 x 30 x 30 cm) containing a tomato plant (± 15 cm high) for 24 h for oviposition. After this period, the plant was removed and placed in another cage where daily assessment was made to check for the appearance of larvae. The first instar larvae of the same age were individualized in Petri dishes (10 cm in height x 2 cm in diameter) containing 3 tomato leaflets with their petioles fixed to a centrifugation microtube with a solution composed of water, potato, dextrose, and agar (PDA) to maintain the turgidity of the vegetable tissue, and evaluated daily until the second larval instar. After reaching the second larval instar, 445 larvae were removed with the aid of a brush with fine, soft bristles, treated with the 1 µL of the LD_{50} of each essential oil (lime: 33.75 µg μL^{-1} petitgrain: 38.78 µg μL^{-1}; bergamot: 35.05 µg μL^{-1}) with the aid of microsyringe and individualized in the same way. The leaflets were replaced by new ones every 3 days. All plates were sealed with PVC plastic film with small holes to allow aeration and prevent the escape of insects.

The experimental design used was completely randomized, with four treatments and 115 repetitions, each one formed by a Petri dish with a treated 2nd instar larva, except for the control that contained 100 repetitions. The sample size was smaller for the control treatment because a relevant mortality rate was not expected and thus without any interference in the quality of the bioassay. Sexing was done in the pupal phase following the method proposed by Genç (2016). Larval and pupal survival, duration of larval instars, pupal and adult stage and total development time were evaluated.

To assess the effects of EOs on surviving adults from treated second instar larvae, newly emerged couples (male and female) from each treatment were separated and maintained in the proportion of 1 couple per Petri dish (1.9 cm height x 10 cm in diameter) covered with perforated PVC plastic film to allow aeration and prevent the escape of insects. Previously, a piece of cotton wool moistened with a solution of 1 part of honey and 1 part of water and also a tomato leaf with 3 leaflets and the petioles fixed in PDA inside a

centrifuge microtube were placed on each plate. The leaflets served as a substrate for oviposition. The number of live insects, the longevity of males and females, daily and total fecundity per female and the percentage of viable eggs were evaluated daily.

The life tables for each treatment were made using the Age-stage, two-sex life table, according to the methodology proposed by Chi (1985, 1988). Biological and demographic data on life history were analyzed using the software TWOSEX-MSChart (Chi 2020). The biological parameters used were Age-stage specific survival rate (Sxj), Age-specific survival rate (lx), fertility by age and stage of development (fx), Age-specific fertility (mx), Age-specific maternity (lxmx); Age-stage life expectancy (exj), Age-stage reproductive value (vxj), net reproductive rate (R_0), intrinsic rate of increase (r), finite rate of increase (λ) and mean generation time (T). The means, variances and standard error of the studied parameters were compared in pairs between treatments by bootstrap method with 100,000 replicates (Efron and Tibshirani 1993). The life table considers the averages of the parameters of survival, life expectancy and fertility until the moment when it reaches age x and stage j.

Statistical analysis

The data related to the screening experiments, dose-time-mortality response and acute toxicity of the major compounds were submitted to the survival analysis of insects using the Weibull model, using the Survival 3.2-7 package (Therneau 2020) in R software (R Core Team 2020); the means were compared by contrast analysis and differentiated into similar groups. The median lethal time (LT_{50}) for each group formed was also calculated.

To determine the LD_{50} and LD_{90} in the 95% confidence interval, logit analysis was used using the DRC package (Ritz 2016) in R software (R Core Team 2020). A binomial generalized linear model (GLM) was adjusted for each EO. To determine the concentrations, the "ED" function was used in a log-logistic model with two parameters to establish the curve:

$$(Y): f(x) = \frac{1}{1+exp\,(b(log\,(x) - log\,(e))\,)}$$

Where the lower limit is 0 and the upper limit is 1; *e* is the inflection point of the dose-response curve and corresponds to the LD_{50} value; *b* is proportional to the slope in the concentration *e*, and *x* corresponds to the concentration value (Ritz et al. 2015). The doses found were different if the confidence limits did not overlap.

The life history data, including the survival, growth, development, longevity and fertility of *T. absoluta* were submitted to the analysis of the two-sex, age-stage life table using the software TWOSEX-MSchart (National Chung Hsing University, Taichung, Taiwan) developed by Chi and Liu (1985) and Chi (1988, 2018). The standard errors of the life history, reproductive and population parameters were estimated via the bootstrap technique using 100,000 resampling (Efron and Tibshirani 1993). The differences among treatments were analysed using the paired bootstrap test at 5% significance level.

For the viability of eggs, the data were adjusted to a GLM with “Quasibinomial” distribution and the averages were compared by Tukey's contrast analysis at 95% probability using the Multcomp package (Hothorn 2008) in R software (R Core Team 2020).

Results

Chemical characterization of EOs

Nine, five and three components were identified in the EOs of *C. aurantifolia* (lime), *C. aurantium* (petitgrain) and *C. aurantium bergamia* (bergamot), respectively. The major component identified in lime oil was α-terpinol (44.74%) and in petitgrain and bergamot oils it was linalyl acetate (Table 1).

Table 1 Relative percentage concentrations of the components in *Citrus aurantifolia* (lime), *Citrus aurantium* (petitgrain) and *Citrus aurantium bergamia* (bergamot) EOs detected in GC-MS analyze.

Essential oil	RI[a]	Compound	Percentages	Method of identification[b]
C. aurantifolia	921	tert-Butylbenzene	2.25	RI, GC-MS
	925	Limonene	11.88	RI, GC-MS
	1010	α-Fenchol	2.41	RI, GC-MS
	1030	3-terpinen-1-ol	4.5	RI, GC-MS
	1040	β-Terpineol	3.18	RI, GC-MS
	1061	2,3,3-Trimethyl-1,4-pentadieno	2.2	RI, GC-MS
	1087	**α-Terpinol***	**44.74**	**RI, GC-MS**
	1094	CTK1F4019 ($C_{10}H_{16}$)	3.88	RI, GC-MS
	1404	CTK5J8343 ($C_{12}H_{20}$)	2.14	RI, GC-MS
		Unknown compounds	22.82	
C. aurantium	998	Linalool	28.04	RI, GC-MS
	1087	α-Terpineol	6.22	RI, GC-MS
	1126	Nerol	0.71	RI, GC-MS
	1154	**Linalyl acetate***	**55.45**	**RI, GC-MS**
	1263	Neryl acetate	6.93	RI, GC-MS
		Unknown compounds	2.65	
C. aurantium bergamia	925	Limonene	4.99	RI, GC-MS
	998	Linalool	29.94	RI, GC-MS
	1153	**Linalyl acetate ***	**58.12**	**RI, GC-MS**
		Unknown compounds	6.95	

[*] The main components of each essential oil are indicated in bold

[a] Retention index on RTX-5MS column relative to homologous series of n-alkanes

[b] Peak identification is based on RI, comparison of retention indices with published data; GC–MS, comparison of mass spectra with those listed in NIST and Adams libraries and in published data

Screening of EOs against *T. absoluta*

In the survival analysis of larvae treated with *Citrus* spp. oils there was significant difference between the treatments ($\chi 2 = 292$; d.f. = 3; $p<0.05$). The oils of *C. aurantifolia*, *C. aurantium* and *Citrus aurantium bergamia* caused 100% insect mortality in 72 h. Three distinct congener groups were formed by contrast analysis, with group 1 being represented by lime and petitgrain oils, with LT_{50} of 13.57 h. Bergamot oil formed group 2 with LT_{50} of 19.34 h. The LT_{50} was higher in the control treatment (acetone), which was >72 h, demonstrating that the essential oils reduced the survival time of the treated insects (Figure 1).

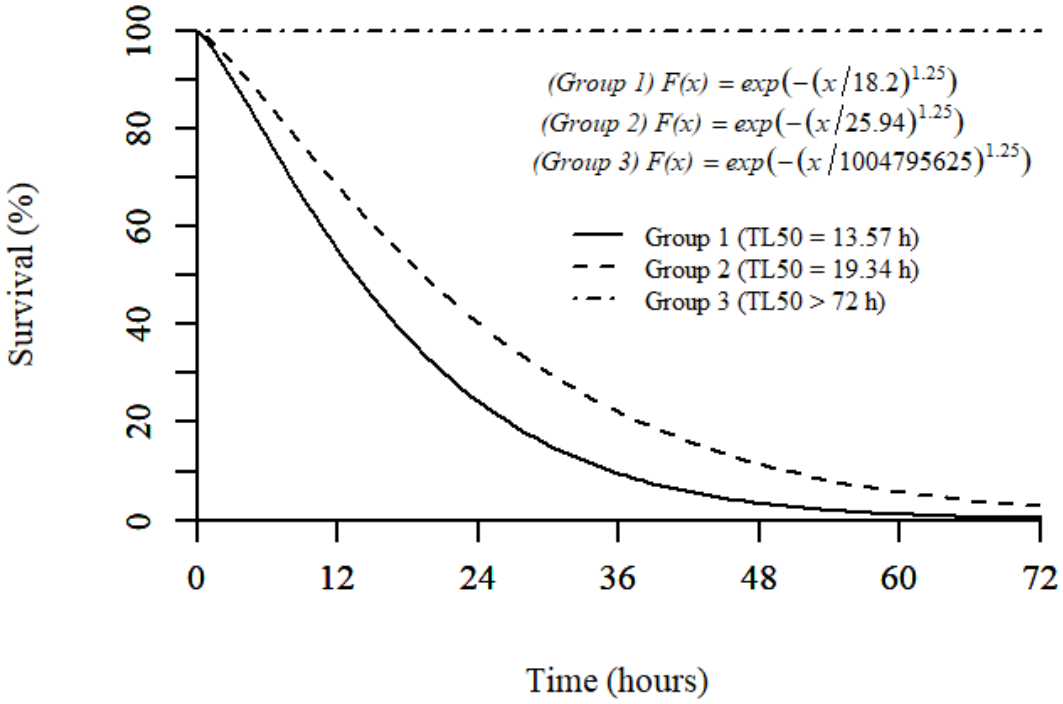

Figure 1. Survival of second instar larvae of *Tuta absoluta* treated with *Citrus aurantifolia* (lime), *Citrus aurantium* (petitgrain) and *Citrus aurantium bergamia* (bergamot) EOs solubilized in acetone at 100 µg μL^{-1}. Groups were formed for treatments with similar means, the **group 1** = lime and petitgrain EOs (LT_{50} 13.57 h); **group 2** = bergamot EO (LT_{50} 19.84 h) and **group 3** = only acetone (LT_{50}>72 h).

EOs dose-time-mortality response of against *T. absoluta*

Lime, petitgrain and bergamot oils showed LD_{50} of 33.75 µg μL^{-1}; 38.78 µg μL^{-1} and 35.05 µg μL^{-1}, respectively, for *T. absoluta* larvae (Table 2). Regarding the survival analysis, six congener groups were formed ($\chi 2 = 677$; d.f. = 18; $p <0.05$). Lime oil was

more toxic at higher doses, and larvae treated with a concentration of 75 µg µL^{-1} showed survival equal to the observed in those treated with the highest concentrations of petitgrain and bergamot (100 µg µL^{-1}) and formed group 1 with LT_{50} of 11.74 h. The petitgrain and bergamot oils showed similar averages at the concentration of 75 µg µL^{-1}, forming group 2 with a LT_{50} of 20.55 h. Group 3 comprised the concentrations of 55 and 41.3 µg µL^{-1} of the lime oil; 41.3 and 31.6 µg µL^{-1} of petitgrain oil and 55; 41.3 and 31.6 µg µL^{-1} of bergamot oil, with a LT_{50} of 46.60 h. Group 4 was formed by doses of 31.6 µg µL^{-1} of lime and petitgrain oils, and within 72 h they did not cause 50% of the population's mortality. The three oils in their lowest doses constituted group 5, with LT_{50} >72 h, and group 6 was constituted only by the control with LT_{50} >72 h (Figure 2).

Table 2. Values of LD_{50} and LD_{90} of *Citrus aurantifolia* (lime), *Citrus aurantium* (petitgrain) and *Citrus aurantium bergamia* (bergamot) EOs to *Tuta absoluta* second instar larvae.

Essential oil	**df**	***χ2***	***p***	***LD$_{50}$**	**CL 95%**	***LD$_{90}$**	**CL 95%**	***b***±SE**
C. aurantifolia	10	12,29	0.27	33.75	29.31 – 38.18	88,59	68.77 – 108.42	-2.23±2.26
C. aurantium	10	10,07	0.43	38.78	33.62 – 43.94	113,36	82.41 – 144.31	-2.05±2.63
C. aurantium bergamia	10	4,16	0.94	35.05	30.43 – 39.68	94,89	72.46 – 117.33	-2.21±2.36

*Doses in µg L^{-1}. CL: confidence limits. [b] is proportional to the slope at the LC50 value χ2 and p values correspond to goodness-of-fit test ** "*b*" = coefficients of the equation f(x)=1/1+exp(*b*(log(x)-log(e)))

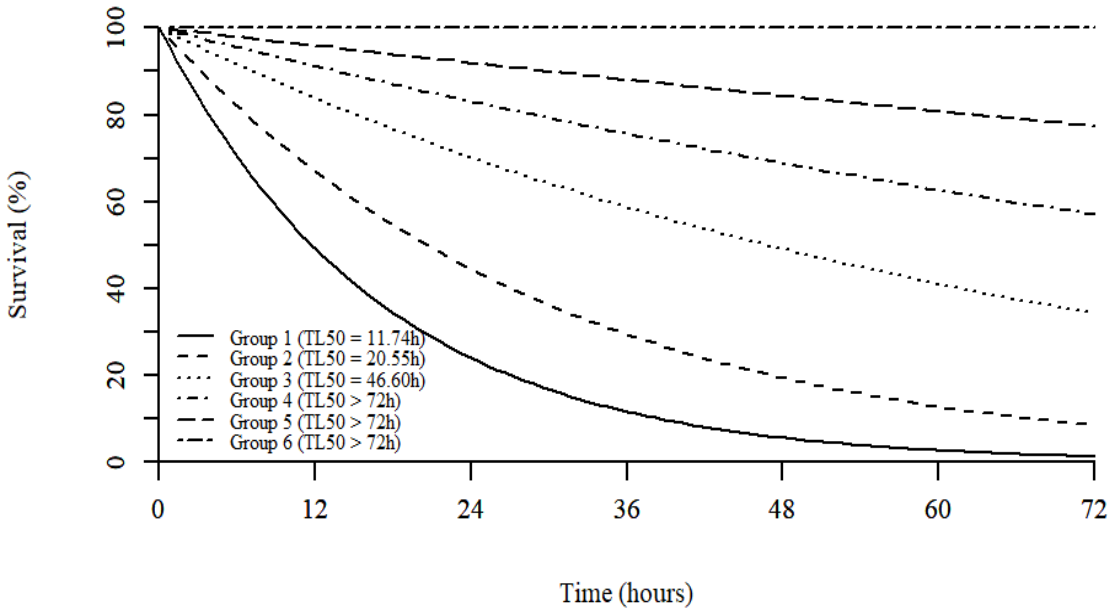

Figure 2. Survival of *Tuta absoluta* exposed to *Citrus aurantifolia* (lime), *Citrus aurantium* (petitgrain) and *Citrus aurantium bergamia* (bergamot) EOs at six different doses. **group 1** = 100, 75 µg µL^{-1} (lime) + 100 µg µL^{-1} (petitgrain and bergamot) ($f(x) = \exp(-(x/16.89)^{1.01})$) ($LT_{50}$ = 11.74 h); **group 2:** 75 µg µL^{-1} (petitgrain and bergamot) ($f(x) = \exp(-(x/29.55)^{1.01})$) ($LT_{50}$ = 20.55 h); **group 3:** 55 and 41.3 µg µL^{-1} (lime and petitgrain) + 55; 41.3 and 31.6 µg µL^{-1} (bergamot) ($f(x) = \exp(-(x/67.17)^{1.01})$) ($LT_{50}$ = 46.6 h); **group 4:** 31.6 µg µL^{-1} (lime and petitgrain) ($f(x) = \exp(-(x/127.40)^{1.01})$) ($LT_{50}$ > 72h); **group 5:** 17.3 µg µL^{-1} (lime, petitgrain and bergamot) ($f(x) = \exp(-(x/275.67)^{1.01})$) ($LT_{50}$ > 72 h); **group 6:** control treatment ($f(x) = \exp(-(x/25305054618)^{1.01})$) ($LT_{50}$ > 72 h).

Acute toxicity of major compounds of EOs against *T. absoluta*

There was a statistically significant difference between survival over time of larvae treated with α-terpineol, linalyl acetate, and the control treatment with acetone ($\chi 2$ = 30.5; d.f. = 2; $p < 0.05$). Although the treatments were separated into three distinct groups by contrast analysis, it was not possible to verify the median lethal time due to the low mortality rate of insects in the treatments (Figure 3).

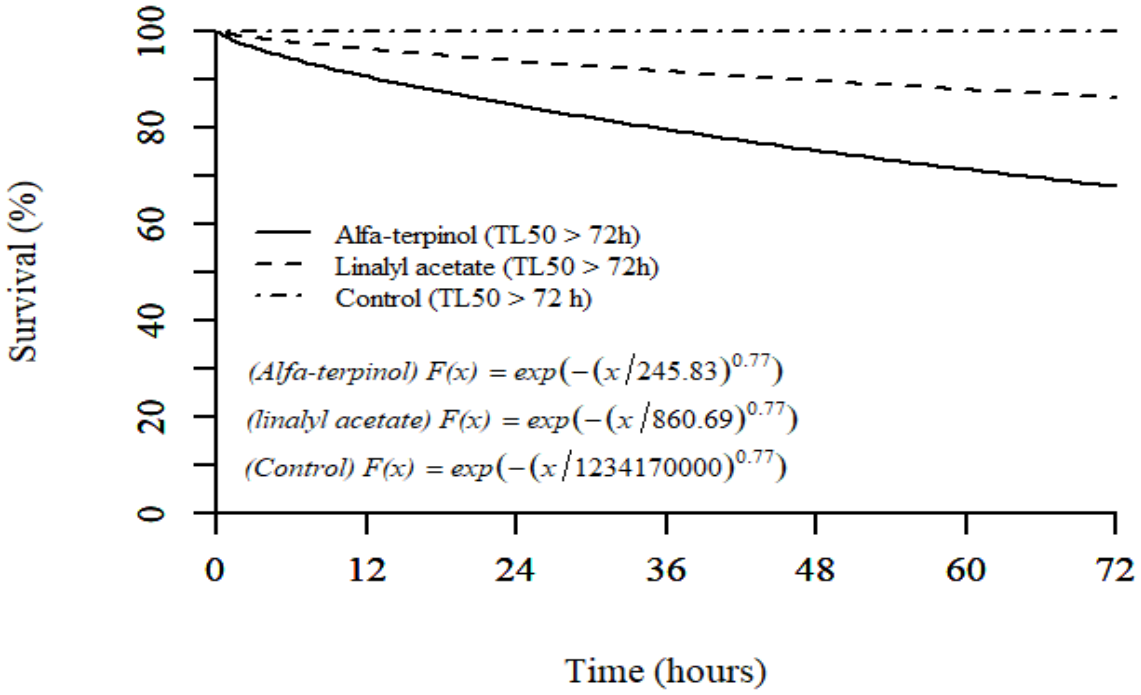

Figure 3. Survival of second instar larvae of *Tuta absoluta* exposed to α-terpinol ($F(x) = \exp(-(x/245.83)^{0,77})$) and linalyl acetate ($F(x) = \exp(-(x/860.69)^{0,77})$) solubiled in acetone (control) ($F(x) = \exp(-(x/1234170000)^{0,77})$).

Life history table of *T. absoluta* treated with LD_{50} of *Citrus* spp.

There were differences in the duration of the larval, and pupal stages for the three EOs. However, the total duration from egg to adult was similar to the control. Regarding the total male longevity, it was observed that insects treated with essential oil of lime had longer longevity (Table 3).

Table 3. Life history parameters of *T. absoluta* treated with LD_{50} of *Citrus aurantifolia* (lime = 33.75 µg μL^{-1}), *Citrus aurantium* (petitgrain =38.78 µg μL^{-1}) and *Citrus aurantium bergamia* (bergamot = 35.05 µg μL^{-1}) EOs.

Parameter	Stage	Control		Lime		Petitgrain		Bergamot	
		n	mean ± SE	n	mean ± SE	n	mean ± SE	n	mean ± SE
Age-stage (days)	Egg	100	3 ± 0 a	115	3 ± 0 a	115	3 ± 0 a	115	3 ± 0 a
	L1	100	2.36 ± 0.08 a	115	2.03 ± 0.04 c	115	2.18 ± 0.04 b	115	2.1 ± 0.03 bc
	L2	84	3.88 ± 0.14 a	66	3.42 ± 0.14 b	53	3.91 ± 0.2 a	87	3.72 ± 0.09 ab
	L3	81	2.07 ± 0.07 a	60	2.12 ± 0.12 a	50	1.72 ± 0.12 b	82	1.83 ± 0.09 b
	L4	78	2.14 ± 0.08 ab	51	2.14 ± 0.14 ab	45	2.47 ± 0.19 a	75	1.99 ± 0.13 b
	Pupa	67	8.48 ± 0.17 b	45	9.53 ± 0.2 a	39	9.03 ± 0.23 ab	53	9.34 ± 0.18 a
	Egg - Pupa	67	21.90 ± 0.21 a	45	22.36 ± 0.28 a	39	22.03 ± 0.42 a	53	22.17 ± 0.24 a
Longevity (days)	Adult	67	14.79 ± 0.48 a	45	14.89 ± 0.65 a	39	14.41 ± 0.68 a	53	15.42 ± 0.88 a
	Female	34	37.38 ± 0.58 a	19	36.37 ± 0.97 a	19	37.58 ± 1.16 a	30	37.73 ± 1.36 a
	Male	33	35.97 ± 0.76 ab	26	37.88 ± 0.95 a	20	35.35 ± 0.54 b	23	37.39 ± 1.16 ab
Life cycle *	Egg - adult	67	36.69 ± 0.48 a	45	37.24 ± 0.69 a	39	36.44 ± 0.65 a	53	37.58 ± 0.91 a

Means in same line followed by different letters differ ($p < 0.05$). Differences between treatments were obtained using the Bootstrap test paired with 100,000 replicates. n = number of specimens at each stage of development. L1 = 1st larval instar; L 2 = 2nd larval instar; L3 = 3rd larval instar and L4 = 4th larval instar.

* Mean of total life history for males and females, in days, only for insects that have become adults.

Survival rate and life expectancy by age-stage of *T. absoluta*

The age-stage survival rate (Sxj) indicates the probability that the insect will survive at age x and stage j. Decreases were observed in the survival of second instar larvae of *T. absoluta* with the EOs of lime (77.39%) and petitgrain (78.26%) when compared to the EO of bergamot (92.17%) and the control (90.0%). This reduction caused by lime and petitgrain EOs was maintained until the third and fourth instars. In the pupal stage, the maximum value for the control treatment was 74%, while for lime, petitgrain and bergamot it was 40.87%, 36.52% and 56.52%, respectively. The EOs showed maximum averages lower than the control treatment regarding the survival of males and females (Figure 4).

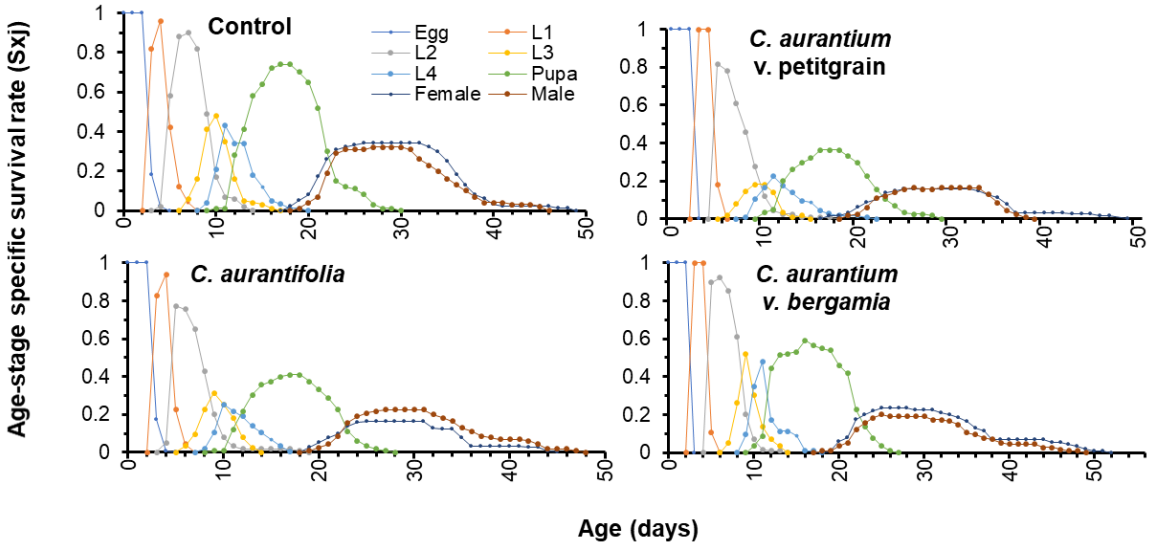

Figure 4. Age-stage specific survival rate (Sxj) of *Tuta absoluta* treated with LD_{50} *Citrus aurantifolia* (lime), *Citrus aurantium* (petitgrain) and *Citrus aurantium bergamia* (bergamot) Eos and only acetone (control).

All EOs reduced life expectancy per stage of development of *T. absoluta* when compared to control in the larval stage. In the pupa stage, the lime and petitgrain EOs did not cause any negative effects, while the bergamot EO reduced life expectancy. Only the petitgrain EO caused a decrease in (exj) in adult males. EOs did not reduce female life expectancy and longevity (Figure 5).

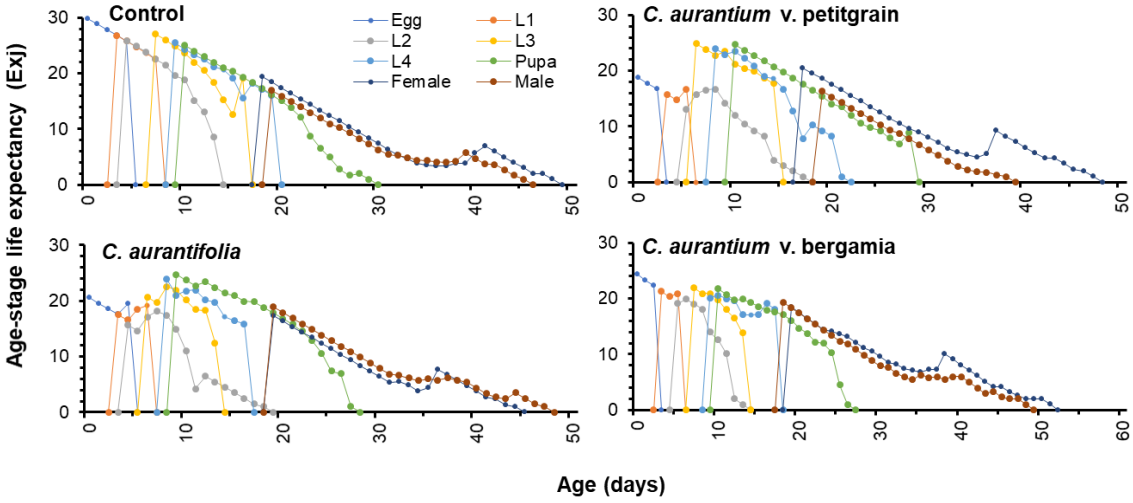

Figure 5. Age-stage life expectancy (exj) of *Tuta absoluta* treated with LD_{50} *Citrus aurantifolia* (lime), *Citrus aurantium* (petitgrain) and *Citrus aurantium bergamia* (bergamot) Eos and only acetone (control).

Reproductive parameters

The total fertility of all females was reduced by the lime and bergamot EOs. When considering the fertility of only the females that oviposited, it was found that the EOs of lime and petitgrain caused a reduction of this biological characteristic, while the EO of bergamot was equal to the other treatments. The total oviposition period was shorter for lime and bergamot EOs, while petitgrain was innocuous. There were no negative effects of treatments in the adult pre-oviposition (APOP) and total pre-opposition (TPOP) periods. The lowest maximum daily fertility (MDF) values were observed in the lime and petitgrain treatments, and the lowest maximum total fertility (MTF) was observed in the lime treatment (Table 4).

Table 4. Reproductive parameters of *Tuta absoluta* exposed to *Citrus aurantifolia* (lime), *Citrus aurantium* (petitgrain) and *Citrus aurantium bergamia* (bergamot) EOs.

Parameter	Control (acetone)		Lime		Petitgrain		Bergamot	
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE
Fecundity (E/F) total	34	46.82 ± 5.76 a	19	24.79 ± 4.05 b	19	30,63 ± 6,1 ab	30	23,1 ± 4,81 b
Fecundity (E/F) *	30	53.07 ± 5.61 a	16	29.00 ± 3.77 b	16	36,38 ± 6,26 b	18	38,5 ± 5,57 ab
Oviposition (days)	30	3.43 ± 0.30 a	16	2.56 ± 0.26 b	16	2,81 ± 0,25 ab	18	2,28 ± 0,24 b
PPOA (days)	30	3.20 ± 0.32 a	16	3.5 ± 0.52 a	16	3,38 ± 0,42 a	18	2,28 ± 0,4 a
PPOT (days)	30	24.67 ± 0.37 a	16	25.38 ± 0.46 a	16	24,88 ± 0,54 a	18	24,33 ± 0,47 a
MFD (E/F)		70		30		46		71
MFT (E/F)		126		54		85		93

* Total females that oviposited; PPOA (days): Pre-oviposition period per adult; PPOT (days): Period of total pre-oviposition; MFD (eggs / female): Maximum daily fertility; MFT (eggs / female): Maximum total fertility.

As for the age-stage reproductive value (vxj) of *T. absoluta*, there were differences in relation to the maximum values of the females, being higher in the control treatment, followed by the petitgrain, lime and bergamot EOs (Figure 6).

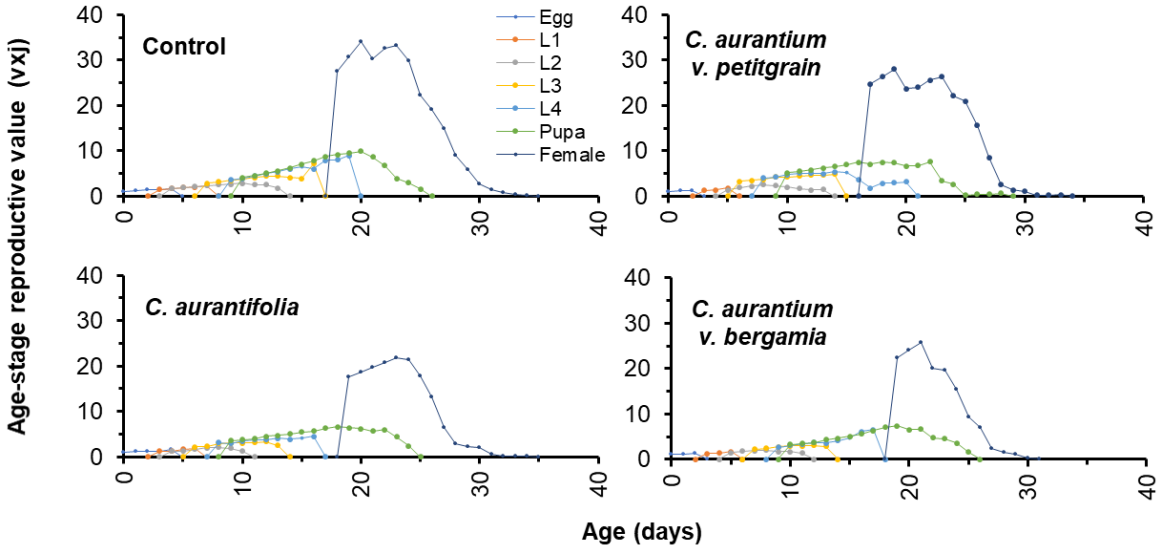

Figure 6. Age-stage reproductive value (vxj) of *Tuta absoluta* treated with LD_{50} *Citrus aurantifolia* (lime), *Citrus aurantium* (petitgrain) and *Citrus aurantium bergamia* (bergamot) Eos and only acetone (control).

The EOs of lime, petitgrain and bergamot caused a marked decrease in the survival rate by specific age (lx) from the fifth day of life of the insects, while in the control treatment the curve slowly decreased over time. The duration of the age-specific maternity period (lxmx) and age-specific fertility were shorter in insects treated with lime and bergamot oils (Figure 7).

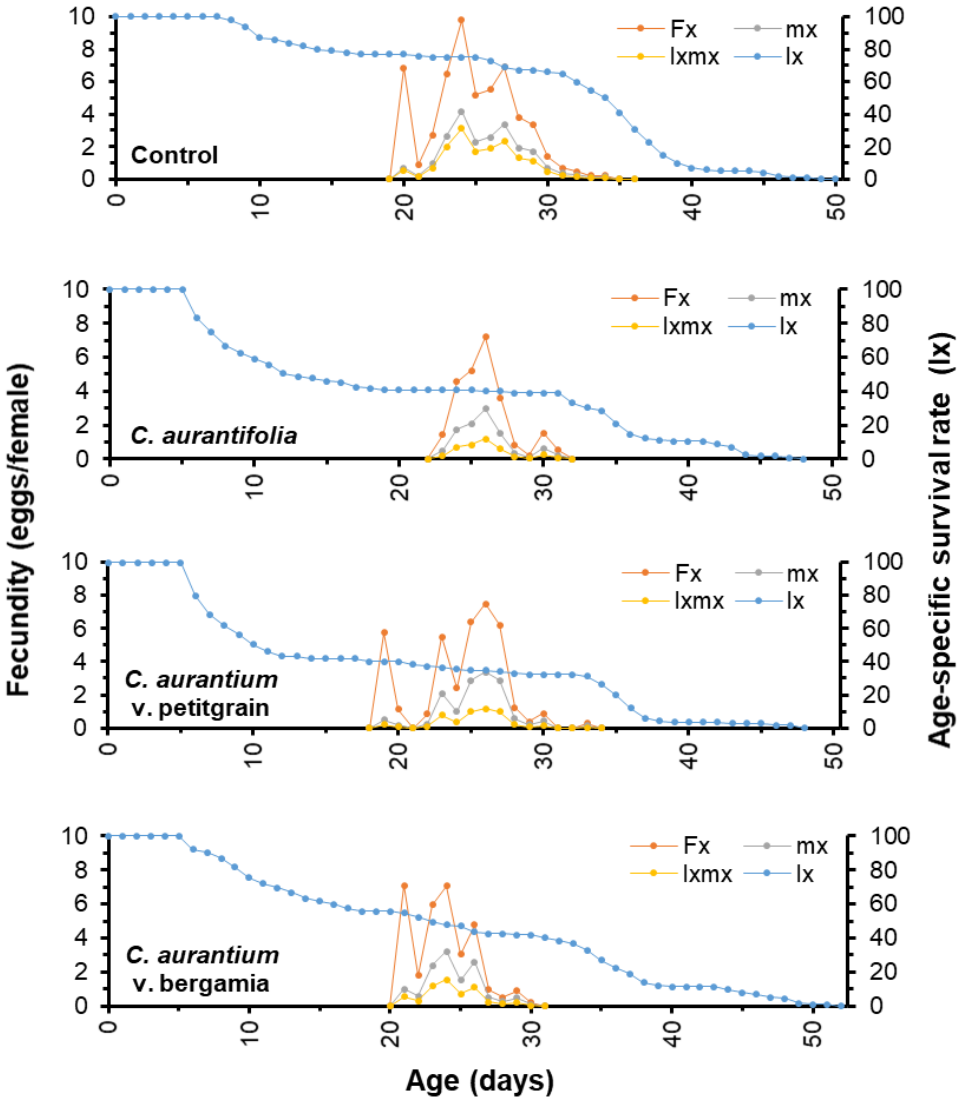

Figure 7 Age-specific survival rate (lx); Age-specific fertility (mx); Age-specific maternity (lxmx); Age-stage specific fertility (fx) of *Tuta absoluta* treated with LD_{50} *Citrus aurantifolia* (lime), *Citrus aurantium* (petitgrain) and *Citrus aurantium bergamia* (bergamot) Eos and only acetone (control).

Regarding the viability of the eggs from the treated females, it was observed that lime oil reduced the viability of the eggs ($\chi 2$ = 291; d.f. = 3; p <0.05), while the other treatments were harmless to this biological characteristic (Figure 8).

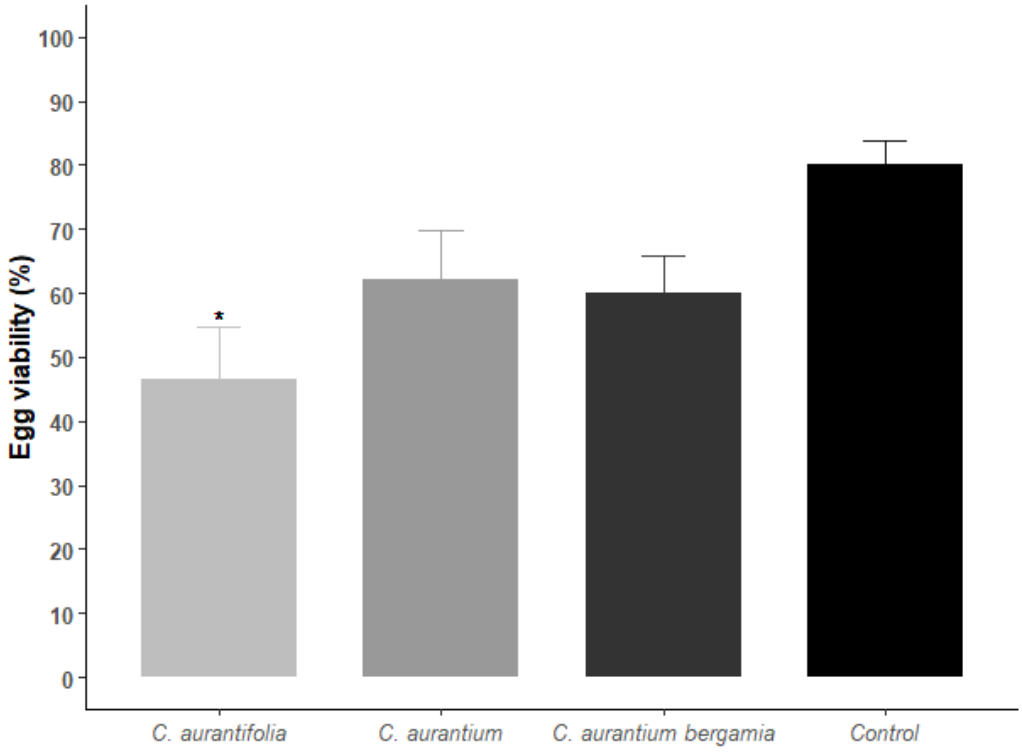

Figure 8. Egg viability (%) of *Tuta absoluta* treated with LD_{50} *Citrus aurantifolia* (lime), *Citrus aurantium* (petitgrain) and *Citrus aurantium bergamia* (bergamot) EOs and only acetone (control). * Differ statistically according to the Tukey test (P < 0.05).

Effects of EOs on demographic parameters

The LD_{50} of all EOs caused a reduction in the intrinsic rate of increase (r), in the finite rate of increase (λ) and in the net reproductive rate (R_0) of *T. absoluta*. Regarding the mean generation time (T), the highest average was found in the lime EO and the lowest average in the bergamot EO (Table 5).

Table 5. Demographic parameters of *T. absoluta* treated with the LD_{50} of the essential oils of *Citrus aurantifolia* (lime), *Citrus aurantium* (petitgrain) and *Citrus aurantium bergamia* (bergamot), and acetone (control).

Parameter	Control	Lime	Petitgrain	Bergamot
intrinsic rate of increase (r)	0.11 ± 0.01a	0.05 ± 0.01b	0.06 ± 0.01b	0.07 ± 0.01b
finite rate of increase (λ)	1.11 ± 0.01a	1.05 ± 0.01b	1.06 ± 0.01b	1.07 ± 0.01b
net reproductive rate (R_0)	15.92 ± 2.94a	4.10 ± 1.07b	5.06 ± 1.45b	6.03 ± 1.56 b
mean generation time (T)	26.18 ± 0.48ab	26.83 ± 0.47a	26.09 ± 0.62ab	25.22 ± 0.47b

intrinsic rate of increase (day-1); finite rate of increase (day-1); R_0, net reproductive rate (offspring per individual); mean generation time (days).

Discussion

In this study it was found that the EOs of *C. aurantifolia* (lime), *C. aurantium* (petitgrain) and *C. aurantium bergamia* (bergamot) have acute toxicity for *T. absoluta.* The composition of the EOs evaluated in this work is directly related to their toxicity, since it is a complex mixture of hydrocarbons, terpenoids and other molecules such as aldehydes, alcohols, esters, and organic acids (Bora et al. 2020). The EOs of Citrus spp. have a broad spectrum of action in the physiology of insects, and studies have shown that the toxicity is partly neurotoxic, acting as inhibitors of acetylcholinesterase (AChE), in the channels of Na^+/K^+ and octopamine receptors (Kostyukovsky et al. 2002; Zarrad et al. 2015; Oboh et al. 2017).

The median lethal time of *T. absoluta* larvae treated with *C. aurantifolia, C. aurantium* (petitgrain) was 13.57 h and *C. aurantium bergamia* of 19.34 h; this rapid action is also possibly linked to mechanisms of action in the nervous system. Few works with these EOs have been done with this pest; however, other studies have also confirmed the toxicity of *C. aurantium* (bitter orange), *C. limon* (lemon), *C. reticulata* (mandarin orange) and *C. sinensis* (sweet orange) oils on *T. absoluta* (Campolo et al. 2017; Zarrad et al. 2017a) and on other arthropod pests (Werdin González et al. 2014; Tacoli et al. 2018; Campolo et al. 2020).

The major compounds identified in the present work were α-terpinol in the EOs of *C. aurantifolia* and linalyl acetate for the EOs of *C. aurantium* petitgrain and *C. aurantium*

bergamia. α-terpinol is a pleasant-smelling monoterpene often found in EOs (Baptistella 2009), few studies with this molecule are described in the literature; the main report was made by Liu et al. (2013) about *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae) which showed a repellent characteristic of this molecule to this pest. Linalyl acetate, on the other hand, is an unsaturated hydrocarbon easily oxidized when in contact with atmospheric oxygen (Calandra and Wang 2017).

Both major compounds found in EOs were also reported by Campolo et al. (2014); Werdin González et al. (2014); Zarrad et al. (2017); Tchameni et al. (2018), but in low concentrations. This difference in concentration of certain EO components in *Citrus* spp. it is the result of the methodology and structure of the plant used for extraction, the effects of the genotype used, the geographical distribution of the plant, the type of soil in which it grows, climatic conditions, stress level and the physiology of the plant (Tadeo et al. 2008). Hili et al. (1997) carried out a comparative study between plants of the genus *Citrus* under different water status and verified variations in the concentrations of major compounds in EOs. The same was verified by Kesterson et al. (1971) who reported variations in the concentration of major compounds in *Citrus* spp. depending on the different maturation stages. Due to this natural variation, it is essential to use GC-EM to characterize the mixture components in studies involving botanical products such as plant extracts and EOs (Koul et al. 2008; Isman and Grieneisen 2014; Bora et al. 2020).

In that study, pure major compounds were not as toxic as the oils. It is necessary to consider that essential oils are complex mixtures of different classes of compounds and that their toxicity may be related to either the major compound, minor compounds, or the synergism of the compounds in the mixture (Bakkali et al. 2008). This is because minor compounds can modulate and amplify the action of the major compounds present in EOs through changes in physical-chemical characteristics, increasing the capacity of cell penetration, fixation, and integument penetration (Karpouhtsis et al. 1998; Cal 2006; Tchameni et al. 2018). Examples of this are molecules that in small quantities can inhibit enzymes such as cytochrome P450, which is responsible for metabolizing other toxic substances in the insect's body (Metcalf 1967; Bernard and Philogfene 1993). The synergism between chemical molecules can be well used in the management of pest

resistance to synthetic and botanical active ingredients as they increase the target's physiological susceptibility to toxic molecules (Bernard and Philogène 1993).

It was observed that the insects exposed to the LD_{50} of the oils of *C. aurantifolia, C. aurantium* and *C. aurantium bergamia* had reduced fertility and duration of the oviposition period; however, they did not show a reduction in the duration of their total life cycle. The EOs decreased the variables that indicate the population growth of *T. absoluta*, such as the intrinsic rate of increase, finite rate of increase and the net reproduction rate. According to Stark and Banks (2003) toxicological analyzes that consider population parameters are more efficient in assessing the impact of the compound on the insect in prolonged periods when compared to studies of only the lethal effect. Therefore, the use of life tables by stage of development for two sexes allows to determine more precisely the population changes of the pest, since it incorporates the dynamic rates of development in time and differentiation of the individual growth stages of both sexes (Chi 1985, 1988).

Studies by Zibaee and Esmaeily (2017) also found negative effects on fertility and demographic parameters of *T. absoluta* when treated with abamectin subdoses. In a study by Kandil et al. (2020) it was demonstrated that subdoses of botanical products decreased the fecundity and viability of *T. absoluta* eggs in a similar way to the present study for the EO of lime. Many studies on the sublethal effects of chemicals are done on beneficial insects and few studies on insect pests (Desneux et al. 2007; Zibaee and Esmaeily 2017). This reduction in reproductive parameters is probably the result of mechanisms that harm the physiological and behavioral aspects of the insects treated (Desneux et al. 2007). Although there are no studies that elucidate the mechanisms of action of EOs on *T. absoluta*, some authors report that subdoses of insecticides can difficult the mating behavior of lepidopterans, leading to a reduction in the eggs produced (Bariola 1984; Beach and Todd 1985).

In the present study it was possible to verify that the EOs of *C. aurantifolia*, *C. aurantium* and *C. aurantium bergamia* are toxic to *T. absoluta*. In addition, when used in subdoses, they affected reproductive and demographic parameters of the pest. All EOs demonstrated that in the future they can be used to control the tomato borer (*T. absoluta)*. The major compounds are not the only ones responsible for the bioactivity of EOs; For this reason, other studies aimed at understanding the effect of α-terpinol and linalyl acetate,

when combined with other compounds in a smaller amount of the mixture, will be important to verify the synergistic effects of the compounds. In addition, studies that seek to understand the mechanisms of action involved in the lethal and sublethal effects of citrus essential oils on the life history of *T. absoluta* are important.

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