



**KAROLINA GOMES DE FIGUEIREDO**

**BIOACTIVITY OF *Cinnamomum* spp. (Lauraceae) ESSENTIAL  
OILS AGAINST *Tuta absoluta* (Meyrick, 1971) (Lepidoptera:  
Gelechiidae) AND SELECTIVITY FOR *Macrolophus basicornis*  
(Stal, 1860) (Hemiptera: Miridae)**

**LAVRAS - MINAS GERAIS**

**2021**

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Dissertation presented to the Federal University of Lavras, as part of the requirements of the Post-Graduate Program in Entomology, to obtain the title of Master.

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**BIOATIVIDADE DE ÓLEOS ESSENCIAIS DE *Cinnamomum* spp. (Lauraceae) PARA *Tuta absoluta* (Meyrick, 1971) (Lepidoptera: Gelechiidae) E SELETIVIDADE PARA *Macrolophus basicornis* (Stal, 1860) (Hemiptera: Miridae)**

Dissertation presented to the Federal University of Lavras, as part of the requirements of the Post-Graduate Program in Entomology, to obtain the title of Master.

Presented in March 26th of 2021.  
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**LAVRAS – MINAS GERAIS**

**2021**

*To my parents Leonardo and Telma for their unconditional love and support during my life  
and my sister Rúbia for complicity.*

*I dedicate.*

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*“In the vastness of space and the immensity of time, it is my joy to share a planet and an epoch with you”*

Carl Sagan

## RESUMO

A traça-do-tomateiro *Tuta absoluta* (Meyrick, 1971) (Lepidoptera: Gelechiidae) é uma das principais pragas causadoras de perdas econômicas em cultivos de solanáceas. O objetivo do trabalho foi avaliar a bioatividade e o ciclo de vida da *T. absoluta* após sua exposição aos óleos essenciais de *Cinnamomum camphora* var. *linalooliferum*, *Cinnamomum camphora* e *Cinnamomum cassia* (Lauraceae) por meio de estudos de tabela de vida para dois sexos por idade e estágio de desenvolvimento, bem como a avaliação dos compostos majoritários cinamaldeído e linalool, o consumo foliar e a suscetibilidade dessa população a inseticidas. Também foi avaliado a toxicidade dos óleos essenciais para o predador *Macrolophus basicornis* (Stal, 1860) (Hemiptera: Miridae), que foram tratados com 1 µL da DL<sub>50</sub> dos óleos para *T. absoluta*. Os óleos essenciais foram solubilizados em acetona nas concentrações de 0,05; 0,10 e 0,07 mg.mL<sup>-1</sup>, que correspondem à DL<sub>50</sub> para *T. absoluta*, respectivamente. As lagartas do tratamento controle foram tratadas apenas com acetona. Lagartas de 2º instar de *T. absoluta* foram tratadas com auxílio de microseringa, de forma que cada uma recebeu 1 µL da solução em seu dorso. Em seguida, foram mantidas em folhas de tomateiro cv Santa Clara dentro de placas de Petri sob condições controladas. O delineamento utilizado foi o inteiramente casualizado com 100 repetições, sendo cada uma formada por uma lagarta. O experimento teve duração de 55 dias, até que os insetos da segunda geração atingissem o segundo instar. A população de *T. absoluta* avaliada apresentou tolerância aos inseticidas fenprotrina, cloridrato de cartape e clorpirifós. Os compostos majoritários cinamaldeído e linalool apresentaram mortalidades de 83,3% e 86,7% de lagartas de *T. absoluta*, sendo possivelmente os responsáveis pela atividade inseticida dos óleos. Os tratamentos formados por óleos essenciais reduziram a duração das fases larval, pupal e adulta de *T. absoluta*, além de reduzir significativamente os parâmetros demográficos como fecundidade, oviposição e viabilidade de ovos e sobrevivência de lagartas de primeiro instar. Quanto aos adultos do predador *M. basicornis*, os óleos essenciais causaram cerca de 50% de mortalidade. Os óleos essenciais de *C. camphora* var. *linalooliferum*, *C. camphora* e *C. cassia* apresentaram grande potencial para uso em programas de manejo da *T. absoluta*, uma vez que foram tóxicos a esse lepidóptero praga e causaram efeito intermediário de até 50% de mortalidade do predador *M. basicornis*.

**Palavras-Chave:** Produtos botânicos. Traça-do-tomateiro. Controle. Seletividade. Mirídeo.



## ABSTRACT

The tomato moth *Tuta absoluta* (Meyrick, 1971) (Lepidoptera: Gelechiidae) is one of the main pests that cause economic losses in solanaceous crops. The objective of this study was to evaluate the bioactivity of the essential oils of *Cinnamomum camphora* var. *linalooliferum*, *Cinnamomum camphora* and *Cinnamomum cassia* (Lauraceae) against *T. absoluta*, and the life cycle of the pest after exposure to these oils, using age-stage, two-sex life table, as well as the evaluation of the major compounds cinnamaldehyde and linalool, leaf consumption and the susceptibility of the population used in the experiments to insecticides. We also evaluated the toxicity of the essential oils to the predator *Macrolophus basicornis* (Stal, 1860) (Hemiptera: Miridae), which were treated with 1  $\mu\text{L}$  of the  $\text{LD}_{50}$  of the oils for *T. absoluta*. The essential oils of *C. camphora* var. *linalooliferum*, *C. camphora* and *C. cassia* were solubilized in acetone at concentrations of 0.05; 0.10 and 0.07  $\text{mg.mL}^{-1}$ , respectively, which correspond to the  $\text{LD}_{50}$  for *T. absoluta*. The caterpillars of the control treatment received acetone only. *T. absoluta* caterpillars of the 2nd instar were treated with 1  $\mu\text{L}$  of the solution applied on their back with a microsyringe. Then, they were kept on tomato leaves cv Santa Clara inside Petri dishes under controlled conditions. The design was completely randomized with 100 replicates, each replicate being a caterpillar. The experiment lasted 55 days, until the second-generation insects reached the second instar. The population of *T. absoluta* evaluated showed tolerance to the insecticides fenpropathrin, cartap hydrochloride and chlorpyrifos. The major compounds cinnamaldehyde and linalool presented mortalities of 83.3% and 86.7% of *T. absoluta* caterpillars, potentially responsible for the insecticidal activity of the oils. The treatments with essential oils reduced the duration of the larval, pupal and adult stages of *T. absoluta*, in addition to significantly reducing demographic parameters such as fecundity, oviposition and viability of eggs and survival of first instar caterpillars. Regarding the adults of the predator *M. basicornis*, essential oils caused about 50% mortality. The essential oils of *C. camphora* var. *linalooliferum*, *C. camphora* and *C. cassia* showed great potential for *T. absoluta* management programs because of their toxicity to this lepidopteran pest and intermediate effect of up to 50% mortality to the predator *M. basicornis*.

**Keywords:** Botanical products. Tomato moth. Control. Selectivity. Myride.

## SUMMARY

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## 1. GENERAL INTRODUCTION

*Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), popularly known as the tomato moth, is considered a key pest of the tomato crop (*Lycopersicon esculentum* Mill.). However, this pest can occasionally occur in other solanaceous crops such as pepper (*Capsicum annuum*), potato (*Solanum tuberosum*) and eggplant (*Solanum melongena*). Initially, the damage is significant mainly in leaves and fruits, but it can also affect stems, buds, and flowers, causing severe losses of harvest, of up to 100%, when control measures are not adopted (DESNEUX et al., 2010; SILVA et al., 2016).

Native to South America, this microlepidoptera was originally described in Peru by Meyrick in 1917 (POVOLNY, 1975; DURIC et al., 2014, BIONDI et al., 2018), and between the 1960s and 1980s this insect dispersed to the other Latin American countries, being found in Bolivia, Brazil, Chile, Colombia, Ecuador, Panama, Paraguay, Uruguay, and Venezuela (DESNEUX et al., 2010). In 2006, *T. absoluta* invaded Spain and spread rapidly throughout Europe and the Mediterranean Sea region, colonizing several continents such as Africa, southern Central America, Middle East and regions of southern Asia (DESNEUX et al., 2011). Currently, *T. absoluta* is no longer an exclusive problem in the American continent, causing a worldwide reduction in fruit production, and consequently promoting an increase in the price of the product (BIONDI et al., 2018).

*T. absoluta* acquired the status of invasive pest due to the short duration of its biological cycle, a relatively wide range of hosts, high adaptability to different climatic conditions, and ability to develop resistance to insecticides (GUEDES; PICANÇO, 2012). In addition, the data obtained through sampling with pheromone traps suggest a high dispersion ability (DESNEUX et al., 2011).

A control method commonly adopted is the use of insecticides from different chemical groups. However, the results are not always satisfactory because, due to the overlap of generations, several applications per cultivation period are necessary to control this pest. Consequently, few active ingredients remain effective in controlling *T. absoluta*, increasing the selection of resistant populations due to the inadequate use of these chemical compounds (LIETTI et al., 2005). Adverse effects on the environment, reduced number of predators and parasitoids, and increased production costs are also observed.

Several authors report the selection of *T. absoluta* populations resistant to chemical products, mainly of the chemical groups of the pyrethroids, abamectin and cartap (HADDI et

al., 2012; GONÇALVES et al., 1994; SIQUEIRA; GUEDES; PICANÇO, 2000; CAMPOS et al., 2014) which causes the population increase of this herbivore, and consequently its damages (PRASTIOLLI; PARRA, 2001).

The use of alternative tactics in the adoption of Integrated Pest Management (IMP) contributes to the reduction of the population levels of the tomato moth in the field (CASTELO BRANCO; FRANÇA, 1995), in addition to the integration of the available control methods (chemical, biological, cultural, and legislative) that are essential for the management of this pest.

The searches for new active ingredients or the use of non-chemical methods for the regulation of pests have become a reality for the success of agricultural systems (TERZIDIS; WILCOCKSON; LEIFERT, 2014). The diversity of active substances derived from plant metabolism, such as secondary metabolism, has motivated the development of several studies involving extracts and essential oils from plants, in view of their diversified biological activities and their important role in the process of developing new therapeutic agents (SILVA, 2014).

In the literature, there are many studies related to essential oils that have a proven fungicidal, acaricidal, insecticidal activity and even in the control of weeds (SILVA et al., 2010).

The Lauraceae family consists of a group of plants with high potential to produce essential oils, endemism in the Amazon rainforest and great commercial potential. In this family, the genus *Cinnamomum* stands out, consisting of about 250 species, many of which are used to produce essential oils important for the world market. Among them, the oils extracted from *Cinnamomum verum*, *Cinnamomum cassia*, *Cinnamomum camphora* and *Cinnamomum zeylanicum* plants are the most commercialized. The commercial value of the oils from genus *Cinnamomum* is based on the species and part of the plant used for its extraction (NOLLET; RATHORE, 2017).

The proof of the insecticidal activity of essential oils and the determination of their major compounds may serve as a basis for the formulation of new products, in which this insecticidal activity can be attributed to the isolated action of a substance, usually the major one, or to the synergistic effect of the constituents present in essential oils (PAPACHRISTOS et al., 2004; OMOLO et al., 2005).

However, one control method should not reduce the efficiency of the other, so it is extremely important that these new studied molecules show physiological selectivity, i.e.,

greater activity as an insecticide on the pest than on its natural enemy, when both enter in direct contact with the insecticide or its residues (PEDIGO, 1988). Botanical products are expected to be more toxic to the pest than to its natural enemy (CHARLESTON et al., 2006). The action of natural enemies, predators, and parasitoids contributes to reducing the need for human intervention in pest control (RIBEIRO et al., 2013; SILVA et al., 2015). Miridae are zoophytophagous insects that belong to the Miridae family, order Hemiptera, suborder Heteroptera (SCHUH, 2013). The use of these predatory stink bugs has contributed significantly to the biological control of pests (BUENO et al., 2012), mainly in the production of vegetables in which they are implemented in protected crops and mass releases for augmentation control have been easily accomplished (CALVO; BOLCKMANS; BELDA, 2012).

A species of miridae that has the potential to control the tomato moth is the predator *Macrolophus basicornis* (Stal, 1860) (Hemiptera: Miridae), that occurs in many countries in South America such as Cuba, Guatemala, Nicaragua, and Venezuela. In Brazil it is found mainly in the states of Goiás, Minas Gerais, Rio de Janeiro, Rio Grande do Sul and Santa Catarina (FERREIRA et al., 2018). Nymphs and adults of this species are often found preying on aphids and lepidopteran caterpillars (HERNANDEZ; HENRY, 2010), in addition to constantly searching for *T. absoluta* eggs in tomato plantations (BUENO et al., 2013; VAN LENTEREN et al., 2017).

Given the importance of pest control in tomato culture and especially the maintenance of natural enemies in this agroecosystem, the agricultural sector needs studies that provide positive economic and ecological results for the control of the tomato pest complex. As a control alternative, the use of these essential oils and the preservation of natural enemies are of great importance and are being studied more and more, to aid the small, medium, and even the large farmers.

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## SECOND PART

### ARTICLE

#### **BIOACTIVITY OF *Cinnamomum* spp. (Lauraceae) ESSENTIAL OILS AGAINST *Tuta absoluta* (Meyrick, 1971) (Lepidoptera: Gelechiidae) AND SELECTIVITY FOR *Macrolophus basicornis* (stal, 1860) (Hemiptera: Miridae)**

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## 1. INTRODUCTION

The tomato moth *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a pest that can cause significant economic losses in tomato and other solanaceous crops worldwide (DESNEUX et al., 2010). The caterpillars of this pest feed on leaf mesophyll, stems, flowers and fruits, which can lead to losses of up to 100% of the production, if control measures are not carried out (CHERIF; VERHEGGEN, 2019).

This lepidopteran was first found and described in Peru in 1917 and arrived quickly in other South American countries; in Brazil, *T. absoluta* was found in tomato plants in the 1980s (GUEDES; PICANÇO, 2012). In a short period of time, this plague spread in Europe in 2006, reaching the Mediterranean Sea and establishing itself in Africa, the Middle East and Asia (BIONDI et al., 2018), being a problem not only in the Americas.

Due to the rapid expansion of this insect in solanaceous crops, there was an increase in the number of insecticide applications (CAMPOS et al., 2017). Synthetic chemical insecticides are usually applied from 10 to 12 times per crop cycle, reaching up to 30 applications (GUEDES; SIQUEIRA, 2013). Despite the various control methods available to manage *T. absoluta*, chemical control is still the most used in open-field and protected crops (GUEDES et al., 2019).

Due to the various applications of insecticides in the tomato crop, the selection of *T. absoluta* populations resistant to various chemical products is recurrent, mainly pyrethroids, abamectin, cartap and organophosphates (CAMPOS et al., 2014; SILVA et al., 2015; HADDI et al., 2017).

The integrated management of *T. absoluta* in tomato aims to decrease the pest population levels in the crop with the integration of the available control methods (chemical, biological, cultural and legislative) that are essential for the production and maintenance of natural enemies in the field (ZAPPALA et al., 2013; BIONDI et al., 2018). Less toxic alternatives for pest control have been advocated, such as the use of essential oils that are products extracted from the secondary metabolism of plants and are easily degraded, reducing the contamination of the environment (NOLLET; RATHORE, 2017).

The genus *Cinnamomun* (Lauraceae) stands out in the market for having more than 250 species and proven antibacterial, antifungal, and insecticidal activities (GUCWA et al., 2018; FIRMINO et al., 2018; DAI et al., 2020; YANG; ISMAN; TAK, 2020). Among the oils extracted from plants of this genus, the most commercialized are from *Cinnamomum verum*

(J. Presl), *Cinnamomum cassia* (L.) J.Presl, *Cinnamomum camphora* (L.) J.Presl and *Cinnamomum zeylanicum* (Blume) (NOLLET; RATHORE, 2017). However, the toxicity of oils or their major compounds often have different effects on natural enemies, depending on the mode of action of the product and the target pest (ISMAN, 2020).

The combination of different control methods must be done in a way that one method does not reduce the efficiency of the other. Therefore, in management programs of arthropod pests, it should be ascertained whether the combination of bioinsecticides and natural enemies is synergistic since the use of biological control has grown widely in Brazil in recent years.

In this context, zoophytophagous Miridae stand out, as they can feed on both arthropods and plant structures such as stems, leaves and fruits at the same stage of development, which may favor their establishment in agricultural crops in periods of absence of prey (CASTAÑÉ et al., 2011). The use of these predatory insects has contributed significantly to the biological control of pests (BUENO et al., 2012), mainly in the production of vegetables under protected cultivation where mass releases for augmentative control have been easily accomplished (ZAPPALA et al., 2013).

Among the zoophytophagous Miridae, the species *Macrolophus basicornis* (Stal, 1860) (Hemiptera: Miridae) has great potential for pest control, especially in solanaceous crops, where nymphs and adults are often found preying on eggs and small caterpillars of *T. absoluta*, aphids and whiteflies (DÍAZ et al., 2014; VAN LENTEREN et al., 2017). For these biological control agents to be successful in the population regulation of arthropod pests, it is of paramount importance that pesticides applied in agricultural crops are harmless to these predators.

As an alternative for tomato pest control, the use of essential oils from plants, together with the preservation of natural enemies are of great importance and are being more studied, aiming to help agricultural producers to maximize the production system.

In this context, the present study aimed to evaluate the lethal and sublethal effects of the essential oils of *C. cassia*, *C. camphora* and *C. camphora* var. *linalooliferum* (Y. Fujita) and their major compounds against *T. absoluta*. Additionally, the toxicity of these essential oils to the predator *M. basicornis* was studied to obtain information for its preservation in agroecosystems.

## 2. MATERIAL AND METHODS

### 2.1 Tomato plants

Tomato seedlings (*Solanum lycopersicum* cv. Santa Clara) were transplanted into plastic pots (5 L) containing Carolina® commercial substrate (composed of peat moss, vermiculite, organic waste, agroindustrial class A organic waste and limestone). The plants were kept in a greenhouse for 30 days, protected from insect infestations, contamination by pathogens and absent of chemical residues, to be used in the breeding of *T. absoluta*.

### 2.2 Obtention of essential oils

The essential oils of howood (*C. camphora* var. *linalooliferum*), camphor (*C. camphora*) and cassia cinnamon (*C. cassia*) were extracted by hydrodistillation, with howood and camphor extracted only from the wood, while Chinese cassia oil was extracted from the bark, leaves and stems. The essential oils came from China and were distributed by Ferquima Indústria e Comércio Ltda, Vargem Grande Paulista - São Paulo, Brazil (Table 1).

**Table 1.** Scientific name, INCI name, plant structure used and extraction method the essential oils evaluated for *Tuta absoluta*.

Scientific name	INCI name	Plant structure used in the extraction of oils	Extraction method
<i>Cinnamomum camphora</i> var. <i>linalooliferum</i>	Cinnamomum Camphora Linalooliferum Wood Oil	Wood	Steam distillation of wood
<i>Cinnamomum camphora</i>	Cinnamomum Camphora Bark Oil	Wood	Steam distillation of wood
<i>Cinnamomum cassia</i>	Cinnamomum Cassia Oil	Leaves, bark and stalk	Steam distillation of leaves, bark and stalk

\* Information provided by the manufacturer, Ferquima Indústria e Comércio LTDA ([www.ferquima.com.br](http://www.ferquima.com.br)).

### 2.3 Chemical characterization of essential oil

All essential oils used in the experiments were characterized in terms of chemical composition by Gas Chromatography coupled to Mass Spectrometry (GC-MS). Qualitative analyzes were carried out in a Shimadzu Gas Chromatograph GCMS-QP2010 Plus in a non-polar capillary column RTX5MS (30 m x 0.25 mm x 0.25  $\mu$ m). The following conditions were used: split mode at an injection rate of 1/20, temperature of 250°C for the injector and ion source and 280 ° C for the interface.

The initial temperature was programmed to 60°C for the first 5 minutes, increasing at a rate of 3°C/min until the final temperature of 240°C, and finally with an isothermal of 5 minutes. The components were identified based on comparisons with the relative retention index using data from a series of n-alkanes (C8-C19), mass spectrum from the equipment database, followed by comparison with retention indices from published data (Adams, 2009).

For the quantitative analyzes, the Shimadzu Gas Chromatograph 2010 with Flame Ionization Detector (GC-FID) was used in an OV-5 column (30 m x 0.25 mm x 0.25  $\mu$ m) under the following conditions: Helium was used as carrier gas at a constant flow of 1 mL/min, injection rate of 1/20, injection volume of 1  $\mu$ l of the oil diluted in ethyl ether, with a temperature detector of 280°C, and the injector at 250°C. The initial temperature of the column was 60°C for 5 minutes, programmed for heating at a rate of 3°C/min until reaching the final temperature of 240°C, completing with an isothermal for 5 minutes.

#### **2.4 *T. absoluta* breeding**

*Tuta absoluta* eggs and caterpillars were collected in tomato crops on the Campus of Universidade Federal de Lavras (UFLA) and placed in acrylic cages (60 x 30 x 30 cm), to start maintenance breeding at the Ecotoxicology and Integrated Pest Management Laboratory (LEMIP) of the Entomology Department of the Universidade Federal de Lavras. To increase the breeding, leaves with *T. absoluta* caterpillars were collected at Agroteste LTDA company (Latitude 21°12' and Longitude 45°03') and added to the breeding. Tomato leaves, with approximately 8 leaflets, were inserted in floral foam moistened daily with water. About 500 adult insects were transferred to a new oviposition cage (60 x 30 x 30 cm), where they remained for 4 days for oviposition and then the eggs were collected for multiplication of the insects in a new cage. The adults were fed with water and honey (1:1) to stimulate oviposition. The population of *T. absoluta* was maintained in the laboratory at a temperature of 24  $\pm$  2°C, relative humidity of 70  $\pm$  10% and 12-hour photoperiod.

## **2.5 Breeding of the predator *M. basicornis***

The adults of *M. basicornis* were collected from horseweed, *Conyza bonariensis* (L.) plants located in the experimental area of coffee at the Universidade Federal de Lavras and placed in acrylic cages (60 x 30 x 30 cm) containing a tobacco plant (*Nicotianna tabacum* L. cv. TNN), with 15 days of transplantation and approximately 15 cm in height, which served as a substrate for oviposition and as a source of food, in a temperature-controlled room as described in sub-item 2.4. The identification of the specimens was made based on the morphological characteristics by an identification key to the species of the Miridae family, proposed by Ferreira and Henry (2011). To reduce inbreeding, and consequently the adverse effects on the progeny of predators used in the study, new insects were obtained at the Laboratory of Insect Biology of the Escola Superior de Agricultura Luiz de Queiroz (ESALQ-USP), Piracicaba - São Paulo. Thus, two populations of predators were used for breeding in the present study.

Eggs of *Ephestia kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) were offered for feeding *ad libitum*. The adults were kept for 7 days in the cages for mating and oviposition, where they were collected using an aspirator coupled to an air compressor and relocated in another cage with the same characteristics mentioned above. Plants with *M. basicornis* eggs were kept until the nymphs hatched, which were fed until they reached adulthood. These steps were cyclical until the insect population increased significantly to carry out the experiments. The adults of *M. basicornis* were transferred in groups of 100 specimens from the tobacco plant to a new cage containing a tomato plant (cv. Santa Clara). After 7 days, they were again transferred to other cages. The nymphs were maintained and fed on the tomato plant, and the population that was evaluated corresponded to the third generation in the laboratory.

## **2.6 Insecticide toxicity against *T. absoluta***

For the insecticide toxicity bioassay against *T. absoluta*, 400 second-instar caterpillars were removed from the breeding after three laboratory generations. The insecticides Cartap BR 500®, Clorpirifós EC®, Danimen 300 EC® and Delegate®, registered for the control of *T. absoluta* by the Ministry of Agriculture, Livestock and Supply - MAPA (AGROFIT, 2021), were tested in the highest dose recommended and twice that dose of each product (Table 2). The insecticides were solubilized in distilled water and the tomato leaflets were

immersed in the chemical solutions for 5 seconds, which allowed the entire coverage of the leaflets.

Then, they were placed in plastic trays containing filter paper to eliminate excess chemical solution from their surfaces. After the leaflets were dry, they were placed in a 5 cm diameter Petri dish with moistened filter paper and then the second-instar caterpillars of *T. absoluta* were transferred with the aid of a paintbrush with soft bristles and the plates were sealed with polyethylene plastic film containing micro-holes to allow aeration. The Petri dishes were kept in the laboratory at a temperature of  $24 \pm 2$  °C, relative humidity of  $70 \pm 10\%$  and 12-hour photoperiod. The experiment consisted of 9 treatments (4 insecticides, two doses of each, and a control composed of distilled water) with 50 replicates, each replicate composed of a caterpillar. The evaluations were carried out after 72 hours of the caterpillars exposure to the residues of the compounds, with the aid of a stereoscope microscope (40x). Insects that did not present movements to the touch of a fine-tipped paintbrush were considered dead.

**Table 2.** Technical name, commercial name, dose and chemical group of the insecticides used in the susceptibility bioassay for *Tuta absoluta*.

Technical name	Commercial name	Dose (ml ou g de c.p.100 L <sup>-1</sup> )*	Chemical group
Cartap hydrochloride	Cartap BR 500 <sup>®</sup>	250 g	Nereistoxin analogues (14)
Chlorpyrifos	Clorpirifós EC <sup>®</sup>	1000 mL	Organophosphate (1B)
Fenprothrin	Danimen 300 EC <sup>®</sup>	150 mL.ha <sup>-1</sup>	Pyrethroids (3A)
Spinetoram	Delegate <sup>®</sup>	16 g.ha <sup>-1</sup>	Spinosyns (5)

\*Maximum dosages of commercial product / 100 L recommended by manufacturers.

c.p. = commercial product name.

Chemical group 14: Channel blockers of nicotinic acetylcholine receptors 1B: Acetylcholinesterase inhibitors; 3A: Sodium channel modulators; 5: Allosteric modulators of nicotinic acetylcholine receptors.

Source: Agrofit – MAPA (2020).

## 2.7 Screening of the bioactivity of essential oils for *T. absoluta*

The essential oils (Table 1) were diluted in acetone at a concentration of 100 mg.mL<sup>-1</sup> and applied topically to the back of *T. absoluta* second instar caterpillars. Each insect received 1µL of the solution with a microsyringe (Hamilton<sup>®</sup> 25 µL). The insects in the control group



were treated with acetone only. The experiment consisted of four treatments, with sixty replications, with five insects per tomato seedling. The experimental design was completely randomized. The mortality assessments of the caterpillars was made at 6, 12, 24, 36, 48 and 72 h after the application of the oils, with the aid of a stereoscopic microscope (40x). Insects that did not present movements to the touch of a fine-tipped paintbrush were considered dead.

## **2.8 Determination of the dose-time-mortality response of essential oils for *T. absoluta***

Caterpillars of *T. absoluta* were treated topically, so that each insect received 1  $\mu\text{L}$  of the solution, following the methodology described in subitem 2.5. Each of the three oils was tested at concentrations of 10; 1; 0.1; 0.01 and 0.001  $\text{mg}\cdot\text{mL}^{-1}$  which were determined by arithmetic progression in previous tests, for obtaining concentration ranges that caused mortality rates between 20 and 100% (FINNEY, 1971). The experiment was carried out in a completely randomized design, formed by 16 treatments (5 concentrations for each essential oil and 1 control treatment). Each treatment consisted of 100 replicates, each replicate consisting of an insect and a tomato leaflet placed in a Petri dish of 5 cm in diameter. The evaluations of insect mortality were performed at intervals of 6, 12, 24, 36, 48 and 72 h after the application of essential oils to calculate the median lethal time ( $\text{LT}_{50}$ ). Mortality assessment was performed with the aid of a stereoscopic microscope (40x). Insects that did not present movements to the touch of a paintbrush with soft bristles were considered dead.

## **2.9 Toxicity of the major compounds of essential oils against *T. absoluta***

The major compounds of the *C. cassia* and *C. camphora* var. *linalooliferum* essential oils (cinnamaldehyde 84.21% pure and linalool 98.75% pure) were acquired by Sigma-Aldrich®. The treatments used consisted of pure substances in a concentration equivalent to the  $\text{LD}_{50}$  of the oils for *T. absoluta*. For the determination of the concentration to be used, the percentage of these substances found in the quantitative analysis by GC-MS was considered. For proportional application, the following formula was used:

$$CST = COE \times TCM(\%)$$

Where: CST is the Concentration to be tested, COE: Concentration of essential oil and TCM: Content of the major compound present in the oil in percentage. The experiment was carried out in a completely randomized design, consisting of 60 replicates per treatment, each

replicate consisting of a second instar caterpillar treated with 1  $\mu$ L of the solution and maintained under conditions of temperature of  $24 \pm 2$  °C, relative humidity of  $70 \pm 10\%$  and 12-h photoperiod. Mortality assessment was performed with the aid of a stereoscopic microscope (40x) after 72 h after the application of treatments. Insects that did not present movements to the touch of a paintbrush with soft bristles were considered dead.

## **2.10 Leaf consumption of *T. absoluta* treated with essential oils and their major compounds**

Second instar caterpillars of *T. absoluta* were treated topically, so that each insect received 1  $\mu$ L of the solution corresponding to the LD<sub>50</sub> of each essential oil and each major compound. The experiment was carried out in a completely randomized design, consisting of 6 treatments (3 essential oils - *C. cassia*; *C. camphora* and *C. camphora* var. *linalooliferum*, 2 major compounds - cinnamaldehyde and linalool and 1 control with acetone) and 60 replicates, each replicate composed of a second instar caterpillar placed in a 10 cm diameter Petri dish with 5 cm<sup>2</sup> piece of a tomato leaf at a temperature of  $24 \pm 2$  °C, relative humidity of  $70 \pm 10\%$  and 12-h photoperiod. Leaf consumption was evaluated 24 h after the application of essential oils and major compounds with the aid of the Image J software.

## **2.11 Life table of *T. absoluta* treated with essential oils**

About 100 eight-days-old couples of *T. absoluta* were kept in an acrylic cage (60 x 30 x 30 cm) containing a tomato plant cv. Santa Clara (15 cm high) for 48 hours for oviposition. With the aid of a magnifying glass (10x), the plants were observed daily to check for the appearance of 2nd instar caterpillars. Subsequently, 400 2nd instar caterpillars were carefully removed with the aid of a soft bristle paintbrush and transferred individually to Petri dishes (2 cm high x 10 in diameter) for topical application of essential oils. All Petri dishes were sealed with plastic PVC film to prevent the escape of insects. Small holes were made in the plastic with an entomological pin to allow gas exchange and moisture stabilization. Tomato leaflets were offered with the petiole wrapped in moistened cotton, being changed every two days to feed the caterpillars inside the plates.

The experimental design was completely randomized, with four treatments and 100 repetitions, with each treatment constituted by the LD<sub>50</sub> of each essential oil, plus the control

treatment that consisted of acetone. Each repetition consisted of a Petri dish with a 2nd instar caterpillar. The bioassay was conducted in an temperature-controlled chamber regulated at a temperature of  $24 \pm 2$  °C, relative humidity of  $70 \pm 10\%$  and 12-h photophase.

To determine the larval and pupal development, the instar change were observed daily, through the visualization of cephalic capsules and or by the size of the caterpillar, with the aid of a stereoscopic microscope (40x). The number and duration of instars, larval and pupal survival, and total time of larval and pupal development of insects were evaluated after the application of essential oils.

To assess the effects of oils on adults from treated caterpillars, about 20 couples of newly emerged adult insects from each treatment were evaluated for their reproduction and longevity. For this purpose, each couple was kept in a Petri dish (2 cm high x 15 cm in diameter) covered with PVC plastic film with small holes made with an entomological pin to prevent the escape of insects and enable gas exchange. Each Petri dish contained a piece of cotton wool moistened with a 1:1 solution of honey and water and a tomato seedling (with 5 leaves) with its stem wrapped in moistened cotton and placed in an Eppendorf, which served as substrate for oviposition.

In this way, the oviposition period, survival, longevity of males and females, the number of eggs laid per female and the proportion of males and females were evaluated to calculate the sexual proportion of the offspring, in addition to the viability of eggs and survival of first instar caterpillars. All data obtained were used to make four life tables referring to the life history traits of insects treated with the essential oils and insects treated only with acetone by the TWO SEX MS-Chart program (CHI, 2020).

## **2.12 Toxicity of essential oils to *M. basicornis***

Adults of the predator *M. basicornis* of 48 h were treated topically; each insect received 1  $\mu$ L of the solution corresponding to the LD<sub>50</sub> of each essential oil and each major compound determined for *T. absoluta*. The experiment was carried out entirely randomized, with 6 treatments (3 essential oils - *C. cassia*; *C. camphora* and *C. camphora* var. *linalooliferum*, 2 major compounds - cinnamaldehyde and linalool, and 1 control with acetone) and 60 replicates (30 males and 30 females), each one constituted by a 48-h old adult of the predator. The insects were placed in Petri dishes of 15 cm in diameter, containing a 10 cm tall tomato seedling and eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) as

*ad libitum* supply of an alternative prey, under temperature conditions of  $24 \pm 2$  °C,  $70 \pm 10\%$  relative humidity and 12-h photoperiod. The test was evaluated 72 h after the application of essential oils, with the aid of a stereoscopic microscope (40x). Insects that did not present movements to the touch of a paintbrush with soft bristles were considered dead.

### 2.13 Data analysis

*Tuta absoluta* susceptibility to insecticides, bioactivity of essential oils, topical application with major compounds bioassay, toxicity of essential oils against *M. basicornis* and mortality of females and males of *M. basicornis* under application of essential oils data were subjected to the Shapiro-Wilk and Bartlett tests to verify the assumptions of normality and homoscedasticity of the analysis of variance (ANOVA). As they did not assume a normal distribution, they were adjusted to the GLM (Generalized Linear Models) using the “binomial” distribution and the means were compared by contrast analysis. Survival data over time were submitted to survival analysis, using the Weibull model, using the Survival package (THERNEAU, 2020). After selecting the most appropriate mathematical model through the analysis of residues, contrast analysis was carried out to verify the similarity between the treatments used to form congener groups. The median lethal time (LT<sub>50</sub>) for each group formed was also calculated. To determine the dose-mortality response and to obtain the median lethal dose (LD<sub>50</sub>), the data were submitted to the Logit analysis, using the drc package (RITZ, 2015).

As the leaf consumption data did not assume a normal distribution, they were adjusted to the GLM (Generalized Linear Models) using the “Quasipoisson” distribution and the means were compared by the Tukey test ( $p < 0.05$ ) using the Multcomp package (HOTHORN, 2015).

The viability data for eggs and first instar caterpillars also did not assume a normal distribution, they were adjusted to the GLM (Generalized Linear Models) using the “Quasibinomial” distribution and the means were compared by the Tukey test ( $p < 0.05$ ) by the Multcomp package (HOTHORN, 2015). All analyzes were performed using the statistical program R: A language and environment for statistical computing (Core Team, 2020).

The processing of data analysis for making life tables was performed using the TWOSEX-MSCHART program (CHI, 2020). The life history data for males and females

were analyzed based on the life-table theory for two sexes by age and development stage (Age-stage, Two-sex life table) proposed by Chi and Liu (1985).

The biological parameters considered, and their respective formulas are: age-stage-specific survival rate ( $S_{xj}$ ) = Probability that a newborn individual will survive until age x and stage j.

$$S_{xj} = \frac{\text{Number of individuals at age } x \text{ and stage } j}{\text{Total of individuals}}$$

Age-specific survival rate ( $l_x$ ) = Proportion of individuals from the initial population that survive until age x.

$$l_x = \frac{\text{Number of individuals alive at age } x}{\text{Total of individuals}}$$

Age-stage-specific life expectancy ( $e_{xj}$ ) = Estimated time that an individual can live at age x and stage j.

$$e_{xt} = \frac{T_x}{S_{xj}} \quad T_x = \frac{(S_{xj} + S_{x+1j})}{2}$$

Age-specific fecundity ( $m_x$ ) = Average number of eggs produced per individual at age x. In this case, the eggs were counted as an estimate of fecundity.

$$m_x = \frac{\text{Number of eggs on the day } x}{\text{Total of individuals on the day } x}$$

Fertility by age-stage of development ( $f_{xj}$ ) = Average fertility of individuals at age x and stage j.

$$f_{xj} = \frac{\text{Number of eggs on the day } x}{\text{Total of alive females on day } x}$$

Distribution of mortality by age-stage of development ( $p_{xj}$ ) = Probability that an individual will die at age x and stage j.

$$p_{xj} = \frac{\text{Number of dead individuals at age } x \text{ and stage } j}{\text{Total of individuals}}$$

Maternity by age ( $l_x m_x$ ) = Number of descendants expected per individual at age  $x$ , taking into account the probability that it will reach that age alive.

$$l_x m_x = l_x \times m_x$$

Reproductive value by age-stage of development ( $v_{xj}$ ) = Contribution of an individual at age  $x$  and stage  $j$  to the future population (offspring).

$$\frac{V_x}{V_0} = \frac{e^{r \cdot x}}{l_x} \cdot \sum_{y=x}^{y(\max)} e^{-r \cdot y} \cdot l_x \cdot m_x$$

Net reproductive rate ( $R_0$ ) = Average number of descendants of an individual throughout his life. The population tends to: increase when  $R_0 > 1$ , decrease when ( $R_0 < 1$ ) or remain stable when  $R_0 = 1$ .

$$R_0 = \sum_{x=0}^{\infty} l_{(x)} m_{(x)}$$

Intrinsic growth rate ( $r$ ) = Refers to the population's ability to increase in number of individuals. The population tends to: increase when  $r > 0$ , decrease when  $r < 0$  or remain stable when  $r = 0$ .

$$r = \frac{\ln R_0}{T}$$

Finite growth rate ( $\lambda$ ) = Average descent of an individual per unit of time. Factor by which a population increases per unit  $x$  of time. The population tends to: increase when  $\lambda > 0$ , decrease when  $\lambda < 0$  or remain stable when  $\lambda = 0$ .

$$\lambda = e^r$$

Average generation time ( $T$ ) = Average generation time. Time elapsed between the birth of the parents and their descendants.

$$T = \frac{\ln R_0}{r}$$

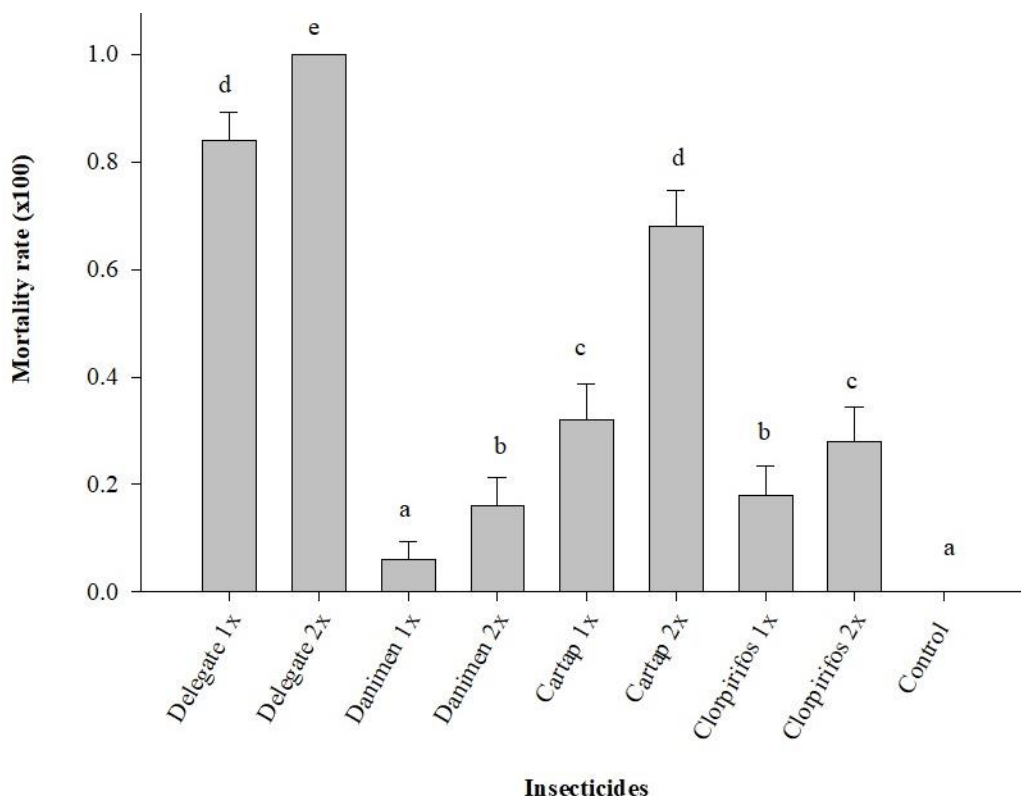
The means and standard errors of the reproductive parameters were estimated using the Bootstrap method, with 100,000 resamples (EFRON; TIBSHIRANI, 1993, HESTERBERG, 2008; HUANG; CHI, 2011; YU et al., 2013; AKKÖPRÜ et al., 2015).

Parameter differences between treatments were analyzed using the paired Bootstrap test, based on the confidence interval (CROWLEY, 1992; HESTERBERG et al., 2005; SMUCKER et al., 2007) by the TWOSEX-MSChart software for Windows (CHI, 2020).

### 3. RESULTS

#### 3.1 Insecticide toxicity for *T. absoluta*

There were differences between treatments ( $\chi^2 = 82,02$ ; d.f. = 7;  $p < 0,05$ ) regarding the mortality of *T. absoluta* caterpillars. Those treated with twice the recommended dose of the insecticide Delegate showed 100% mortality, having the highest control efficiency. However, at the recommended dose, this insecticide caused a lower percentage of mortality, not differing from Cartap at twice the recommended dose, with an average mortality rate of 68%. The insecticide Cartap used in the recommended dose ( $250 \text{ g} \cdot 100 \text{ L}^{-1}$ ) caused 32% of mortality, as well as the insecticide Chlorpyrifos at twice the recommended dose, with mortality rate of 28%. Chlorpyrifos at the recommended dose caused 18% mortality, similarly to Danimien at twice the recommended dose, with an average of 16%. Danimien applied at the recommended dose caused 6% mortality, being the same as the control treatment composed only of distilled water (Figure 1).



**Figure 1.** Mortality of second instar caterpillars of *Tuta absoluta* treated with insecticides in the recommended dose (1x), with twice the recommended dose (2x) and by the control with distilled water. Means followed by the same letter do not differ by Tukey test ( $p < 0,05$ ).

#### 3.2 Chemical characterization of the essential oils



Ten, twelve and five components have been identified in the essential oils of *C. cassia* (Chinese cassia), *C. camphora* (camphor) and *C. camphora* var. *linalooliferum* (howood), respectively. The major component identified in the oil of *C. cassia* was cinnamaldehyde (84.21%), in the oil of *C. camphora* was 1,8-Cineol (66.74%) and in the oil of *C. camphora* var. *linalooliferum* was linalool (98.75%) (Table 3).

**Table 3.** Relative composition (%) of the components identified in the gas chromatography analysis coupled with mass spectrometry of the essential oils of *Cinnamomum cassia*, *Cinnamomum camphora* and *Cinnamomum camphora* var. *linalooliferum*.

Óleo essencial	Compostos	RI <sup>a</sup>	RI <sup>b</sup>	Porcentagens*
<i>Cinnamomum cassia</i>	Benzaldehyde	959	960	0.75
	Phenyl ethyl alcohol	1114	1108	0.33
	Borneol	1166	1169	0.13
	Cinnamaldehyde <Z>	1220	1219	0.30
	Anisaldehyde <o->	1244	1242	0.38
	<b>Cinnamaldehyde &lt;E&gt;</b>	<b>1282</b>	<b>1270</b>	<b>84.21</b>
	α-Copaene	1375	1376	0.38
	Coumarin	1439	1434	1.31
	Cinnamy acetate <E>	1449	1446	2.82
<i>Cinnamomum camphora</i>	Cinnamaldehyde<(E)>-p-methoxy	1537	1564	7.98
	α-Thujunene	927	930	0.11
	α-Pinene	934	939	6.24
	α-Fenchene	948	952	0.37
	Sabinene	974	975	3.06
	β-Pinene	977	979	1.11
	Myrcene	992	990	2.28
	α-Phellandrene	1006	1002	0.39
	α-Terpinene	1017	1017	1.91
	o-Cymene	1027	1026	9.95
	<b>1,8-Cineole</b>	<b>1034</b>	<b>1031</b>	<b>66.74</b>
γ-Terpinene	1060	1059	1.71	
Terpinolene	1089	1088	0.28	
<i>Cinnamomum camphora</i> var. <i>linalooliferum</i>	Eucalyptol	1031	1026	0.18
	cis-Linalool oxide	1073	1072	0.28
	trans-Linalool oxide	1089	1084	0.37
	<b>Linalool</b>	<b>1108</b>	<b>1096</b>	<b>98.75</b>
	Camphor	1145	1146	0.17

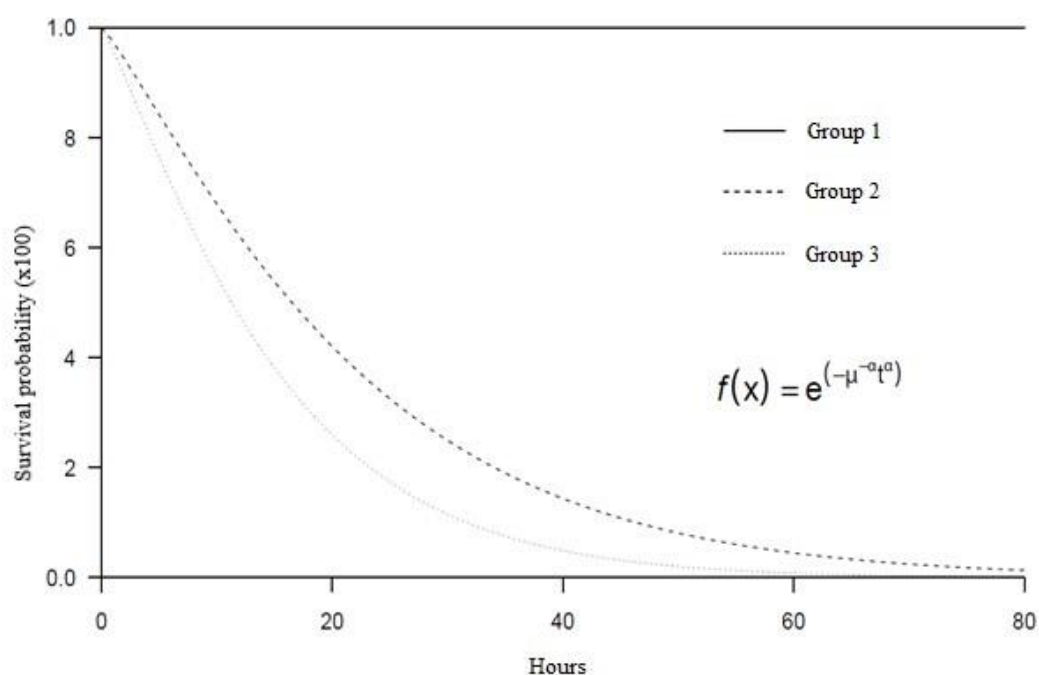
RI<sup>a</sup>: Relative retention index values calculated using the column RTX-5 (GC-MS) and the series of n-alkanes C8–C19.

RI<sup>b</sup>: relative retention indexes published with a column DB-5.

\*Averages of relative compositions calculated using the OV-5 column (GC-FID) and the C8–C19 n-alkane series.

### 3.3 Bioactivity of the essential oils

The essential oils of *C. cassia*, *C. camphora* and *C. camphora* var. *linalooliferum* caused 100% mortality of *T. absoluta* caterpillars at the concentration of 100 mg.mL<sup>-1</sup> of acetone at the end of the evaluation period ( $\chi^2 = 250.5$ ; d.f. = 3;  $p < 0.05$ ). The survival analysis allowed the formation of three congener groups (Figure 2), and group 1 were formed only by acetone, with 100% survival and LT<sub>50</sub> greater than 72 h. Group 2 was formed by the essential oils of *C. cassia* and *C. camphora* and the time needed to cause the mortality of half the population was 16.5 h. Group 3 consisted only of the essential oil of *C. camphora* var. *linalooliferum* with the lowest LT<sub>50</sub>, which was 11.5 h.



**Figure 2.** Survival analysis of second instar caterpillars of *Tuta absoluta* submitted to topical application of the *Cinnamomum* spp. essential oils solution. Group 1: acetone  $f(x) = \exp(-(2408090628)^{-1.162791} * x^{1.162791})$  (LT<sub>50</sub> > 72 h), Group 2: *Cinnamomum cassia* e *Cinnamomum camphora*  $f(x) = \exp(- (22.59661)^{-1.162791} * x^{1.162791})$  (LT<sub>50</sub> = 16,5 h) and Group 3: *Cinnamomum camphora* var. *linalooliferum*  $f(x) = \exp(- (15.44214)^{-1.162791} * x^{1.162791})$  (LT<sub>50</sub> = 11,5 h).

### 3.4 Determination of the dose-time-mortality response of essential oils for *T. absoluta*

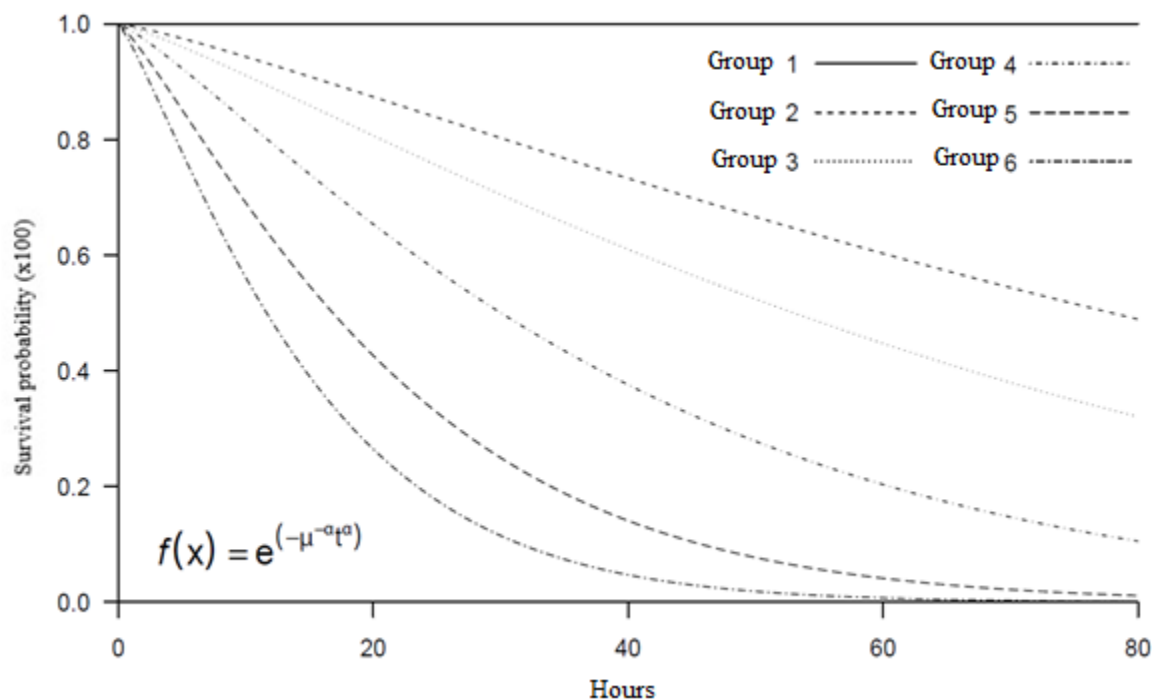
The caterpillars were more susceptible to the essential oil of *C. camphora* var. *linalooliferum*, when compared to other oils (Table 4). *Cinnamomum cassia* presented intermediate LD<sub>50</sub> when compared to the other oils and *C. camphora* caused the lowest mortality of *T. absoluta* caterpillars in the evaluation period with LD<sub>50</sub> twice as high as the value for *C. camphora* var. *linalooliferum* ( $\chi^2 = 318.85$ ; d.f. = 15;  $p < 0.05$ ). The same pattern

of mortality was found for the LD<sub>90</sub> of essential oils. The survival analysis after topical application of the oils allowed the formation of six congener groups ( $\chi^2 = 384.25$ ; g.l. = 15;  $p < 0.05$ ).

**Table 4.** Lethal doses of the essential oils of *Cinnamomum cassia*, *Cinnamomum camphora* and *Cinnamomum camphora* var. *linalooliferum* for *Tuta absoluta*.

Treatments	n	$\chi^2$	p	*b	*e	LD <sub>50</sub> (mg.mL <sup>-1</sup> )	LD <sub>90</sub> (mg.mL <sup>-1</sup> )
<i>C. cassia</i>	100	92.851	0.600	-0.584	0.06	0.07±0.03	2.93±2.33
<i>C. camphora</i>	100	84.096	0.821	-0.676	0.09	0.10±0.04	2.51±1.79
<i>C. camphora</i> var. <i>linalooliferum</i>	100	47.162	1.000	-1.176	0.05	0.05±0.02	0.35±0.18

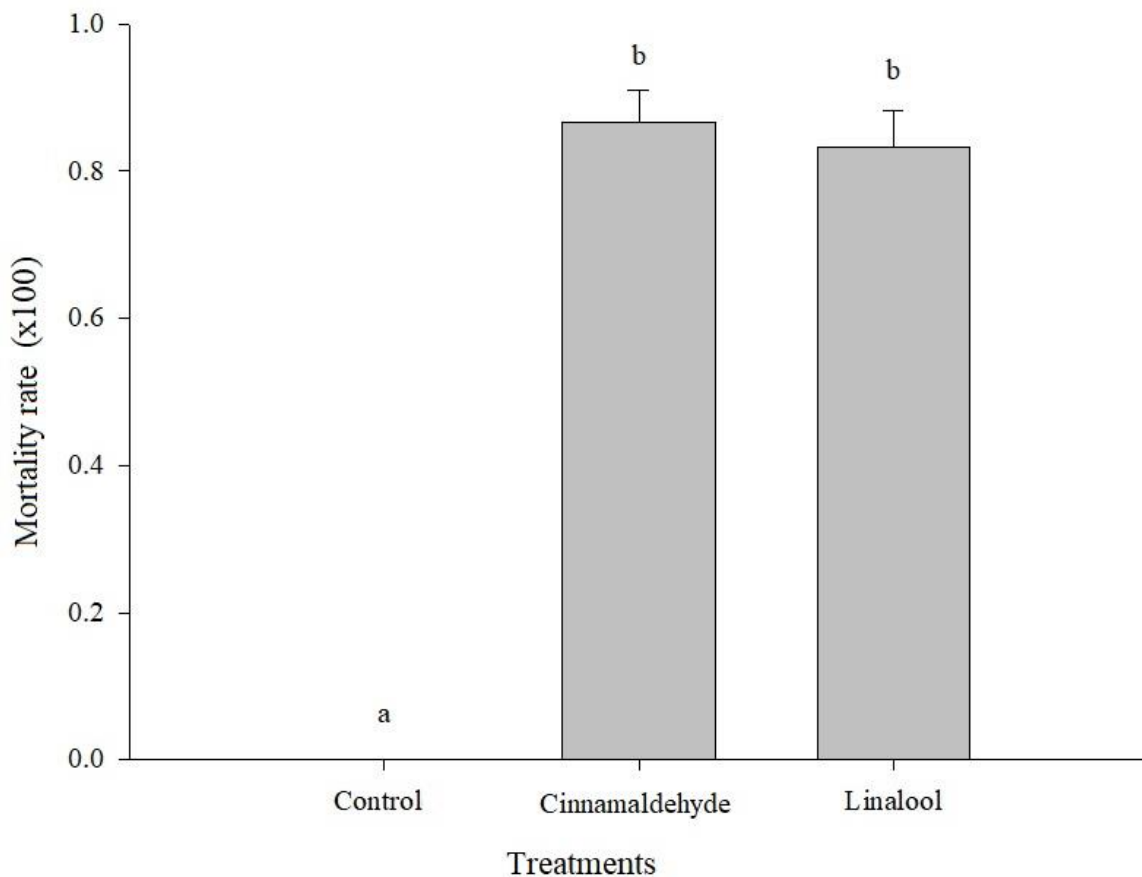
Group 1 consisted of the control treatment with acetone, with LT<sub>50</sub> greater than 72 h. Group 2 was formed only by the treatment *C. camphora* in the concentration of 0.001 mg.mL<sup>-1</sup>, with LT<sub>50</sub> greater than 72 h. Group 3 consisted of the oils of *C. camphora* and *C. cassia* at a concentration of 0.01 mg.mL<sup>-1</sup> and the oils of *C. cassia* and *C. camphora* var. *linalooliferum* at a concentration of 0.001 mg.mL<sup>-1</sup> with a LT<sub>50</sub> of 53 hours. For group 4, a LT<sub>50</sub> of 30 h was verified, and this group was composed by the oil of *C. camphora* var. *linalooliferum* at a concentration of 0.01 mg.mL<sup>-1</sup>, by the oils of *C. camphora*, *C. cassia* and *C. camphora* var. *linalooliferum* at a concentration of 0.1 mg.mL<sup>-1</sup> and by the oils of *C. camphora* and *C. cassia* at a concentration of 1 mg.mL<sup>-1</sup>. Group 5 was formed by the oils of *C. camphora* and *C. cassia* at a concentration of 10 mg.mL<sup>-1</sup> and by the oil of *C. camphora* var. *linalooliferum* at 1 mg.mL<sup>-1</sup> with LT<sub>50</sub> of 17 h. Group 6 presented a LT<sub>50</sub> of 11.5 h and was formed by the oil of *C. camphora* var. *linalooliferum* at a concentration of 10 mg.mL<sup>-1</sup> (Figure 3).



**Figure 3.** Survival curves of second instar caterpillars of *Tuta absoluta* treated with different concentrations of three essential oils. Group 1 = acetone  $f(x) = \exp(-323108908)^{-1.201923} \cdot x^{1.201923}$  ( $LT_{50} > 72$  h), Group 2 = *Cinnamomum camphora* ( $0,001 \text{ mg.mL}^{-1}$ )  $f(x) = \exp(-105.7206)^{-1.201923} \cdot x^{1.201923}$  ( $LT_{50} > 72$  h), Group 3 = *Cinnamomum camphora* and *Cinnamomum cassia* ( $0.01 \text{ mg.mL}^{-1}$ ), *Cinnamomum cassia* and *Cinnamomum camphora* var. *linalooliferum* ( $0.001 \text{ mg.mL}^{-1}$ )  $f(x) = \exp(-71.85858)^{-1.201923} \cdot x^{1.201923}$  ( $LT_{50} = 53$  h), Group 4 = *Cinnamomum camphora* var. *linalooliferum* ( $0.01 \text{ mg.mL}^{-1}$ ), *Cinnamomum camphora*, *Cinnamomum cassia* and *Cinnamomum camphora* var. *linalooliferum* ( $0,1 \text{ mg.mL}^{-1}$ ) and *Cinnamomum camphora* and *Cinnamomum cassia* ( $1 \text{ mg.mL}^{-1}$ )  $f(x) = \exp(-40.707)^{-1.201923} \cdot x^{1.201923}$  ( $LT_{50} = 30$  h), Group 5 = *Cinnamomum camphora* and *Cinnamomum cassia* ( $10 \text{ mg.mL}^{-1}$ ) and *Cinnamomum camphora* var. *linalooliferum* ( $1 \text{ mg.mL}^{-1}$ )  $f(x) = \exp(-22.81687)^{-1.201923} \cdot x^{1.201923}$  ( $LT_{50} = 17$  h) and Group 6 = *Cinnamomum camphora* var. *linalooliferum* ( $10 \text{ mg.mL}^{-1}$ )  $f(x) = \exp(-15.76355)^{-1.201923} \cdot x^{1.201923}$  ( $LT_{50} = 11,5$  h).

### 3.5 Topical application bioassay of major compounds of essential oils in second instar caterpillars of *T. absoluta*

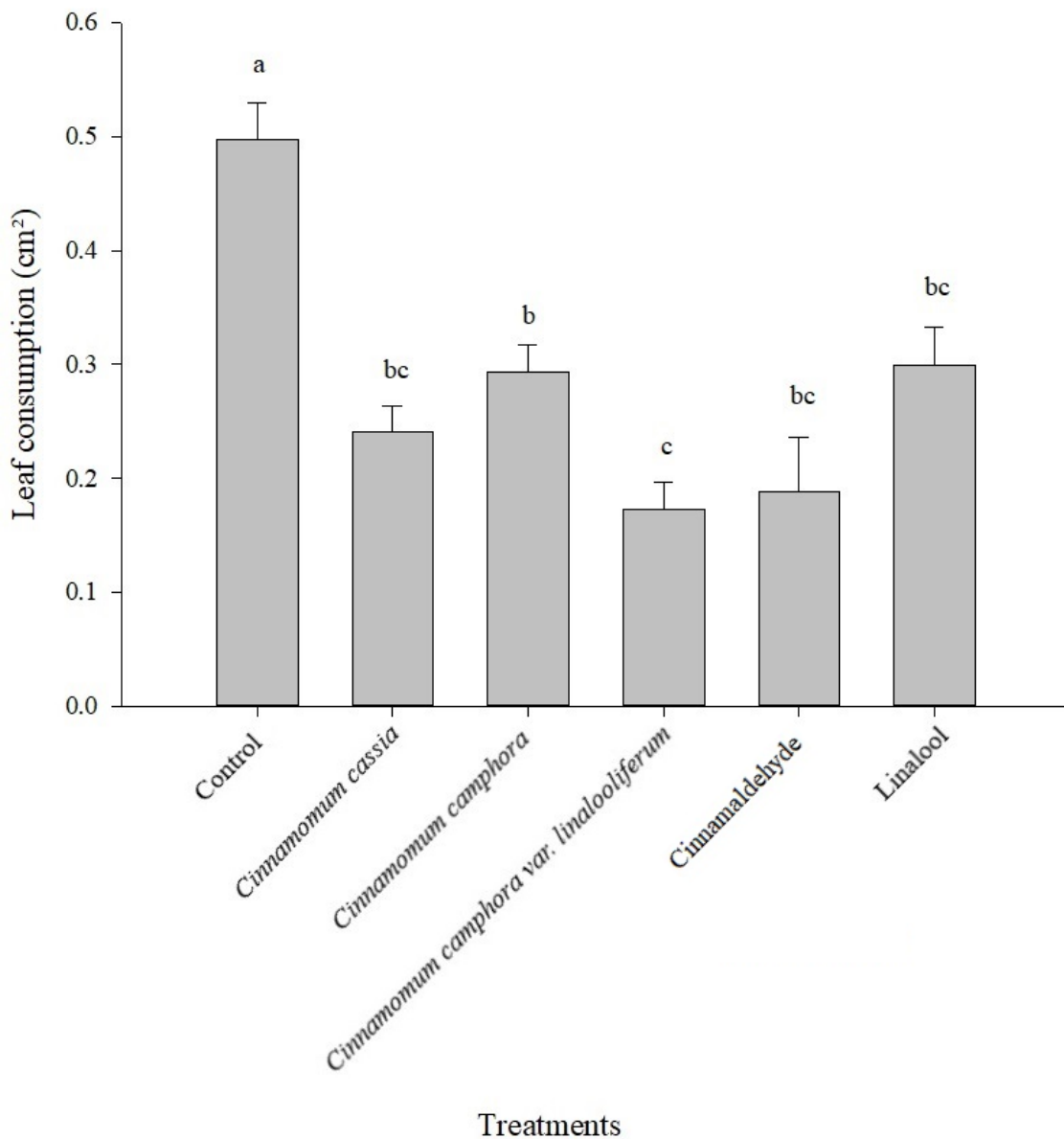
Differences were found between treatments ( $F = 107.04$ ; d.f. = 2;  $p < 0.05$ ) regarding the mortality of *T. absoluta* caterpillars; those that received the application of the major compounds cinnamaldehyde and linalool presented mortality rates of 83.3 and 86.7% (Figure 4).



**Figure 4.** Mortality of second instar caterpillars of *Tuta absoluta* at 72 h after being treated with the major compounds cinnamaldehyde and linalool from the essential oils of *Cinnamomum cassia*, *Cinnamomum camphora* var. *linalooliferum* and with acetone (control). Means followed by the same letter do not differ, using the Tukey test ( $p < 0.05$ ).

### 3.6 Leaf consumption of *T. absoluta* treated with essential oils and major compounds

After 24 hours of offering leaf sections for caterpillars that received the treatment via topical application, leaf consumption in the control treatment (acetone) was 0.5 cm<sup>2</sup>. Caterpillars treated with *C. camphora* showed an average consumption of 0.29 cm<sup>2</sup>, higher than the caterpillars treated with the oil of *C. camphora* var. *linalooliferum*, which had an average consumption of 0.17 cm<sup>2</sup> ( $F = 18,17$ ; d.f. = 5;  $p < 0.05$ ). Caterpillars treated with the essential oil of *C. cassia* and with the major compounds cinnamaldehyde and linalool showed an average consumption of leaf mesophyll of 0.24; 0.18 and 0.30 cm<sup>2</sup>, respectively, not differing from treatments with oils of *C. camphora* and *C. camphora* var. *linalooliferum*.



**Figure 5.** Leaf consumption of second instar caterpillars of *Tuta absoluta* at 24 h of topical application of compounds and control treatment with acetone. Means followed by the same letter do not differ, using the Tukey test ( $p < 0.05$ ).

### 3.7 Life table of *T. absoluta* treated with essential oils

#### 3.7.1 Effects of essential oils on the life cycle of *T. absoluta*

Second-instar caterpillars treated with essential oils showed different development periods, with the shortest time observed during the change from second to third instar was in the treatment with *C. cassia* essential oil. Differences occurred for larval stages L3 and L4 in all treatments ( $p < 0.05$ ). An increase in the pupal period was observed for the *C. camphora*

treatment. Insects exposed to *C. camphora* var. *linalooliferum* essential oil showed shorter total time of the immature stage; however, differences in the period of adulthood were observed for all treatments, in which the lowest average was found for the treatment of *C. camphora* (Table 5).

**Table 5.** Effects of essential oils from plants of the genus *Cinnamomum* spp. in the development of *Tuta absoluta*.

Parameters	Stages	Control		<i>Cinnamomum. cassia</i>		<i>Cinnamomum camphora</i>		<i>Cinnamomum camphora</i> var. <i>linalooliferum</i>	
		N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE
Development time (days)	Egg	100	3.00 ± 0 a	100	3.00 ± 0 a	100	3.00 ± 0 a	100	3.00 ± 0 a
	L1	100	2.00 ± 0 a	100	2.00 ± 0 a	100	2.00 ± 0 a	100	2.00 ± 0 a
	L2	98	2.22 ± 0.09 a	88	1.28 ± 0.06 d	85	1.65 ± 0.11 c	82	1.89 ± 0.03 b
	L3	94	1.36 ± 0.06 c	77	2.31 ± 0.07 b	70	2.96 ± 0.11 a	82	1.06 ± 0.03 d
	L4	94	2.06 ± 0.03 b	69	2.45 ± 0.06 a	66	1.89 ± 0.04 c	62	1.65 ± 0.07 d
	Pupa	94	9.66 ± 0.07 b	69	9.52 ± 0.07 b	60	12.17 ± 0.21 a	48	9.96 ± 0.20 b
	Egg - Pupa	94	20.36 ± 0.05 c	69	20.57 ± 0.06 b	60	23.75 ± 0.22 a	48	19.56 ± 0.18 d
Longevity (days)	Adult	94	16.15 ± 0.30 a	69	8.48 ± 0.33 c	60	6.80 ± 0.49 d	48	11.77 ± 0.37 b
	Female	42	36.86 ± 0.56 a	26	30.69 ± 0.17 d	31	30.94 ± 0.85 c	27	31.93 ± 0.31 b
	Male	52	36.23 ± 0.25 a	43	28.05 ± 0.47 c	29	30.14 ± 0.58 c	21	30.57 ± 0.66 b
Life cycle*	Egg - Adult	94	36.51 ± 0.29 a	69	29.04 ± 0.34 c	60	30.55 ± 1.10 b	48	31.33 ± 0.35 b

Averages on the same line followed by different letters differ from each other ( $p < 0.05$ ). Differences between treatments were obtained using the Bootstrap test paired with 100.000 replicates.

N = number of specimens at each stage of development.

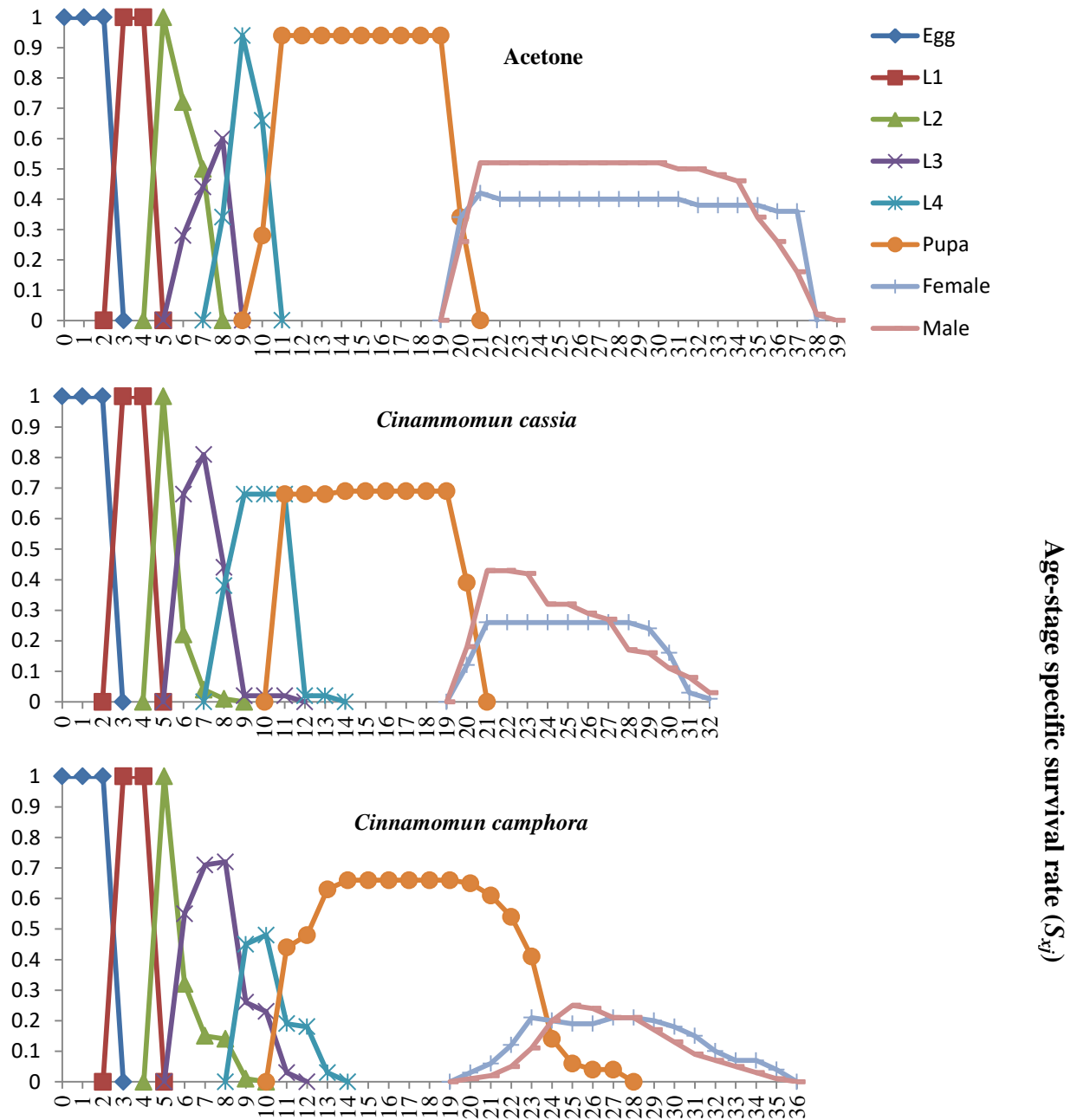
L1 = 1st instar caterpillar, L2 = 2nd instar caterpillar, L3 = 3rd instar caterpillar, L4 = 4th instar caterpillar.

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

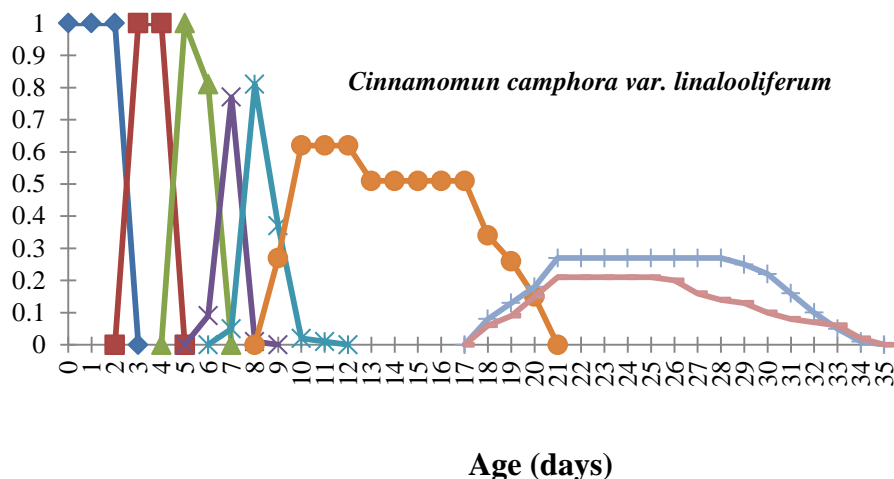
Higher mortality rates were observed between the second and fourth instars, and in the fourth instar most insects reached adulthood. It should be noted that in all treatments, the insects that reached the pupal stage were able to reach the adult stage. The proportion of females and males determined for each treatment was: acetone (1.24♀:1♂), *C. cassia* (1.66♀:1♂), *C. camphora* (1♀:0.94♂) and *Cinnamomum camphora* var. *linalooliferum* (1♀:0.78♂). Differences in the longevity of females and males were observed between treatments, with females treated with *C. cassia* oil having lower longevity, while for males the lower longevity was found for *C. cassia* and *C. camphora* oils.

### 3.7.2 Survival rate and life expectancy of *T. absoluta*

The lowest age-stage-specific survival rate ( $S_{xj}$ ) was obtained in the larval stages (Figure 6). *Cinnamomum camphora* caused the highest insect mortality during larval development. However, *C. cassia* oil decreased the total life cycle of insects, resulting in decreased female longevity. Females and males of the treatment with *C. camphora* var. *linalooliferum* oil emerged before the specimens of the other treatments, on the 17th day, because the period from egg to pupa was shorter than in the other treatments.

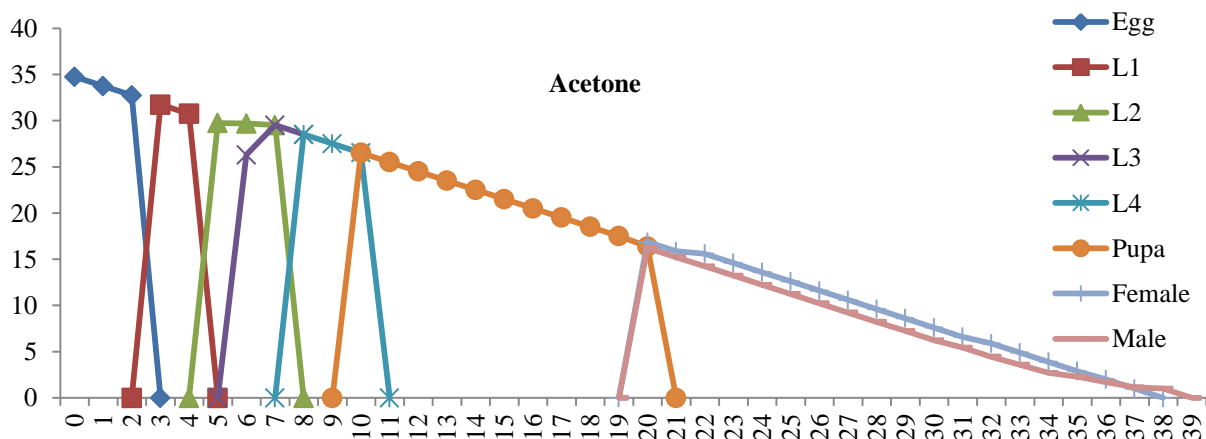


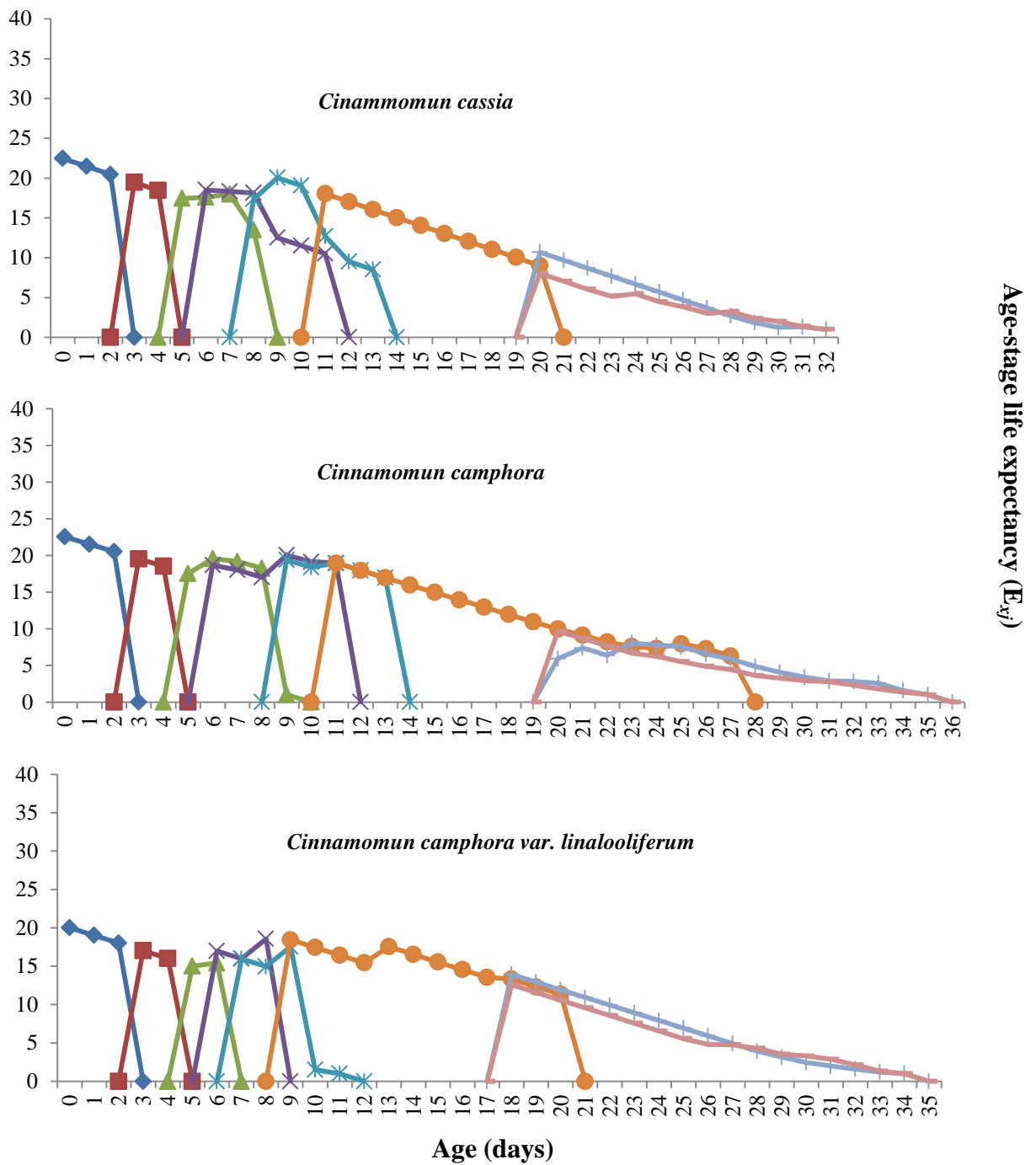




**Figure 6.** Survival rate by age-specific stage ( $S_{xj}$ ) of *Tuta absoluta* in the control treatments (acetone), *Cinnamomum cassia*, *Cinnamomum camphora* and *Cinnamomum camphora* var. *linalooliferum*.

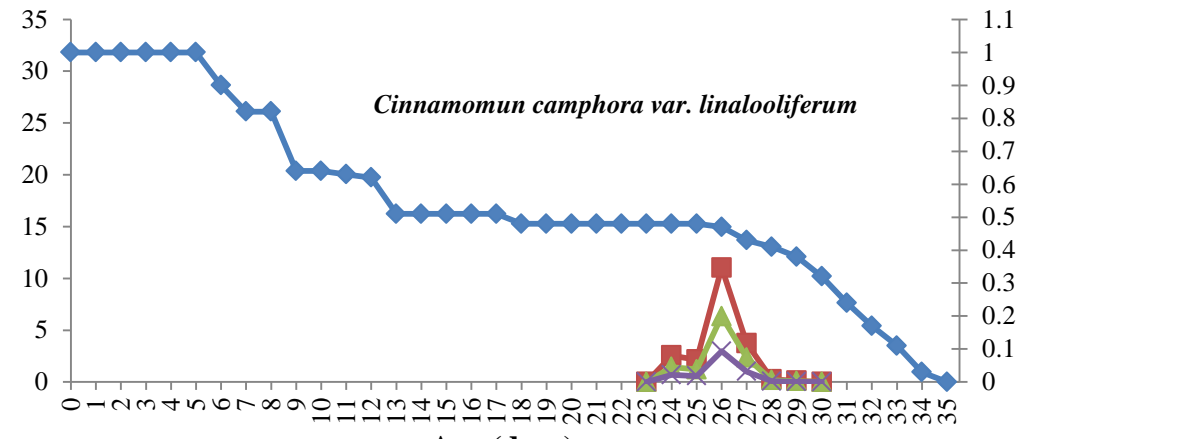
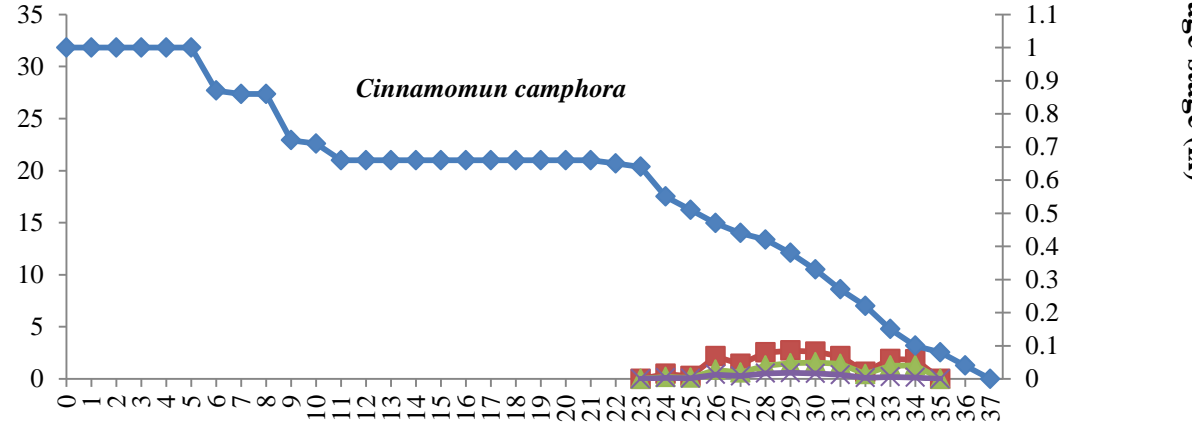
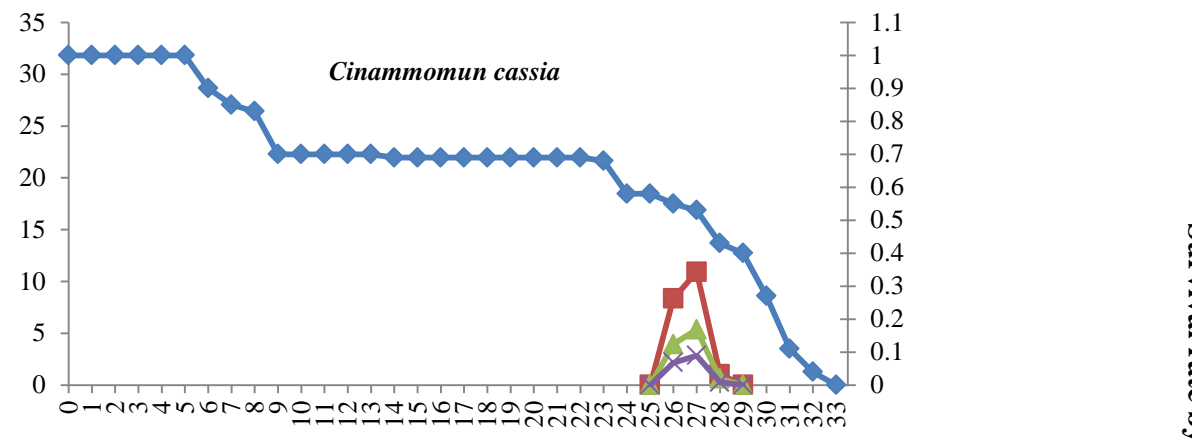
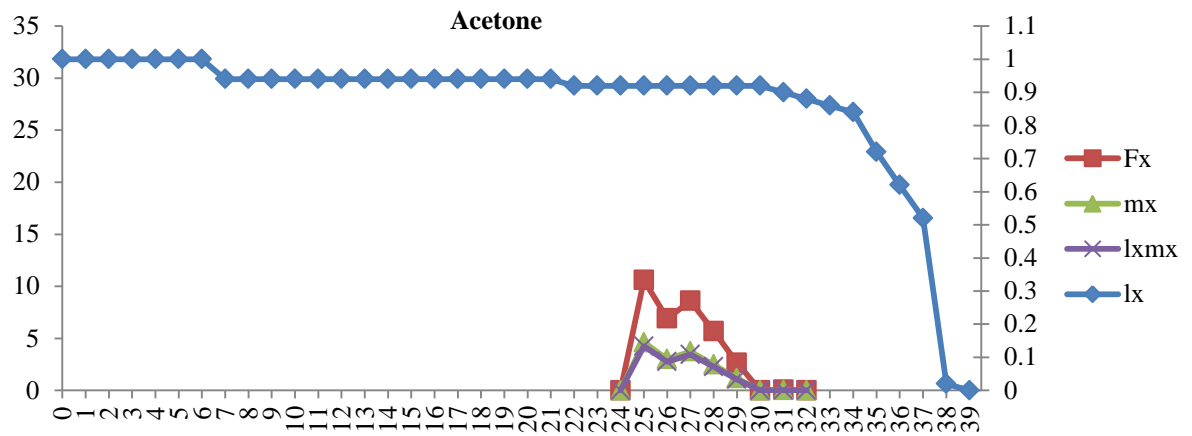
For the control (acetone), *C. cassia*, *C. camphora* and *C. camphora* var. *linalooliferum* the highest age-stage specific life expectancy ( $e_{xj}$ ) was observed for the egg phase (34.74, 22.46, 22.53 and 20.02 days, on day 1, respectively). The oil of *C. camphora* var. *linalooliferum* presented the lowest values for the  $e_{xj}$  parameter at the beginning of each stage of development of *T. absoluta*. For males and females, the lowest life expectancy was observed for *C. camphora* oil. Females had a higher life expectancy in all treatments, with a gradual decline in this parameter in adulthood for both sexes (Figure 7).





**Figure 7.** Life expectancy by age-stage ( $E_{xj}$ ) of *Tuta absoluta* in the control treatments (acetone), *Cinnamomum cassia*, *Cinnamomum camphora* and *Cinnamomum camphora var. linalooliferum*.

The age-specific survival rate ( $lx$ ) is a simplified form of  $S_{xj}$ , used to describe changes in population survival according to age. The curve of this parameter reduced more evidently in the initial stages of development of the pest in the treatment with the *C. camphora var. linalooliferum* oil (Figure 8).



Age (days)

**Figure 9.** Survival rate by specific age-stage ( $l_x$ ), fertility by specific age-stage ( $f_x$ ), fertility by specific age ( $m_x$ ) and maternity by specific age ( $l_x m_x$ ) for *Tuta absoluta* in control treatments (acetone), *Cinnamomum cassia*, *Cinnamomum camphora* and *Cinnamomum camphora* var. *linalooliferum*.

### 3.7.3 Reproductive parameters

The lowest fecundity of *T. absoluta* was observed in the treatment with *C. camphora*. There was an increase in the adult pre-oviposition period (APOP) in the treatments of *C. cassia* and *C. camphora* var. *linalooliferum* and total pre-oviposition period (TPOP) in the treatment with *C. camphora*. Females from parents treated with essential oil of *C. cassia* and *C. camphora* var. *linalooliferum* showed a shorter oviposition period, and the fecundity of these females treated with both oils was reduced. The lowest maximum daily fecundity (MFD) and maximum total fecundity (MTF) were found in treatments with essential oils (Table 6).

**Table 6.** Reproductive parameters of *Tuta absoluta* in different treatments. Maximum daily fertility (MDF), maximum total fertility (MTF), adult pre-oviposition period (APOP) and total pre-oviposition period (TPOP).

Parameters	Control		<i>Cinnamomum cassia</i>		<i>Cinnamomum camphora</i>		<i>Cinnamomum camphora</i> var. <i>linalooliferum</i>	
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE
<b>Total fecundity</b>	42	32.86 ± 1.84 a	26	20.31 ± 2.32 b	31	10.52 ± 1.72 c	27	19.85 ± 1.87 b
<b>Fecundity (E/F)*</b>	40	34.50 ± 1.51 a	26	20.31 ± 2.32 b	20	16.30 ± 1.50 b	27	19.85 ± 1.87 b
<b>Oviposition (days)</b>	40	3.70 ± 0.13 a	26	1.50 ± 0.10 c	20	2.35 ± 0.18 b	27	1.59 ± 0.12 c
<b>APOP (days)</b>	40	5.20 ± 0.10 b	26	6 ± 0.14 a	20	4.40 ± 0.21 c	27	6.30 ± 0.21 a
<b>TPOP (days)</b>	40	25.35 ± 0.09 d	26	26.54 ± 0.10 b	20	28.25 ± 0.50 a	27	25.85 ± 0.17 c
<b>MDF (E/F)</b>	-	35	-	35	-	15	-	42
<b>MTF (E/F)</b>	-	51	-	46	-	27	-	42

\*Total of females that oviposited.

Averages on the same line followed by different letters are significantly different with  $p < 0.05$ .

Differences between treatments were obtained using the Bootstrap test paired with 100.000 replicates.

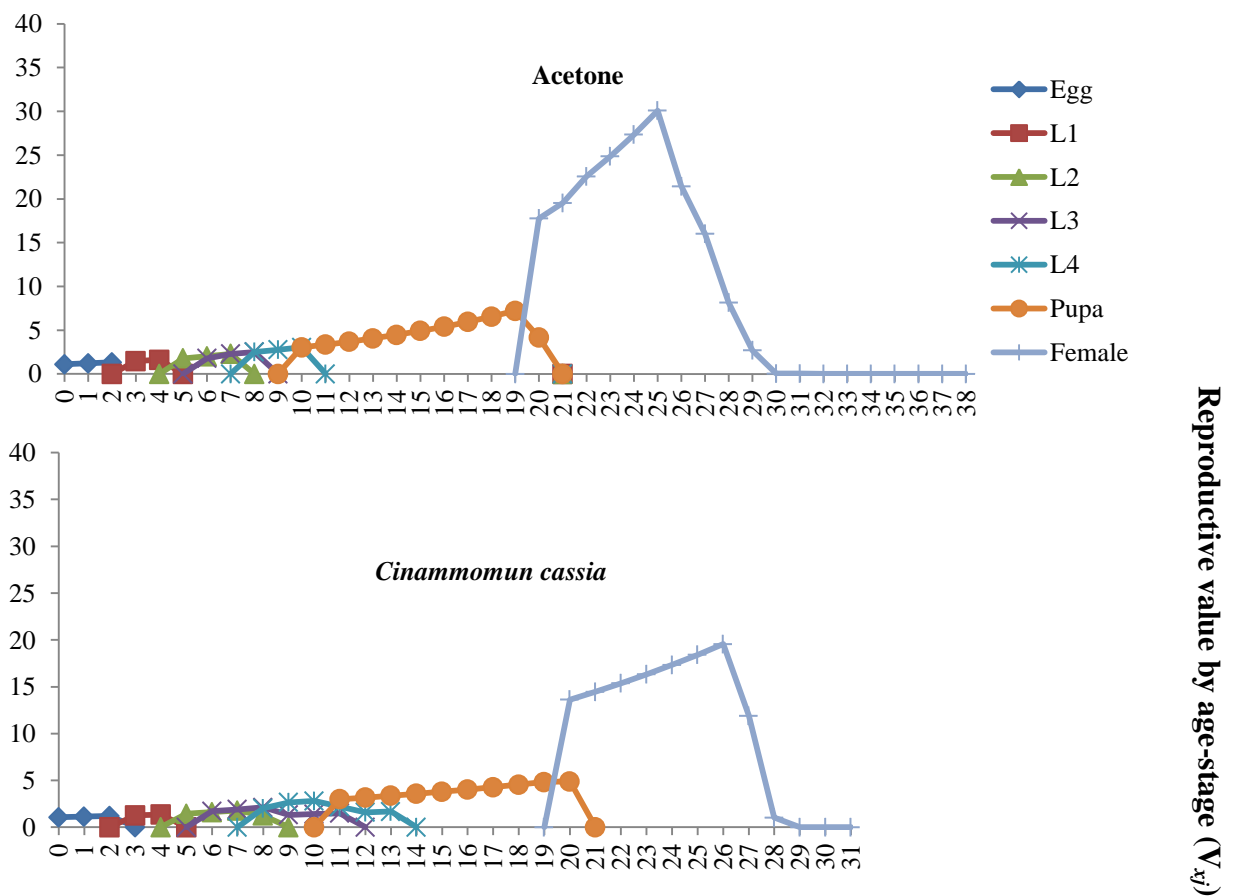
(E/F) = eggs per female.

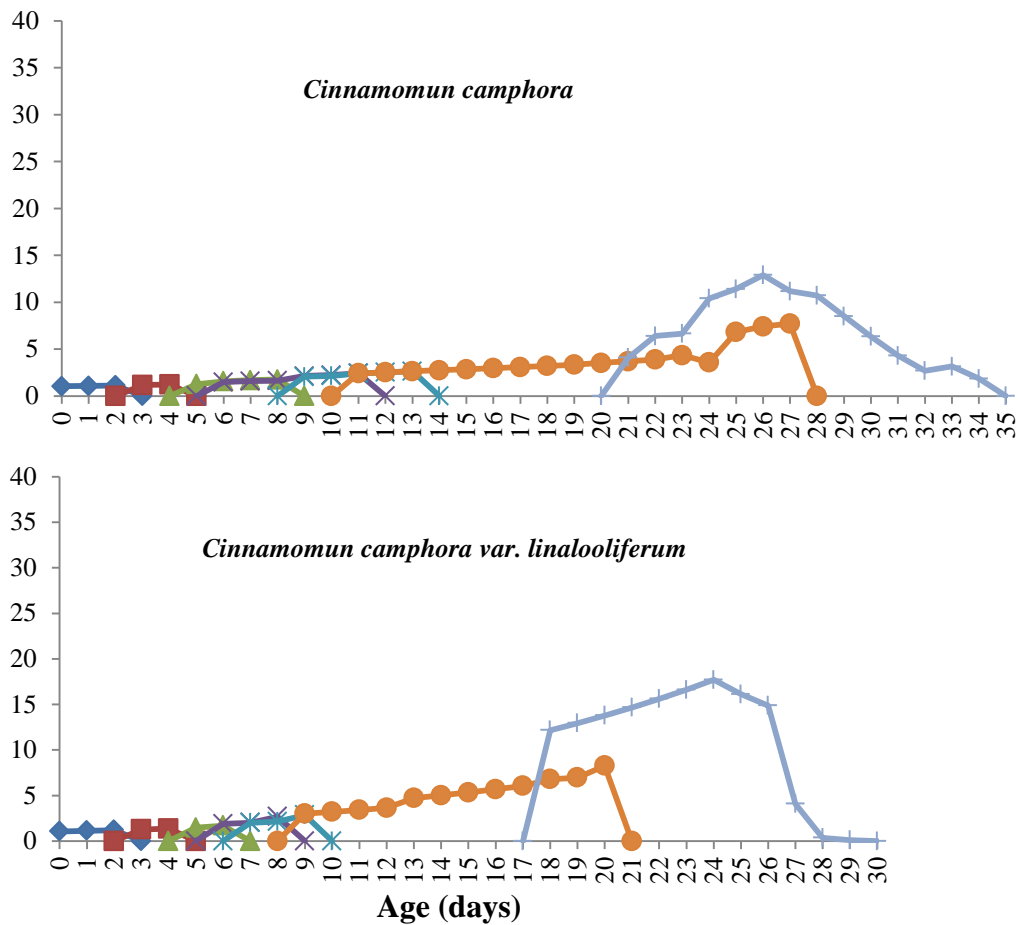
N = number of specimens for each parameter.

Through the parameter  $f_{xj}$  it was possible to visualize when the first oviposition occurred, the peak and last fecundity event, in addition to the average daily number of eggs per female (E/F). After the peak of fertility there was a gradual decline in this parameter, indicating a decrease in the number of offspring produced per female with increasing age,

except for treatment with *C. camphora*, in which no peak was observed, but instead a small increase in fertility from the 25th to the 35th day, a period near the end of the pest's life cycle (Figure 9).

The age-stage specific reproductive value ( $v_{xj}$ ) does not consider the presence of males. In the control treatment (acetone), the population growth period occurred from the 19th to the 30th day, when the highest values of  $v_{xj}$  occurred between the 22nd and 26th day, with a reproductive peak on the 25th day. For *C. cassia* essential oil, the population growth period occurred from the 19th to the 29th days, and the highest values of  $v_{xj}$  were verified between the 24th and 27th days, with a population peak on the 26th day. In the treatment based on essential oil of *C. camphora*, the period of population growth was observed between the 20th and 35th days, with higher values of  $v_{xj}$  between 24th and 28th days, with a peak on the 26th day. For the treatment with *C. camphora* var. *linalooliferum* essential oil the period of population growth was observed between the 17th and 30th days, with the highest values of  $v_{xj}$  between 22nd and 26th days, with a peak on the 24th day (Figure 10).





**Figure 10.** Reproductive value by age-stage ( $v_{xj}$ ) of *Tuta absoluta* in the control treatments (acetone), *Cinnamomum cassia*, *Cinnamomum camphora* and *Cinnamomum camphora* var. *linalooliferum*.

### 3.7.4 Population parameters

Regarding the intrinsic rate of increase ( $r$ ) and finite rate of increase ( $\lambda$ ) the highest values were found for the control (acetone), followed by *C. camphora* and *C. camphora* var. *linalooliferum*. *C. cassia* oil did not differ from *C. camphora* and *C. camphora* var. *linalooliferum*. For the net reproductive rate ( $R_0$ ), only the control obtained a higher value and the other treatments did not differ significantly from each other. As for the mean generation time ( $T$ ), the highest values were found for *C. camphora*, followed by the control and *C. cassia* oil that did not differ from each other, and the shortest time was found for *C. camphora* var. *linalooliferum*. This result indicates that the progeny from parents treated with *C. camphora* needs 2.47 and 3.06 days more than the progeny from parents of the control and *C. camphora* var. *linalooliferum* treatments, respectively, to complete a generation (Table 7).

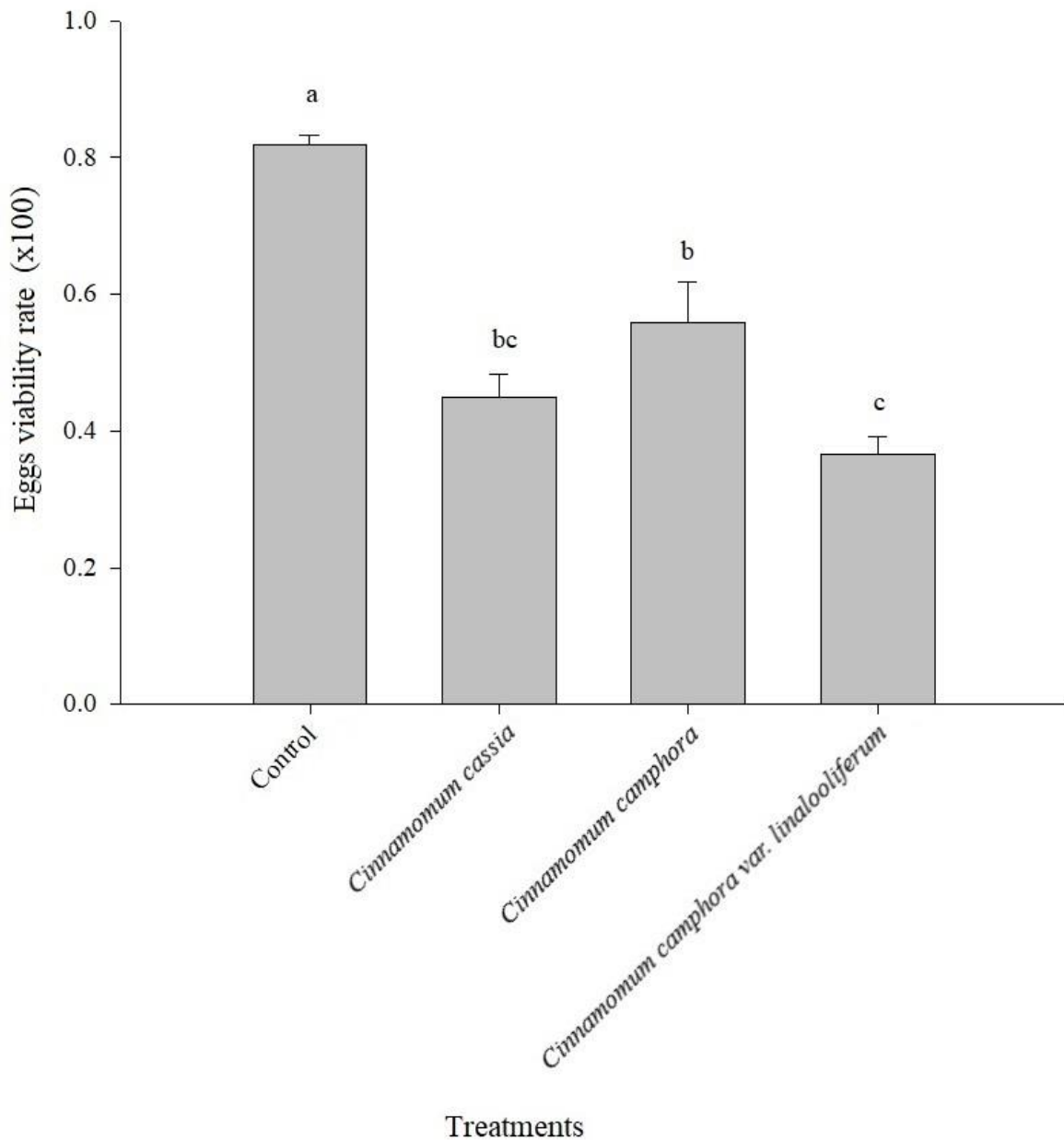
**Table 7.** Population parameters of *Tuta absoluta* treated with essential oils from plants of the genus *Cinnamomum* spp.

Demographic parameters	Control Mean ± SE	<i>Cinnamomum</i> <i>cassia</i> Mean ± SE	<i>Cinnamomum</i> <i>camphora</i> Mean ± SE	<i>Cinnamomum</i> <i>camphora</i> var. <i>linalooliferum</i> Mean ± SE
<b>Intrinsic growth rate (r)</b>	0.10 ± 0.004 a	0.06 ± 0.008 bc	0.04 ± 0.008 c	0.06 ± 0.007 b
<b>Finite growth rate (λ)</b>	1.10 ± 0.005 a	1.06 ± 0.008 bc	1.04 ± 0.008 c	1.06 ± 0.007 b
<b>Net reproduction rate (R0)</b>	13.8 ± 1.79 a	5.28 ± 1.07 b	3.26 ± 0.72 b	5.36 ± 1.01 b
<b>Average generation time (T)</b>	27.43 ± 0.07 b	27.63 ± 0.11 b	29.90 ± 0.54 a	26.84 ± 0.22 c

Averages on the same line followed by different letters are significantly different with  $p < 0.05$ . Differences between treatments were obtained using the Bootstrap test paired with 100.000 replicates.

### 3.7.5 Viability of eggs and survival of first instar caterpillars

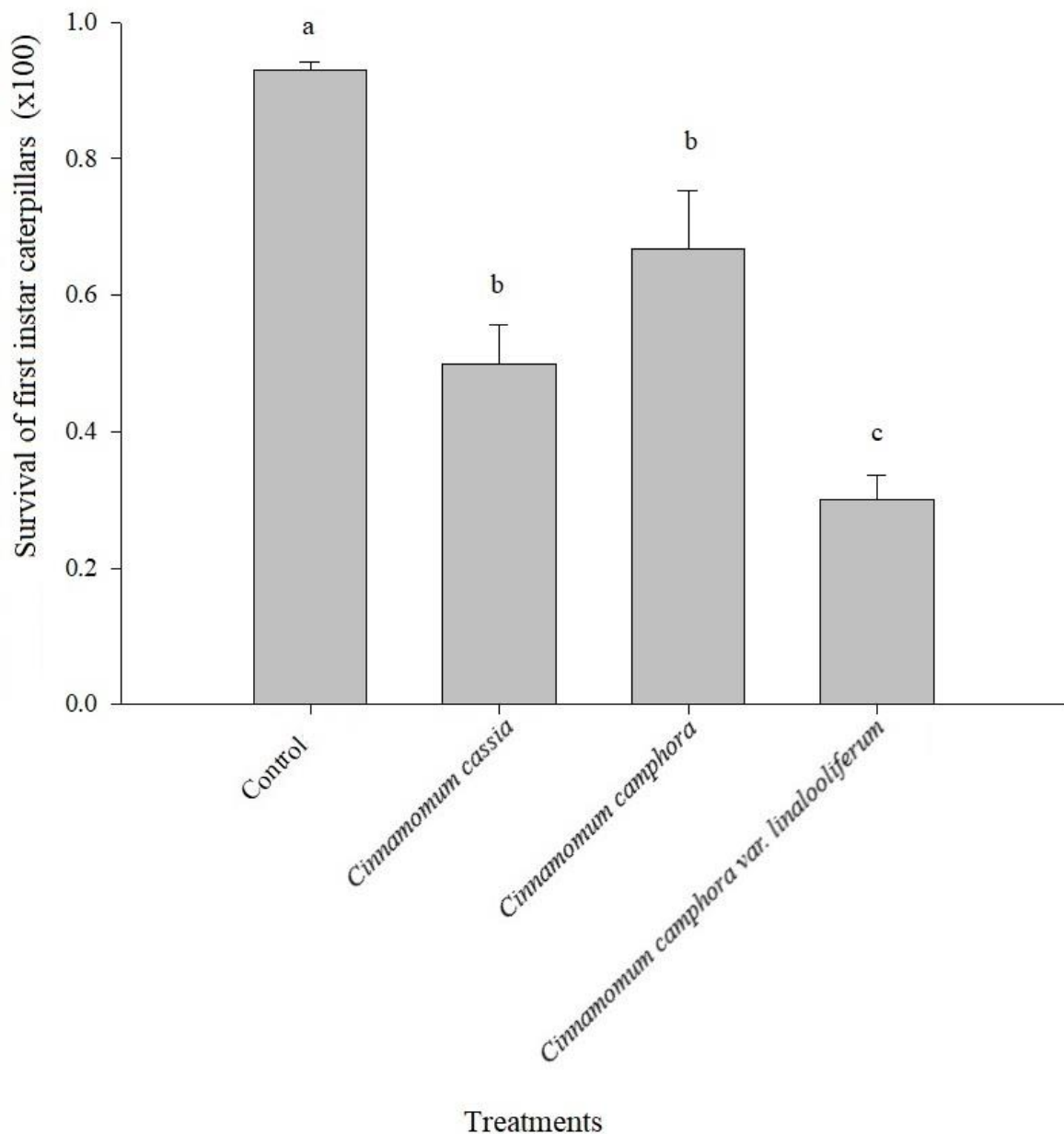
The second generation from parents treated with essential oils and acetone were evaluated for the viability of eggs and survival of first instar caterpillars. For eggs from acetone treatment, viability of 82% was verified, differing from the other treatments ( $F = 51.12$ ; d.f. = 4;  $p < 0.05$ ). In the treatment with the oil of *C. camphora* the viability of the eggs was 56% and of *C. camphora* var. *linalooliferum* was 37%. The essential oil of *C. cassia* presented 45% of viable eggs, not differing from the oils of *C. camphora* and *C. camphora* var. *linalooliferum* (Figure 11).



**Figure 11.** Viability of *Tuta absoluta* eggs from parents treated with essential oils of *Cinnamomum cassia*, *Cinnamomum camphora* and *Cinnamomum camphora* var. *linalooliferum* and with acetone (control).

Survival was also assessed for caterpillars that reached the second instar. It was found that 93% of caterpillars from parents treated with acetone only reached the second instar ( $F = 45.32$ ; d.f. = 4;  $p < 0.05$ ). No differences were found in the average survival of first instar caterpillars treated with *C. cassia* and *C. camphora* oils, with averages of 50 and 77% survival. The lowest survival was found for caterpillars from parents treated with the *C. camphora* var. *linalooliferum* oil, with only 30% of the insects reaching the second instar (Figure 12).

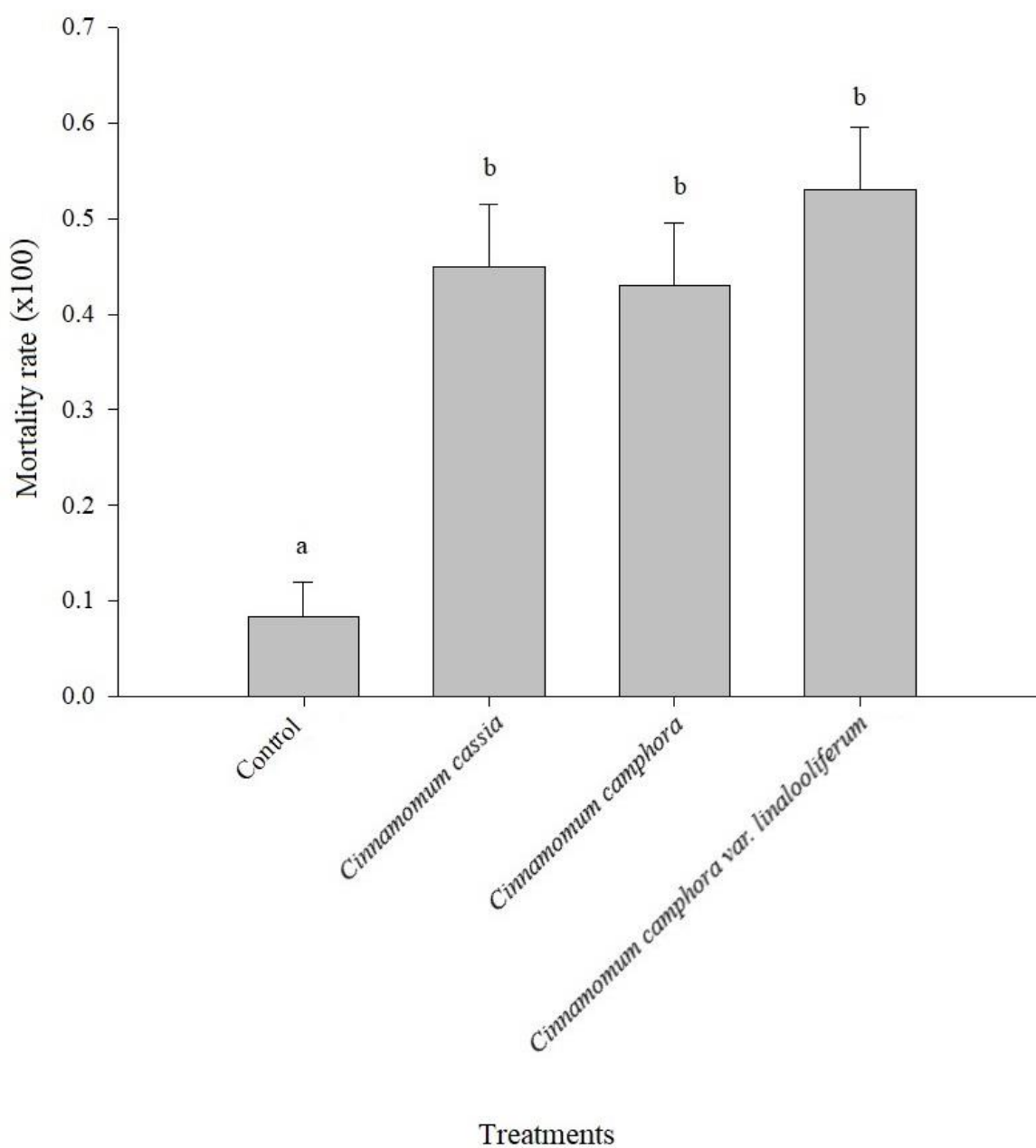




**Figure 12.** Survival of first instar caterpillars of *Tuta absoluta* from parents treated with essential oils of *Cinnamomum cassia*, *Cinnamomum camphora* and *Cinnamomum camphora* var. *linalooliferum* and with acetone (control).

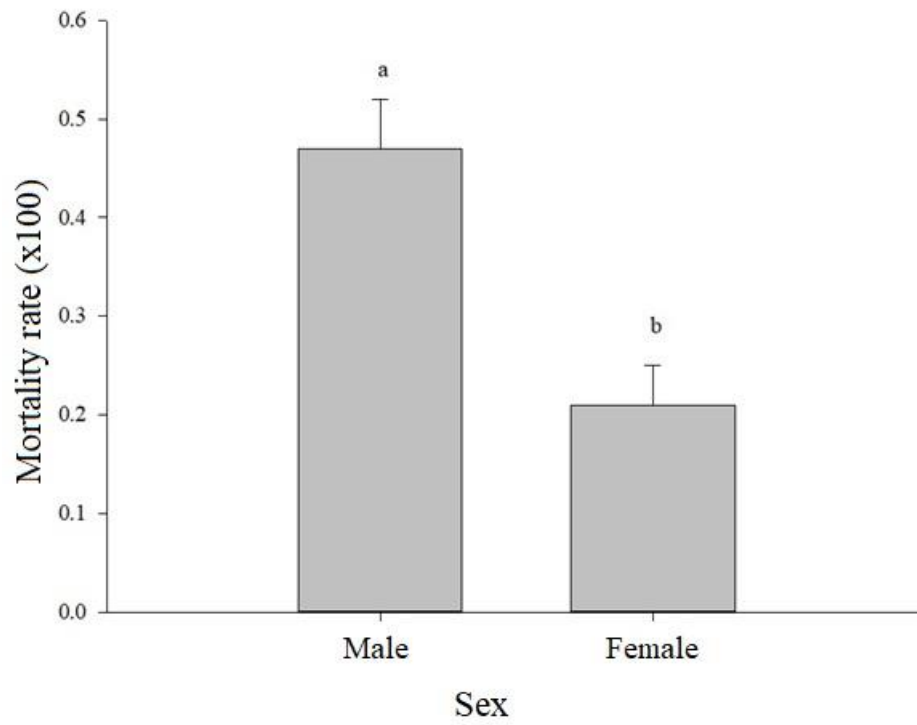
### 3.8 Toxicity of essential oils to *M. basicornis*

72 h after the application of essential oils in the LD<sub>50</sub> for *T. absoluta*, the predator mortality rate was evaluated. The lowest mortality rate was observed for the treatment consisting only of acetone. The treatments with the essential oils of *C. camphora*, *C. cassia* and *C. camphora* var. *linalooliferum* had an intermediate mortality rate with averages of 43%, 45% and 53%, respectively ( $F = 37.7$ ; d.f. = 3;  $p < 0.05$ ) (Figure 13).



**Figure 13.** Adult mortality of *Macrolophus basicornis* 72 h after application of the LD<sub>50</sub> of essential oils of *Cinnamomum cassia*, *Cinnamomum camphora* and *Cinnamomum camphora* var. *linalooliferum* determined for *Tuta absoluta* and acetone (control).

In this experiment there was also a difference in mortality between females and males of *M. basicornis* with the application of essential oils. Males had a 47% mortality rate while females had a 21% mortality rate ( $F = 16.24$ ; d.f.= 1;  $p < 0.05$ ) (Figure 14).



**Figure 14.** *Macrolophus basicornis* male and female mortality 72 h after application of the LD<sub>50</sub> of essential oils of *Cinnamomum cassia*, *Cinnamomum camphora* and *Cinnamomum camphora* var. *linalooliferum* determined for *Tuta absoluta*.

#### 4. DISCUSSION

There are several records of the inefficiency of control of *T. absoluta* with insecticides in Brazil. The indiscriminate use of chemical products has led to the selection of resistant populations, since in some producing regions tomato growers carry out 20 insecticide applications per crop cycle, not considering the action thresholds and, in most cases, with calendar applications, i.e., regardless of the presence or absence of the pest (GUEDES; PICANÇO, 2012).

The resistance of *T. absoluta* populations to the chemical group of organophosphates has been reported in Iran, Greece, Italy, Brazil, Argentina and Chile (ZIMBAEE et al., 2018; RODITAKIS; SKARMOUTSOU; STAURAKAKI, 2013; HADDI et al., 2017; LIETTI; BOTTO; ALZOGARAY, 2005), since products from this chemical group have been used for several decades causing high selection pressure and, in many cases, are not considered selective to natural enemies of *T. absoluta*. Moradi et al. (2019) and Haddi et al. (2017) described that in resistant lepidopteran pests there is a conformational change of the binding site, weakening it and preventing the action of the insecticide Chlorpyrifos. These results corroborate those of the present study, in which the connection between Chlorpyrifos and its site of action may have not occurred, since there was low mortality from insects, even when the compound was applied at twice the recommended dose.

Since the 1990s, resistance to cartap hydrochloride has been studied in Brazil, as it is the pioneer insecticide in *T. absoluta* control and is widely used (SIQUEIRA; GUEDES; PICANÇO 2000). The population of *T. absoluta* exposed to the recommended dose of cartap hydrochloride in this study showed low mortality, but when twice the recommended dose was applied, there was a significant increase in the mortality of this pest. Resistance levels from 1.5 to 6.4 times for this insecticide were verified in several tomato producing regions in Brazil, emphasizing the importance of rotating chemicals with different modes of action to avoid the selection of resistant populations (SILVA et al., 2016).

Pyrethroid insecticides were commercially registered for the control of *T. absoluta* in the 1980s and twenty years after their use, the first records of resistant populations of this pest were reported (SIQUEIRA; GUEDES; PICANÇO 2000). Although in Brazil, pyrethroids are no longer widely used to control *T. absoluta*, they are still used by small producers due to their low prices and lower toxicity to mammals. In addition to reducing efficiency, the intensive use of these products can accelerate the selection of *T. absoluta* resistance since they

have a broad spectrum of action and are very toxic to natural enemies, which are able to indiscriminately prey or parasitize susceptible and resistant pest specimens.

The results of low mortality of this pest under application of fenpropathrin obtained in the present study corroborate those of Silva et al. (2015), in which the application of products from the pyrethroids group proved to be ineffective in the control of *T. absoluta*, suggesting that tolerance to pyrethroids is present in several populations of this pest in Brazil (HADDI et al., 2017).

Spinosyns are a chemical group of insecticides formulated through the fermentation of the soil bacterium *Saccharopolyspora spinosa* (Mertz and Yao, 1990) (Actinomycetes: Pseudonocardiales) and is registered for several agricultural crops under organic cultivation in Brazil. Spinosyns were recently introduced in Brazil and due to its exclusive mode of action, competing with the neurotransmitter acetylcholine in the production of successive stimuli, causing hyperexcitability and collapse of nerve cells and leading to insect death, it causes high mortality rates. Thus, it has also caused high mortality of insect populations resistant to analogous compounds (RACKE, 2007). In the present study, Spinosad caused a high mortality of *T. absoluta* caterpillars, demonstrating the susceptibility of the evaluated population. Campos et al. (2014) observed that under spinosyn selection pressure, the selection of resistant tomato moth populations occurred quickly. However, with the removal of selection pressure, susceptibility was restored in just eight generations. This fact shows a high relationship between susceptibility to spinosyns and the number of sprays received by populations at field level (REYES et al., 2012).

In comparison with synthetic chemical insecticides, essential oils can be less harmful to the environment, because they degrade more quickly and because they are less toxic to natural enemies, which delays the appearance of resistant insect populations. However, for a product to be used in pest control, its toxicity and safety must be evaluated (HADDI et al., 2020; PAVELA; BENELLI, 2016).

This is the first study regarding the toxicity of essential oils from plants of the Lauraceae family against *T. absoluta* and its effects on the predator *M. basicornis*. However, studies evaluating the toxicity of essential oils from plants of other botanical families for this pest are found in the literature.

In a study by Campolo et al. (2017) the toxicity of citrus oils to *T. absoluta* was evaluated. The oils were efficient, and when emulsified in nanoformulations they caused higher mortality of this insect. In another study, the essential oil of *Carum copticum*

(Apiaceae) had a toxic effect on *T. absoluta* and its major compound thymol caused greater mortality of this insect in its immature stage (PIRI et al., 2020).

The insecticidal activity of the essential oils of the Lauraceae family may be related to their major compounds such as aldehydes (cinnamaldehyde), phenols and alcohols (LI; KONG; WU, 2013). In the present study, lethal and sublethal activities of the three essential oils evaluated were demonstrated. The essential oil of *C. cassia* has cinnamaldehyde (84.21%) as a major compound (Table 6), corroborating with other studies which proved that high levels of this compound in the oil are responsible for the greater insecticidal activity (JEON et al., 2017; LIU et al., 2014). The mechanism of action of cinnamaldehyde is associated with the formation of Schiff bases with membrane proteins through reaction with the free carbonyl group. Schiff's bases can destroy cell membranes and prevent the transport of substances across the plasma membrane, in addition to ceasing breathing and inducing starvation, causing the death of the insect (GONZÁLEZ-AGUILAR et al., 2011; JEON et al., 2017). These observations are in accordance with the results found in the present study, where both *C. cassia* oil and the major isolated compound, cinnamaldehyde, caused a decrease in the leaf consumption of *T. absoluta* caterpillars and reduced their total life cycle.

It was found that *T. absoluta* caterpillars treated with *C. camphora* oil had shorter longevity. The main constituent of this oil is 1.8-cineole (66.74%), also known as eucalyptol (LEE et al., 2006; ISMAN, 2015). The essential oil of *C. camphora* presents a mixture of several molecules with modes of action that are still unknown (HADDI et al., 2020). Some authors have already reported the insecticidal activity of this oil to other insects, and the toxicity has been related to other major compounds. Qualitative and quantitative variations in the chemical composition of essential oils can occur due to numerous factors, such as cultivation method, part of the plant used for extraction, influence of the genotype used, geographic distribution of the plant, type of soil in which it was cultivated, climatic conditions and level of stress, which affect the physiology of the plant (GUO et al., 2016).

Chen et al. (2014) found that the oil extracted from *C. camphora* leaves showed high toxicity when applied directly to the tobacco beetle *Lasioderma serricornis* (Fabricius, 1792) (Coleoptera: Anobiidae). The insecticidal activity of the essential oil of *C. camphora* extracted from the bark, stem, leaves and fruits is known to control stored grain pests, showing fumigating activity and causing mortality when applied directly to beetles (GUO et al., 2016). The oil of *C. camphora* was evaluated for the control of insect vectors of human diseases. Xu et al. (2020) concluded that the essential oil of *C. camphora* had great potential

in the control of *Anopheles stephensi* (Liston, 1901) (Diptera: Culicidae), the main vector of malaria in Asia.

The oil of *C. camphora* var. *linalooliferum* has linalool as a major compound (98.75%). This monoterpene acts in the competitive inhibition of the enzyme acetylcholinesterase (LÓPEZ; PASCUAL-VILLALOBOS, 2010), prevents the degradation of the acetylcholine neurotransmitter, causing hyperexcitation of movements due to the excessive nerve impulses, resulting in difficulty to breathe and feed (LÓPEZ; PASCUAL-VAL, 2015). In the present study, *T. absoluta* caterpillars treated with this essential oil at a concentration of 10 mg.mL<sup>-1</sup> showed a LT<sub>50</sub> of 11.5 h. This rapid action is possibly related to effects on the nervous system and, for this reason, there was a low leaf consumption of *T. absoluta* caterpillars treated with the major compound linalool.

There are studies in the literature that report the effect of linalool against stored grain pests. Kheloul et al. (2020) found that *Tribolium confusum* larvae (Jacquelin du Val, 1863) (Coleoptera: Tenebrionidae) treated with this compound showed low survival and reduced emergence of adult insects. Kamanula et al. (2017) evidenced the contact toxicity of this molecule for the maize weevil, *Sitophilus zeamais* (Motschulsky, 1855) (Coleoptera: Curculionidae), to which 10 mg.mL<sup>-1</sup> of linalool caused 100% mortality 48 h after the adult insects were treated.

Bordin et al. (2021) found in fumigation tests that the essential oils of *C. cassia* and *C. camphora* var. *linalooliferum* caused mortality of 96% and 61% of the parasitic poultry red mite *Dermanyssus gallinae* (De Geer, 1778) (Acari: Dermanyssidae); however, when the major compounds were tested pure, they caused less mortality, indicating that the insecticidal activity occurs due to several constituents of the oil mixture and not just from the major compound.

It was observed that insects treated with LD<sub>50</sub> of the three essential oils (*C. cassia*, *C. camphora* e *C. camphora* var. *linalooliferum*) had shorter life cycles compared to the control; because of this, females had reduced fecundity and oviposition period duration. Essential oils also reduced the variables of demographic parameters, such as the intrinsic rate of increase, finite rate of increase and the basic reproduction rate. Younes, Zohdy and Fathy (2018) evaluated two microbial biopesticides in the population parameters of *T. absoluta*, in which both, the bacterium *Bacillus thuringiensis* subsp. *kurstaki* and the entomopathogenic fungus *Beauveria bassiana* negatively affected the life cycle and demographic parameters of *T. absoluta*, in addition to having an acute effect on this insect pest.

In the literature, studies that evaluated only the lethal effect of botanical insecticides for insect pests are common; however, the analysis of the toxicity of compounds in underdoses through life tables can show the effects on biological parameters and population growth of insects (STARK; BANKS, 2003). The results obtained in the present study corroborate those of Kandil; Abdel-Kerim and Moustafa (2020), who underdosed two bioinsecticides from *Bacillus thuringiensis* on *T. absoluta* caterpillars and verified, through the life table, a reduction in the development of this insect. Another study also found lower fertility rates and shorter duration of the biological cycle of *T. absoluta* caterpillars with underdoses of the insecticide acaricide abamectin (ZIBAEE; ESMAEILY, 2017).

Although there are many essential oils evaluated for the control of arthropod pests, there are few studies of their effects on tomato moth. In addition, few studies have evaluated the toxicity of new bioinsecticides to natural enemies (DESNEUX; DECOURTYE; DELPUECH. 2007). In the present work, the toxicity of the essential oils of *C. cassia*, *C. camphora* and *C. camphora* var. *linalooliferum* for the predator *M. basicornis* was evaluated because natural enemies can be highly sensitive depending on the dose, exposure time and product type (BIONDI et al., 2013). It was observed that the LD<sub>50</sub> of the essential oils estimated for *T. absoluta* caused intermediate mortality of *M. basicornis* and that the males were more negatively affected by the oils than the females. Mesak (2020) found that females of *M. basicornis* treated with the insecticide flubendiamide had a higher life expectancy than males. Soares et al. (2019) found that the botanical insecticide Prev-Am® applied at half the concentration was able to repel *T. absoluta* and keep the predator *Nesidiocoris tenuis* (Reuter, 1895) (Hemiptera: Miridae) in the environment, demonstrating compatibility between biological control and the use of botanical products. New studies are necessary to generate information that allows the integration of biological and chemical control methods, in the search for more toxic formulations for the pest and selective for natural enemies.



## 5. CONCLUSION

*T. absoluta* population evaluated is tolerant to synthetic insecticides and susceptible to the essential oils of *C. cassia*, *C. camphora* and *C. camphora* var. *linalooliferum*. The essential oils studied are toxic to the tomato moth, as it reduces the duration of the life cycle and the ability to leave descendants of this insect pest. The major components cinnamaldehyde and linalool from the oils of *C. cassia* and *C. camphora* var. *linalooliferum* are responsible for the insecticidal activity against *T. absoluta*, however further studies are needed to understand its mode of action. All essential oils tested in this study demonstrate high potential to be used in the management of *T. absoluta*, since they presented high toxicity to this pest and an intermediate effect of up to 50% mortality of the predator *M. basicornis*.

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