

***Cinnamomum camphora* var. *linaloolifera* essential oil on pest control: Its effect on *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae)**

Óleo essencial de *Cinnamomum camphora* var. *linaloolifera* no controle de pragas: Seu efeito sobre *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae)

Aceite esencial de *Cinnamomum camphora* var. *linaloolifera* en el control de plagas: Su efecto sobre *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae)

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Camila Bonatto Vicenço

ORCID: <https://orcid.org/0000-0003-0713-7707>
University of Caxias do Sul, Brazil
E-mail: cbvicenc@ucs.br

Wendel Paulo Silvestre

ORCID: <https://orcid.org/0000-0002-9376-6405>
University of Caxias do Sul, Brazil
E-mail: wpsilvestre@ucs.br

Gabriel Fernandes Pauletti

ORCID: <https://orcid.org/0000-0001-9850-3682>
University of Caxias do Sul, Brazil
E-mail: gfpaulet@ucs.br

Neiva Monteiro de Barros

ORCID: <https://orcid.org/0000-0002-6748-3428>
University of Caxias do Sul, Brazil
E-mail: nmbarros@ucs.br

Joséli Schwambach

ORCID: <https://orcid.org/0000-0001-6785-4164>
University of Caxias do Sul, Brazil
E-mail: jschwambach@ucs.br

Abstract

This study evaluated the insecticidal effect of *Cinnamomum camphora* var. *linaloolifera* essential oil and its major compound, linalool, on *Trialeurodes vaporariorum*. Essential oil treatments were applied at 0.5, 1.0, 1.5, and 2.0% v/v on eggs, nymphs, and adult individuals and linalool equivalent concentrations on nymphs of *T. vaporariorum*. The negative controls water and Tween-80[®] (0.5% v/v) and a positive control (spiromesifen 0.05% v/v for eggs and nymphs; pymetrozine 0.04% m/v for adults) were also used. The essential oil of *C. camphora* at 2.0% v/v caused egg mortality of 49.7%; same concentration caused the highest nymph mortality (88.5%). For adults, the essential oil at 2.0% v/v caused the highest mortality (40.0%) after 48 h, not changing from 48 to 72 h. The essential oil and linalool performed similarly relative to the nymphs, whose mortalities have not differed statistically, with exception of the concentrations of 0.5 and 1.0% v/v. Regarding the chemical control, the concentration of 2.0% v/v has had similar mortality for nymphs; for eggs and adults, the essential oil caused lower mortality (49.4 and 40.0%) than the synthetic pesticide (65.0 and 72.0%). The essential oil of *C. camphora* may be a potential control alternative for *T. vaporariorum*, especially regarding the Integrated Pest Management (IPM) practices.

Keywords: Alternative control; Greenhouse whitefly; Linalool; Terpenes; Bioinsecticide.

Resumo

Este trabalho avaliou o efeito inseticida do óleo essencial de *Cinnamomum camphora* var. *linaloolifera* e seu compostos majoritário, o linalol, sobre *Trialeurodes vaporariorum*. Tratamentos contendo óleo essencial nas concentrações de 0,5; 1,0; 1,5 e 2,0% v/v foram aplicados sobre ovos, ninfas e indivíduos adultos de *T. vaporariorum*. Os controles negativos foram água e Tween-80[®] (0,5% v/v) mais um controle positivo (espiromesifeno 0,05% v/v para ovos e ninfas; pimetozina 0,04% m/v para adultos) foram também usados. O óleo essencial de *C. camphora* a 2,0% v/v causou mortalidade de ovos de 49,7%; a mesma concentração causou a maior mortalidade de ninfas (88,5%). Para os adultos, o óleo essencial a 2,0% v/v causou a maior porcentagem de mortalidade (40,0%) após 48 h, não se alterando entre 48 e 72 h. O óleo essencial e o linalol tiveram desempenho semelhante com relação às ninfas, cujas mortalidades não diferiram estatisticamente, com exceção das concentrações de 0,5 e 1,0% v/v. Em relação ao controle químico, a concentração de 2,0% v/v apresentou mortalidade similar para ninfas; para ovos e adultos, o óleo essencial causou menores mortalidades (49,4 e 40,0%) do que o pesticida sintético (65,0 e 72,0%). O óleo essencial de *C. camphora*

pode ser uma alternativa de controle para *T. vaporariorum*, especialmente tendo em vista as práticas do Manejo Integrado de Pragas (MIP).

Palavras-chave: Controle alternativo; Mosca-branca-de-casa-de-vegetação; Linalol; Terpenos; Bioinseticida.

Resumen

Este trabajo evaluó el efecto insecticida del aceite esencial de *Cinnamomum camphora* var. *linaloolifera* y su compuesto principal, linalool, en *Trialeurodes vaporariorum*. Tratamientos que contienen aceite esencial en concentraciones de 0,5; 1,0; Se aplicaron 1,5 y 2,0% v/v a huevos, ninfas e individuos adultos de *T. vaporariorum*. Los controles negativos fueron agua y también se utilizó Tween-80® (0,5% v/v) más un control positivo (espiromesifeno 0,05% v/v para huevos y ninfas; pimetozina 0,04% p/v para adultos). El aceite esencial de *C. camphora* al 2,0% v/v provocó una mortalidad de huevos del 49,7%; la misma concentración causó la mayor mortalidad de ninfas (88,5%). Para los adultos, el aceite esencial al 2,0% v/v causó el mayor porcentaje de mortalidad (40,0%) después de 48 h, y no cambió entre 48 y 72 h. El aceite esencial y el linalol se comportaron de manera similar en relación a las ninfas, cuyas mortalidades no difirieron estadísticamente, con la excepción de concentraciones de 0,5 y 1,0% v/v. En relación al control químico, la concentración de 2,0% v/v presentó similar mortalidad para ninfas; para huevos y adultos, el aceite esencial causó una menor mortalidad (49,4 y 40,0%) que el plaguicida sintético (65,0 y 72,0%). El aceite esencial de *C. camphora* puede ser un control alternativo para *T. vaporariorum*, especialmente considerando las prácticas de Manejo Integrado de Plagas (MIP).

Palabras clave: Control alternativo; Mosca blanca de invernadero; Linalol; Terpenos; Bioinsecticida.

1. Introduction

Trialeurodes vaporariorum (Westwood, 1856) is considered the main pest in ornamental and vegetables in protected cultivations (Lourenção et al., 2008). More than 250 species of plants are susceptible to this insect (Zapata et al., 2016), whose attacks cause economic losses to the agricultural sector (CABI, 2017).

T. vaporariorum feeds on sap (phloem) by introducing its buccal apparatus in plant leaves, causing mechanical damage to the plant tissues. The damage is characterized by localized spots and yellowing and fall of leaves; if the infestation reaches critical levels, wilting and reduction of the growth of the host plants may also be seen (Capinera, 2008).

Most of the phloem ingested is not metabolized by *T. vaporariorum*, being excreted as honeydew, a sugary substance rich in carbohydrates (Henneberry et al., 1999). The honeydew covers fruits and leaves and serves as food to a saprophyte fungus, rendering the plant surface blackish and sticky (Perring et al. 2018). This indirect damage fosters the development of a fungus (*Capnodium* sp.), which harms the leaves' physiological processes, causing them to wilt and fall, hastening the development cycle of the cultivation (Barbosa et al., 2002). *T. vaporariorum* may also act as a vector in transmitting viral diseases, which causes severe damage and production loss in several kinds of vegetables (Capinera, 2008).

This insect's management is carried out mainly by using synthetic pesticides (Michereff Filho & Inoue-Nagata, 2015). In general, large-spectrum pesticides are used without considering the negative impacts that come from the excessive use of chemical control because, in many cases, several applications are necessary, which results in excessive use of synthetic molecules. Hence, pesticide-resistant individuals of *T. vaporariorum* are continuously selected (Erdogan et al., 2012; Ovčarenko et al., 2014).

The integration of several control techniques may be useful in the reduction of *T. vaporariorum* infestations. Natural products, such as plant extracts and essential oils, may be used as tools to help control this pest. Essential oils could be considered as large-spectrum pesticides (have several modes of action) and low risk (due to the quick volatilization and limited field permanence) (Koul et al., 2008). Unlike synthetic pesticides, essential oils may act by several mechanisms, reducing the probability of the individuals becoming resistant due to the diverse composition of active compounds in essential oils (Pavela & Benelli, 2016).

Essential oils are complex mixtures of naturally occurring compounds, which are secondary metabolites of plant metabolism and may contain more than 300 compounds at different concentrations (Sell, 2006). Several factors determine the chemical composition of essential oils, both biotic and abiotic (luminosity, water stress, attack by pests and pathogens), which

may be correlated (Morais, 2009; Pandey et al., 2014; Campos et al., 2019; Silvestre et al., 2019).

Many essential oils obtained from plants of several botanical families show some insecticidal activity or repellent on arthropods. In general, essential oils may be absorbed by the cuticle, inhaled, or ingested by the insects (Regnault-Roger & Hamraoui, 1995). Inside the several families, the genus *Cinnamomum* highlights itself, which is already cited in the literature as presenting biological activity (Nerio et al., 2010).

The *Cinnamomum* genus, belonging to the Lauraceae family, is constituted by approximately 330 species; many produce essential oils. The essential oil of *Cinnamomum camphora* Ness and Eberm var. *linaloolifera* Fujita, commonly known as 'ho-sho', is cited in the literature as having antimicrobial, antioxidant, and insecticidal activities (Liu et al., 2006; Cansian et al., 2010; Gilles et al., 2010; Wang et al., 2011; Tomazoni et al., 2017).

The chemical composition of *C. camphora* essential oil is already presented and discussed in the literature; its major compound, linalool, is a monoterpene whose repellent and insecticidal properties were addressed by other researchers, suggesting the potential use to control *T. vaporariorum* (Cansian et al., 2010, 2015; Tambwe et al., 2014).

This work aimed to evaluate the chemical composition of the essential oil of *C. camphora* var. *linaloolifera* and investigate the effect of the essential oils on eggs, nymphs, and adults of *T. vaporariorum*, also evaluating the activity of linalool on nymphs of this pest, aiming to get an alternative form of control for this insect.

2. Methodology

The experiments and the evaluation of the insecticidal activity of the *C. camphora* essential oil on *T. vaporariorum* were carried out in the Laboratory of Pest Management of the University of Caxias do Sul, Caxias do Sul, RS, Brazil, from August 2017 to November 2019.

2.1 Breeding of *T. vaporariorum* individuals

A population of *T. vaporariorum* was collected from tomato plants (*Solanum lycopersicum*) in Santa Lúcia do Piaí (geographical coordinates: 29°12'54.9" S; 50°59'2.5" W), district of the municipality of Caxias do Sul, RS, Brazil. The colony was kept in bean plants (*Phaseolus vulgaris* L.), in transparent acrylic boxes inside a greenhouse in the Institute of Biotechnology of UCS.

The *T. vaporariorum* individuals were identified, relative to the biotype, by PCR (polymerase chain reaction) of the mtCOI region and posterior RFLP (Restriction Fragment Length Polymorphism); all carried out in the Universidade Estadual Paulista (UNESP).

2.2 Plant material for essential oil extraction

The plant material of *Cinnamomum camphora* var. *linaloolifera* was collected in the Institute of Biotechnology of UCS (geographical coordinates: 29°09'47.8"S 51°08'37.4"W). After the collection, the material underwent a visual inspection with the removal of inorganics and decaying plant material. The leaves were separated and dried at room temperature (22-25 °C) for four days in a kiln with forced air circulation.

2.3 Essential oil extraction and synthetic major compound

The essential oil was extracted by steam distillation for two hours. The volume of obtained essential oils was measured using a 25 mL glass graduated cylinder. The essential oil was kept in amber flasks, and 0.5 mL was sent to chromatographic analysis. The material was stored under refrigeration at 4±2 °C until use in the bioassays.

The synthetic compound linalool (CAS 78-70-6, purity of 99.2%) was purchased from Merck (Germany) and was kept

under refrigeration at 4 ± 2 °C until use in the bioassays.

2.4 Chromatographic analysis

The analyses were carried out in the Analytical Central of the Institute of Biotechnology of the UCS, based on the procedures described by Silvestre et al. (2019) and Vicensó et al. (2020). The GC-FID analysis was carried out using a Hewlett Packard 6890 Series gas chromatograph, equipped with an HP-Chemstation data processing unit. An HP-Innowax column (30 m x 320 μ m i.d.) with 0.50 μ m film thickness (Hewlett Packard, Palo Alto, USA) was used. Temperature programming was: 40 °C for 8 min, from 40 °C to 180 °C at 3 °C·min⁻¹, from 180 to 230 °C at 20 °C·min⁻¹, 230 °C for 10 min; injector temperature of 250 °C; split ratio 1:50, FID detector at 250 °C; hydrogen as carrier gas at 34 kPa, injected volume of 1 μ L, diluted in hexane (1:10).

The GC/MS analysis was carried out using a gas chromatograph, coupled with an HP 6890/MSD5973 mass spectrometer, equipped with an HP-Chemstation and the Wiley 275 spectra library. An HP-Innowax fused silica capillary column (30 m x 320 μ m i.d.) with 0.50 μ m film thickness was used. The temperature program used was the same as GC-FID; interface at 280 °C; split ratio 1:100; helium as carrier gas at 56 kPa; flow ratio of 1.0 mL·min⁻¹; ionization energy of 70 eV; injected volume of 1 μ L diluted in hexane (1:10).

The essential oils components were identified by comparison between the obtained spectra and the ones from the Wiley library (GC/MS) and by comparison of the calculated linear retention indexes (LRI's) and their literature references. The LRI values were calculated using the Van den Dool and Kratz equation and a C8-C30 alkane series solution as standard.

2.5 Bioassays

Tests were performed with eggs, nymphs, and adult individuals of *T. vaporariorum* had an experimental design completely randomized. All tests were carried out in an air-conditioned room at 25 ± 2 °C, $50\pm 5\%$ RH, and a photoperiod of 14:10 h (light:dark).

For the three stages of *T. vaporariorum*, the treatments were the concentration of essential oil and negative and positive controls. Five concentrations of *C. camphora* essential oil were tested: 0.1, 0.5, 1.0, 1.5, and 2.0% v/v. Also, treatments with the major compound were carried out with nymphs. The same concentrations were used but adjusted to the major compound's content in the essential oil, according to Equation 1.

$$C = D \times \frac{T}{100} \quad (1)$$

Being 'C' the adjusted concentration, 'D' the theoretical dose, and 'T' the major compound's content in the essential oil.

All treatments (essential oil and major compound) were emulsified using Tween-80[®] (0.5% v/v). The recommended pesticides for the control of the *T. vaporariorum* were used as positive controls. For the adult phase, it was used pymetrozine in the recommended dose of 0.04 wt.% (Chess[®], Syngenta), and for the juvenile phase (eggs and nymphs) it was used spiromesifen in the recommended dose of 0.05% v/v. (Oberon[®], Bayer S.A.). Two treatments were used as negative controls: water and Tween-80[®] 0.5% v/v.

For the tests with the eggs of *T. vaporariorum*, tomato plants (*S. lycopersicum* cv. Micro-Tom) with 30 days old underwent visual inspection to exclude plants with any sign of disease or infestation. The selected plants had their leaves removed, except for one leaf. After, 20 adult individuals, not sexed, were transferred from the colony using a manual aspirator. The plants were covered using a voile fabric to avoid insect escape, and they were kept ovipositing for 48 h. After this period,

the adults were removed, and the leaves with the eggs were treated in the abaxial direction with the essential oil/major compound solutions using a manual sprayer. The treated plants were kept in an air-conditioned room as previously described and the mortality/toxicity evaluations were carried out after eight days. It was counted the number of individuals that reached the first nymphal stage. It was used five replicates for each treatment, being each replicate composed of a plant.

For the tests with the nymphs of *T. vaporariorum*, 20 adult individuals were transferred using a manual aspirator to tomato plants (*S. lycopersicum* cv. Micro-Tom) with 30 days old. The plants were covered using a voile fabric to avoid insect escape, and they were kept ovipositing for 48 h. After this period, the adults were removed, and the plants were kept in an air-conditioned room as previously described. When the nymphs reached the second nymphal stage (which was observed using a magnifier), the treatments were sprayed in the abaxial direction of the leaves using a hand sprayer. The plants were kept in an air-conditioned room, and the evaluation of the mortality/toxicity was carried out after eight days by counting the number of nymphs that reached the fourth nymphal stage and the dead ones. It was used five replicates for each treatment, being each replicate composed of a plant.

For the test with the adult individuals of *T. vaporariorum*, it was used the methodology described by Müller et al. (2018). Leaves of tomato plant (*S. lycopersicum* cv. Micro-Tom) with 30 days old were cut and washed with tap water. After, both leaves sides were sprayed with 1 mL of treatments and kept 30 min away from sunlight to dry on an absorbent paper. Each leaf was put in glass flasks (7 cm of diameter and 9 cm of height) with moistened floral foam. Each flask was infested with five adult individuals of *T. vaporariorum* with the age of 5-7 days by using a manual aspirator. The flasks were closed using black TNT fabric and kept in an air-conditioned room. The mortality percentage of the individuals was evaluated in 24, 48, and 72 h after infestation. It was used five replicates for each treatment and five individuals for each replicate, totaling 25 individuals for each treatment.

2.6 Statistical analysis

Data normality was evaluated using the Kolmogorov-Smirnov test. The parametric data were analyzed by analysis of variance (ANOVA), followed by least significant difference (LSD) test at a 5% significance level ($p < 0.05$). The Spearman correlation coefficient was also evaluated at a 5% significance level, aiming to verify possible relationships between mortality data and the concentrations of essential oil/major compound. The data that presented significant correlations were used to generate polynomial regressions to observe the data behavior. The statistical analyses were carried out using the Statistical Package for the Social Sciences software (SPSS for Windows 17.0) (Green & Salkind, 2005).

3. Results

3.1 Essential oil composition and yield

The essential oil obtained from the dried leaves of *C. camphora* has had a yield of 1.60% v/w. Sixteen compounds were identified in the essential oil, being linalool the major compound, with 92.34 wt.% (Table 1). Relative to chemical classes, oxygenated monoterpenes composed most of the essential oil (94.61 wt.%), followed by small amounts of sesquiterpenes (2.96 wt.%) and monoterpenes (1.16 wt.%).

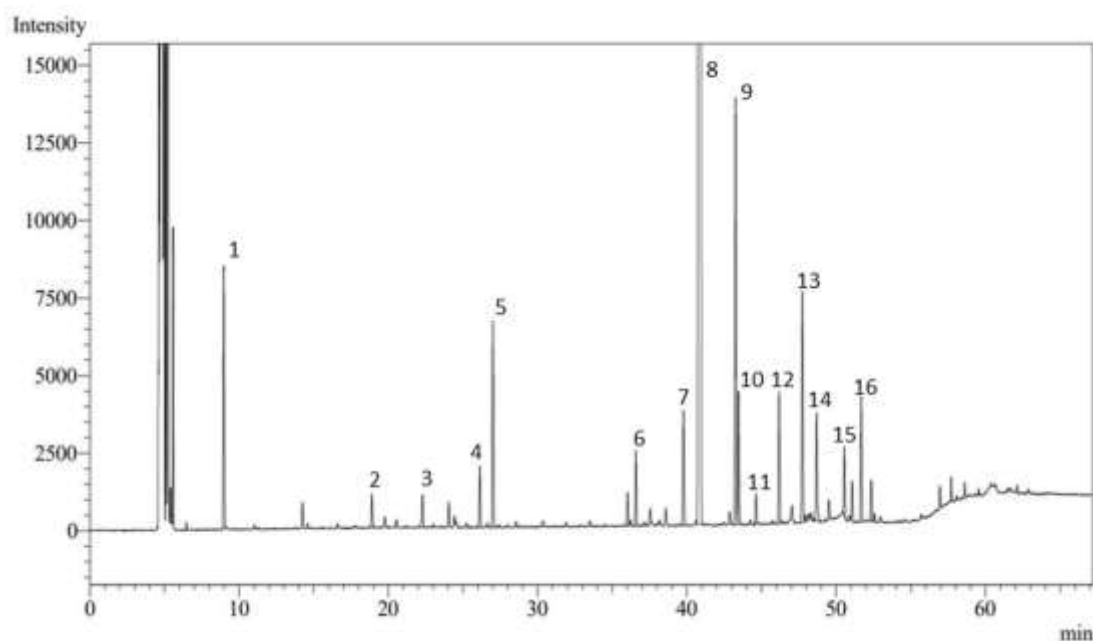
Table 1. Chromatographic analysis (GC-FID and GC/MS) of *Cinnamomum camphora* var. *linaloolifera* essential oil, obtained by steam distillation.

Compound	IRL Calc.	IRL Lit.	Content (wt.%)
β -pinene	1107	1108	0.11
Myrcene	1169	1167	0.16
γ -terpinene	1241	1240	0.21
o-cymene	1258	1254	0.68
<i>cis</i> -linalool oxide	1444	1441	0.08
1-octen-3-ol	1456	1460	0.21
Camphor	1528	1531	0.42
Linalool	1555	1551	92.34
β -caryophyllene	1611	1610	1.84
terpinen-4-ol	1616	1612	0.82
γ -elemene	1645	1644	0.11
germacrene-D	1724	1726	1.01
cuminal	1798	1794	0.07
E,Z-2,6-dimethyl-3,5,7-octatriene-2-ol	1812	1815	0.26
E,E-2,6-dimethyl-3,5,7-octatriene-2-ol	1829	1830	0.43
p -cymen-8-ol	1848	1850	0.19
Hydrocarbon monoterpenes			1.16
Oxygenated monoterpenes			94.61
Hydrocarbon sesquiterpenes			2.96
Others			0.21
Not identified			1.05

IRL Calc.: calculated linear retention index; IRL Lit.: linear retention index according to literature data (NIST).
Source: Authors (2021).

The chromatogram of the FID analysis of the *C. camphora* essential oil, along with peak identification, is presented in Figure 1.

Figure 1. Chromatogram of the essential oil of *Cinnamomum camphora* var. *linaloolifera*.



1 – β -pinene; 2- myrcene; 3 – γ -terpinene; 4 – o-cymene; 5 – *cis*-linalool oxide; 6 – 1-octen-3-ol; 7 – camphor; 8 – linalool; 9 – β -caryophyllene; 10 – terpinen-4-ol; 11 – γ -elemene; 12 – germacrene-D; 13 – cuminal; 14 – E,Z-2,6-dimethyl-3,5,7-octatrien-2-ol; 15 – p -cymen-8-ol; 16 – E,E-2,6-dimethyl-3,5,7-octatrien-2-ol.
Source: Authors (2021).

3.2 Bioassays

It was possible to observe a decrease in the number of nymphs that emerged with an increase in essential oil concentration. There was a difference in the mortality of eggs from the concentration of 0.1% v/v of *C. camphora* essential oil, differing from the negative controls (water and Tween-80®). However, essential oil treatments presented a lower mortality rate of eggs than the positive control (spiromesifen 0.05% v/v). The mortality data of eggs and nymphs of *T. vaporariorum* are presented in Table 2.

Table 2. Mortality percentage of eggs and nymphs of *T. vaporariorum* treated with different concentrations of the essential oil of *Cinnamomum camphora* var. *linaloolifera*.

Treatments	Mortality (%)	
	Eggs	Nymphs
Water	5.49 e	1.76 e
Tween-80 0.5% v/v	6.22 e	2.50 e
Spiromesifen 0.05% v/v	65.00 a	98.32 a
0.1% v/v	23.13 d	8.50 e
0.5% v/v	36.37 c	29.24 d
1.0% v/v	45.55 bc	78.00 c
1.5% v/v	30.91 b	83.44 bc
2.0% v/v	49.37 b	88.54 ab
LSD (5%)	10.462	10.534
F-value	34.80	132.97
p-value	<0.0001	<0.0001
Coefficient of variation (%)	23.33	16.76

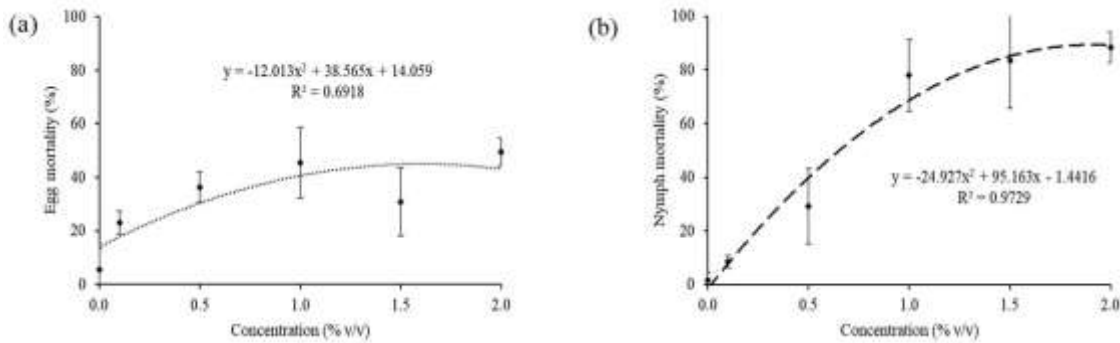
Means followed by the same letter in column do not differ statistically by least significant difference (LSD) test at 5% probability. Source: Authors (2021).

The number of eggs that have not hatched has not differed statistically in the essential oil concentrations of 1.0, 1.5, and 2.0% v/v, whose mortality varied between 30.9 and 49.4%. The essential oil concentration of 2.0% v/v had a poorer performance than the positive control (spiromesifen 0.05% v/v).

For the nymphs (Table 2), it was possible to observe that the concentrations of 1.5 and 2.0% v/v have not differed statistically; there was no difference of the concentration of 2.0% v/v relative to the chemical control (88.5 and 98.3% of individuals mortality, respectively). Also, the concentration of 1.5% v/v has not differed from the concentration of 1.0% v/v, causing nymph mortalities of 78.0 and 83.4%, respectively. The essential oil concentration of 0.1% v/v has not differed from the two negative controls (water and Tween-80®).

The Spearman correlation coefficient between the essential oil concentration of the eggs and nymphs' mortality were 0.7854 and 0.8991, respectively; both were significant at a 5% probability. Figure 2 presents the regression graphs between the essential oil concentration and the egg and nymph mortality of *T. vaporariorum*.

Figure 2. Regression graphs of the mortality of eggs (a) and nymphs (b) of *T. vaporariorum* as a function of *C. camphora* essential oil concentration.



Source: Authors (2021).

It was observed that the mortality of eggs and nymphs followed a quadratic pattern relative to the dose of essential oil, with determination coefficients (R^2) of 0.6918 for eggs and 0.9729 for nymphs. It is important to note the difference between the egg and nymph mortalities (60 and 90% for essential oil concentration of 2.0% v/v), although the regression's overall aspect was similar for both bioassays.

The evaluation of the adult individuals of *T. vaporariorum* (Table 3) showed a small increase in mortality (12.0%) at the concentration of 2.0% v/v in the first 24 h, being the only treatment that differed from the others. The mortality percentage after 48 h increased to 40% at the same concentration. This value has not increased, remaining unchanged after 72 h. Noteworthy, insecticide pymetrozine caused insect mortality only after 48 h (64.0%); this percentage increased to 72.0% after 72 h.

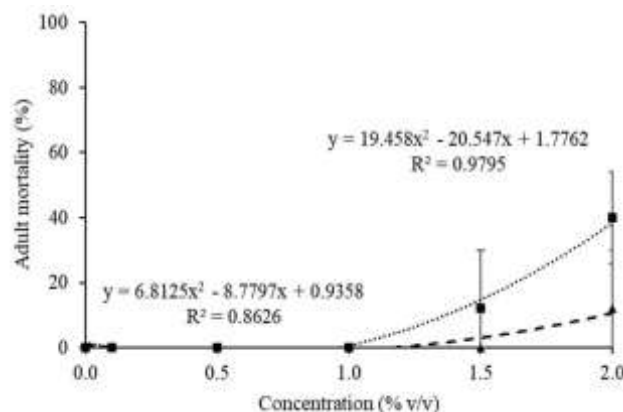
Table 3. Mortality percentage of adult individuals of *T. vaporariorum* treated with different concentrations of *Cinnamomum camphora* var. *linaloolifera* essential oil, in different evaluation times.

Treatments	Mortality (%)		
	24 h	48 h	72 h
Water	0.00 a	0.00 d	0.00 c
Tween-80 0.5% v/v	0.00 a	0.00 d	0.00 c
Pymetrozine 0.04% m/v	0.00 a	64.00 a	72.00 a
0.1% v/v	0.00 a	0.00 d	0.00 c
0.5% v/v	0.00 a	0.00 d	0.00 c
1.0% v/v	0.00 a	0.00 d	0.00 c
1.5% v/v	0.00 a	12.00 c	12.00 c
2.0% v/v	12.00 b	40.00 b	40.00 b
LSD (5%)	8.148	11.157	13.201
F-value	2.25	39.60	34.05
p-value	0.0557	<0.0001	<0.0001
Coefficient of variation (%)	150.00	59.73	66.11

Means followed by the same letter in column do not differ statistically by least significant difference (LSD) test at 5% probability. Source: Authors (2021).

The determination of the Spearman correlation coefficient indicated the existence of three significant correlations between the mortalities of adult individuals at 24 (0.4167), 48, and 72 h (0.7553 in both). Figure 3 presents the regression data of the adult mortality relative to the evaluation times and the concentration of *C. camphora* essential oil.

Figure 3. Regression graphs of the mortality of adult individuals of *T. vaporariorum* after 24 (dashed line) and 48/72 h (dotted line) as a function of the concentration of *C. camphora* essential oil.



Source: Authors (2021).

The bioassay that compared the essential oil of *C. camphora* and linalool, its major compound, (Table 4), showed that linalool caused higher mortality percentages of nymphs at the concentrations of 0.5 and 1.0% v/v when compared to the essential oil.

Table 4. *T. vaporariorum* nymph mortality percentage treated with *Cinnamomum camphora* var. *linaloolifera* essential oil and linalool, its major compound.

Treatment	<i>C. camphora</i>	Linalool
Water	1.76 Ad	1.76 Ac
Tween-80 0.5% v/v	2.50 Ad	2.50 Ac
Spiromesifen 0.05% v/v	98.32 Aa	98.32 Aa
0.5% v/v*	29.24 Bc	55.56 Ab
1.0% v/v*	65.65 Bb	78.00 Ab
1.5% v/v*	83.44 Ab	90.76 Aa
2.0% v/v*	88.54 Aab	87.70 Aa
Factor	F-value	p-value
Essential oil kind	1.86	0.178
Concentration	208.61	<0.0001
Interaction between factors	4.37	0.0011
LSD (5%)	11.341	
Coefficient of variation (%)	15.98	

Means followed by the same letter, uppercase in row (OE kind) and lowercase in column (concentration) do not differ statistically by the least significant difference (LSD) test at 5%. * - The concentration of the major compound was adjusted according to its content in the essential oil.

Source: Authors (2021).

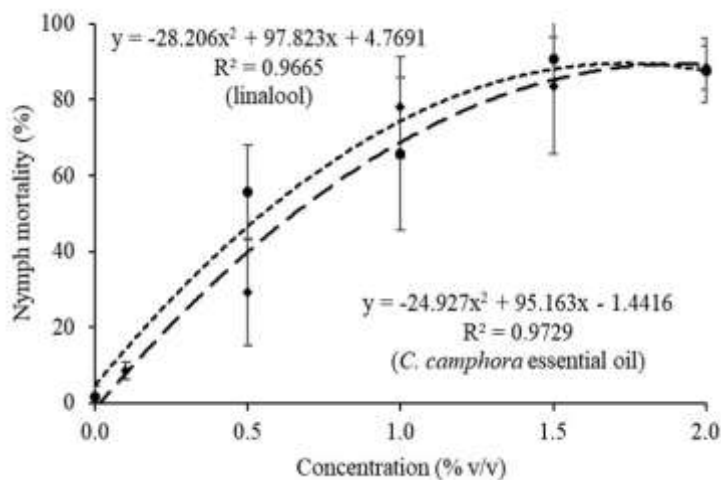
However, at higher concentrations (from 1.5% v/v on), no statistical difference was observed between the two treatments. Moreover, essential oil from 1.5% v/v on, linalool and chemical control (spiromesifen) have had a similar performance, in which the mortality percentages of *T. vaporariorum* nymphs were above 90% for both treatments.

The test also indicated that linalool was the primary insecticidal agent in the essential oil of *C. camphora* var. *linaloolifera*. As the concentration of essential oil increased, the mortality percentage increased considerably; from 1.5% v/v on, the essential oil's toxic effect has not differed from the one of linalool. These results may indicate that the major compound (linalool) concentration in the essential oil was sufficient to suppress possible minor compounds' antagonistic effects.

The Spearman correlation coefficient indicated a significant correlation between nymph mortality and linalool concentration (0.8443), beyond the already established correlation with *C. camphora* essential oil (0.8891). Figure 4 presents the

regression graphs of *T. vaporariorum* nymph mortality relative to both linalool and *C. camphora* essential oil concentrations.

Figure 4. Regression graphs of *T. vaporariorum* nymph mortality as a function of the concentration of *C. camphora* essential oil (dashed line) and linalool (dotted line).



Source: Authors (2021).

Evaluating the graph shows that *T. vaporariorum* nymphs' mortality data exposed to both the essential oil and linalool fitted similarly, with a quadratic fit. It indicates both treatments presented the same toxic effect on nymphs of this pest, with mortalities ranging from 85 to 90% for a concentration of 2.0% v/v.

4. Discussion

4.1 Essential oil yield and composition

Relative to *C. camphora* essential oil, other authors reported similar results, which also characterized linalool as the major compound of this essential oil in contents ranging from 84 to 95 wt.% (Cansian et al., 2010, 2015; Nenaah & Ibrahim, 2011; Tomazoni et al., 2017).

The compounds found in the essential oil are classified into two main chemical groups, varying according to the metabolic pathway from which they originated: terpenes (mono-, sesqui-, and diterpenes) and, in lower contents, phenylpropanoids (Regnault-Roger et al., 2012). In plants, monoterpenes act as defense agents against pathogens due to allelochemical and repellent activities, among other functions (Chand et al., 2017).

The physiological expression of secondary metabolism of plants may differ due to the stage of development (Hansted et al., 1994; Raguso & Pichersky, 1999). The proportion of monoterpenes, for example, depends on circadian rhythm and varies according to the plant stage (Clark & Menary, 1981). Some plant species also have different chemotypes, i.e., they are botanically and genetically identical. However, they differ in the level of gene expression, which is linked to the activation of several metabolic pathways due to specific alleles' presence or activation (Regnault-Roger et al., 2012).

Essential oil composition also may be changed during the extraction process. Water kind and quality, acidity, and extraction temperature may cause oxidation and hydrolysis of terpenes. The storage conditions are also quite crucial since the degradation possibilities are many. These essential oil changes occur mostly by polymerization and oxidation reactions (Simões et al., 2017).

4.2 Insecticidal activity of *C. camphora* essential oil on *T. vaporariorum*

Literature is scarce about the effect of the essential oil of *C. camphora* in the juvenile phase (eggs and nymphs) of *T. vaporariorum*, neither other species, having only studies relative to the adult phase. The essential oil of *C. camphora* has had toxicity on *Sitophilus zeamais*, causing 100% mortality at the concentration of 0.114% v/v (Cansian et al., 2015).

The behavior observed in the present work may indicate that, at higher concentrations (1.5 and 2.0% v/v), the effect of the essential oil on egg and nymph mortality becomes constant, not increasing in higher concentrations. Pereira et al. (2018), using essential oil from the fruits of *Zanthoxylum riedelianum* to control second-instar nymphs of *Bemisia tabaci*, reported a log-logistic fit of the data in function of the concentration, with mortalities in the range of 50-80%.

Essential oils contain several compounds that can exert repellent and insecticidal mechanisms. Many studies have demonstrated that these compounds have biological activity and can cause adverse effects on several pests (Dhifi et al., 2016). The toxicity of essential oil on insects, nematodes, fungi, bacteria, and other organisms may be attributed to the terpenic compounds present in these oils (Regnault-Roger et al., 2012; Pavella & Benelli, 2016). The quick action of these natural products against insects may be indicative of neurotoxic action. There is evidence of interference of terpenes with the neuromodulator octopamine or GABA-modulated calcium channels (Priestley et al., 2003; Isman, 2006). They can also affect acetylcholinesterase, octopamine, sodium channels, among other mechanisms (Huignard et al., 2008; Regnault-Roger et al., 2012; Campos et al., 2018). However, the essential oils' action capacity is poorly understood, since the toxic effect may differ according to the quality and quantity of the compounds present in the essential oil (Campos et al., 2018).

Considering the crescent necessity of the development and use of more environmentally friendly molecules, the *C. camphora* essential oil presents itself as a feasible alternative due to the relatively high oil yield (about 2.0% v/w) and the high content of linalool (the *linaloolifera* variety). The use of natural molecules over synthetic ones is also interesting due to the biodegradability, low presence of residues, and a slower resistance development by the target pests (Cansian et al., 2015; Pavella & Benelli, 2016; Campos et al., 2018).

According to the EPA (Environmental Protection Agency, 2019), to register a pesticide, the substance must cause at least 90% of the target individuals' mortality. In the present study, the chemical pesticides caused a *T. vaporariorum* egg mortality of 65% and an adult mortality of 72% after 72 h of evaluation, indicating that this pest may have developed some resistance mechanism against these pesticides (spiromesifen and pymetrozine). Zhou et al. (2019) reported a linear increase in the mortality of *Aphis pomi* with time when exposed to small concentrations of *Dracocephalum integrifolium* essential oil; however, as the essential oil concentration increased, the effect was more pronounced, reaching higher mortalities at the same time.

Taking into account the IPM method, a comprehensive control using several mechanisms (natural predators, natural and synthetic chemicals, management procedures) also helps in hindering resistance development and increasing the overall control efficiency without excessive use of any of the control methods (Nerio et al., 2012; Regnault-Roger et al., 2012). In this sense, the leaf essential oil of *C. camphora* as a control tool in the integrated management of *T. vaporariorum* may be a valuable and important tool in promoting more eco-friendly and efficient management of the cultures affected by this insect.

5. Conclusion

The chemical analysis of the essential oil of *C. camphora* showed linalool as the major compound, with 92.34 wt.%. According to the results, the *C. camphora* essential oil presented a moderate activity on eggs and adult individuals of *T. vaporariorum* and was successful in controlling the nymphs of this pest. Thus, this essential oil may be an effective alternative and an option in the management of this insect, especially in light of the MIP procedures.

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