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Article

# Lethal Effect and Two-Sex Life Table of *Phthorimaea absoluta* (Meyrick) (Lepidoptera: Gelechiidae) Treated with *Melaleuca alternifolia* and *Eucalyptus staigeriana* Essential Oils

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## Abstract

The tomato leafminer *Phthorimaea absoluta* is an important pest of solanaceous plants and is considered a key pest of tomato plants. This pest is rapidly dispersed, difficult to control, and resistant to the main synthetic insecticides. The objective was to determine the lethal and sublethal effects of three essential oils (Eos) of *Melaleuca alternifolia* and *Eucalyptus staigeriana* against *P. absoluta*. We evaluated the effects of the EOs during the life cycle of the pest using an age-stage, two-sex life table. The treatments were solubilized in acetone and applied to the back of the caterpillars. The EOs of *M. alternifolia* and *E. staigeriana* reduced *P. absoluta* longevity to 22.2 and 15.9 days and negatively impacted demographic parameters. *Melaleuca alternifolia* notably decreased the intrinsic rate of increase. Chromatographic findings revealed the presence of 19 compounds in the *M. alternifolia* EO, with terpinen-4-ol,  $\gamma$ -terpinene and  $\alpha$ -terpinene being the most abundant. On the other hand, *E. staigeriana* presented 25 constituents, with the compounds limonene, geranial and neral being the most abundant. The results obtained demonstrate the insecticidal potential of *M. alternifolia* and *E. staigeriana* EOs in the control of *P. absoluta*.

**Keywords:** South American tomato pinworm; Botanical insecticides; IPM

## 1. Introduction

The South American tomato pinworm, *Phthorimaea absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), is a highly invasive pest of Solanaceae and is recognized as one of the most significant threats to tomato (*Solanum lycopersicum* L.) production globally, having been reported in more than 90 countries [1,2]. In Asia, its presence has already been confirmed in Korea, with the first detection reported in Jeonbuk Province [3]. Owing to its high reproductive potential, rapid generational turnover, and ability to complete several generations annually [4,5], infestations can result in substantial yield losses, ranging from 50% to total crop failure [6].

The rapid expansion of *P. absoluta* in Solanaceae worldwide, which led to a significant increase in the use of synthetic insecticides [8]. The indiscriminate use of synthetic chemical insecticides led to the rapid selection of *P. absoluta* populations resistant to the main chemical classes, such as organophosphates, pyrethroids and diamides [9–11]. In addition, insecticides can cause negative impacts on natural enemies and the contamination of the environment and enable the resurgence of secondary pests [12–14]. Due to the adverse effects of synthetic insecticides, studies that seek

alternatives to control *P. absoluta* should be encouraged, and in this context, there is a growing demand for the development of botanical insecticides to control this pest [15–17].

Botanical insecticides are plant products that have activity against insect pests and may originate from dried and ground plant structures, crude extracts or isolated components of the secondary metabolism of plants [17]. Botanical products can cause lethal (mortality) and sublethal effects, such as effects on behavior, development, and reproduction and interfering with metabolic pathways in arthropod pests [17–20]. Natural insecticides are most often easily degradable and are considered environmentally friendly products [21,22]. In addition, the raw materials are available in large quantities and at low cost, and many plant derivatives have several mechanisms of action still unexplored [23]. Among botanical insecticides, essential oils (EOs) exhibit bioactivity against several groups of insect pests and have been gaining prominence among biopesticides [17].

The use of plant EOs represents a promising strategy within integrated pest management (IPM) programs, as they are rich in bioactive compounds and exhibit insecticidal, repellent, and oviposition-detering properties [24–26]. In addition to their efficacy, EOs generally pose low environmental risks due to their volatility, rapid degradation, and minimal soil persistence, reducing the likelihood of resistance development in pest populations owing to their multiple modes of action [25]. These oils are typically complex mixtures of terpenoids, whose biological effects often result from synergistic or additive interactions among constituents [26]. In *P. absoluta*, various EOs have been shown to induce adult and larval mortality [27], exhibit repellency [28], and inhibit oviposition behavior [29]. Despite these benefits, EOs derived from Myrtaceae species remain underexplored in the management of tomato leafminers [15]. Recent studies have further confirmed the fumigant toxicity [30] and behavioral effects of selected plant EOs on *P. absoluta*, as well as their safety to natural predators such as *Macrolophus pygmaeus* (Rambor, 1839) (Hemiptera: Miridae) [31].

The EOs of plants of the Myrtaceae family represent a class of volatile secondary metabolites containing terpenes and aromatic compounds as the main components [32]. These plants are gaining increasing interest in IPM programs due to their ovicidal, larvicidal, and adulticidal action; repellent activity; and fumigant and contact toxicity [33]. The *Eucalyptus staigeriana* F. Muell. ex Bailey and *Melaleuca alternifolia* Cheel are among the species of the family Myrtaceae with insecticidal activity. The EOs from the leaves of plants of the genus *Eucalyptus* are commercially used in the pharmaceutical, cosmetic and food industries [34]. The EO bioactivity of *E. staigeriana* has been reported for different insect orders. For example, in insects of the order Coleoptera, a harmful effect was observed on the oviposition and emergence of *Zabrotes subfasciatus* (Both, 1833) (Bruchidae) and *Calosobruchus maculatus* (Fabricius, 1775) (Chrysomelidae) [35] as well as repellency to *C. maculatus* [36] and *Sitophilus zeamais* (Mots, 1885) (Curculionidae) [37]. In addition, the survival of *Lutzomyia longipalpis* (Lutz and Neiva, 1912) (Diptera: Psychodidae) [38] and the reproductive parameters of *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) were affected [39]. On the other hand, EOs from the stems and leaves of *M. alternifolia*, which are rich in terpenes, have natural pharmacological properties [40]. In addition, in insect pests, these EOs have an inhibitory effect on feeding [41,42], a lethal effect, and on behavior [43], as well as potential in the control of stored grain pests, due to their fumigant action [41]. However, studies evaluating the potential of these EOs in controlling *P. absoluta* are still scarce.

In this study, we assessed the insecticidal potential of *E. staigeriana* and *M. alternifolia* EOs against *P. absoluta*. Initially, both oils were chemically characterized to determine their main constituents. Then, their effect was inferred from the dose–response curves and life history parameters of *P. absoluta*. The results offer valuable insights into the lethal and sublethal effects of these EOs on *P. absoluta*, contributing to the understanding of their impact on pest population dynamics. This study provides relevant information on the applicability of the tested EOs to control a pest of global importance.

## 2. Materials and Methods

### 2.1. Biological Material and Experimental Conditions

The rearing was established by using eggs and caterpillars of *P. absoluta* collected from tomato plants grown on the Campus of the Federal University of Lavras (UFLA) and in the field of the company Agroteste LTDA (21°12' S, 45°03' W), with no history of application of synthetic chemical pesticides. After collection, the insects were transferred to acrylic cages (60 × 30 × 30 cm) containing tomato (*S. lycopersicum* cv. Santa Clara) branches for feeding.

After emergence, approximately 500 adults were transferred to a new cage containing tomato shoots that served as oviposition substrates. The adults were offered an aqueous solution of honey (1:1) soaked in moistened cotton wool as a food source. The branches containing the eggs were removed every four days and placed in acrylic cages for maintenance in the laboratory, aiming to provide insects for the bioassays. From the second generation onwards, second-instar and same-generation caterpillars were used in the bioassays. The rearing of *P. absoluta* and the bioassays were conducted under controlled temperature (25 ± 2°C), relative humidity (70 ± 10%), and photophase (12h) conditions.

### 2.2. Obtention and Chemical Characterization of EOs

The EOs of *M. alternifolia* and *E. staigeriana* were purchased from Empresa Ferquima Indústria e Comércio Ltd.a., Vargem Grande Paulista, São Paulo - Brazil. The chromatographic parameters and equipment used to analyze the EOs were the same as those described in the established methodology [44]. All analyses were performed in triplicate. The analyte concentrations were expressed as the mean relative area percentage of the chromatographic peaks ± standard deviation (n = 3).

### 2.3. Acute Toxicity of EOs in a Topical Application Against *P. absoluta*

The EOs of *M. alternifolia* and *E. staigeriana* were previously diluted in acetone and applied topically to the dorsum of second-instar *P. absoluta* caterpillars at a dose of 120 µL EO.caterpillar<sup>-1</sup>. Each caterpillar received 1 µL of the solution on its back using a microsyringe (Hamilton® 25 µL). Untreated control insects were treated with acetone alone. A completely randomized design was used, with 60 caterpillars per treatment, each replicate consisting of a single treated caterpillar placed in a 5 cm diameter Petri dish containing a leaflet of tomato plant *S. lycopersicum* cv. Santa Clara for feeding the insects. A piece of filter paper moistened with distilled water was placed under the leaflet to maintain its turgidity. The experiment was repeated twice on different days, totaling 120 replicates per treatment. The mortality evaluations of the caterpillars were performed at 6, 12, 24, 36, 48, and 72 h after the application of the treatments using a stereoscopic microscope (40x). A dead caterpillar was considered one that did not respond to touch with a soft-bristled brush, remaining still.

### 2.4. Dose–Response and Time–Response Bioassays of EOs

The EO from *M. alternifolia* was applied to *P. absoluta* caterpillars at doses of 30, 40, 55, 74 and 100 µg EO.caterpillar<sup>-1</sup>, and *E. staigeriana* EO was applied at doses of 50, 62, 78, 97 and 120 µg EO.caterpillar<sup>-1</sup>. These doses were determined by means of previous tests and by arithmetic progression, aiming to obtain average percentages of mortality between 20 and 90% [45]. The negative control treatment was acetone. All treatments were applied topically to the back of second-instar *P. absoluta* caterpillars as described in subitem 2.3. A completely randomized design was used, with 60 replicates per treatment, each represented by a treated caterpillar kept in a Petri dish containing one tomato leaflet. The assays were repeated twice.

### 2.5. Effects of Sublethal Doses of EOs on the Life History Parameters of *P. absoluta*

For the life table bioassay, approximately 200 adult couples of *P. absoluta* 72h after emergence were kept in acrylic cages (60 × 30 × 30 cm) containing tomato plants cv. Santa Clara (15 cm tall) for 48h in order to oviposition. The plants were observed daily to verify the appearance of 2nd instar caterpillars when they were removed with the aid of a brush and subjected to the application of the

treatments. The EOs were used at doses equivalent to the previously estimated LD<sub>50</sub> (lethal dose capable of killing 50% of the insect population) (subitem 2.4). The treatments consisted of acetone (negative control), *M. alternifolia* (76.5 µL EO.caterpillar<sup>-1</sup>) and *E. staigeriana* (78.5 µL EO.caterpillar<sup>-1</sup>), which were applied to the dorsum of the caterpillars with the aid of a microsyringe (subitem 2.3). Then, the caterpillars were individualized in Petri dishes (2 cm high × 5 cm in diameter) and fed every 72h with leaflets of tomato plants that contained their petioles wrapped in cotton moistened with distilled water and inserted into a microtube. All plates were sealed with perforated PVC film to allow gas exchange and moisture stabilization. The experimental design used was completely randomized, with 3 treatments and 100 replicates, each consisting of a Petri dish with a treated second-instar caterpillar. The duration of the instars, larval and pupal survival, and duration of the larval and pupal stages of the insects were evaluated daily after the topical application of the EOs.

To evaluate the effects of the oils on the reproduction and longevity of adults from treated caterpillars, couples were formed with newly emerged insects (< 48 h of age). Each pair was placed in a Petri dish (2 cm high × 15 cm 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 soaked in a 1:1 aqueous solution of a tomato plant (five 5 leaflets) with its stem wrapped in moistened cotton and placed in a microtube, which served as a substrate for oviposition. The oviposition period, survival, and longevity of males and females, as well as fecundity, were recorded.

## 2.6. Statistical Analyses

Data on insect survival over time were analyzed using the nonparametric Kaplan–Meier estimator and subjected to the log-rank test using the survival package [45]. The survival curves were compared using the pairwise multiple comparison test. The median lethal time (LT<sub>50</sub>), i.e., the time needed to cause 50% mortality in the population was also estimated for each treatment. To determine the median lethal dose (LD<sub>50</sub>), the data were subjected to logit analysis using the drc package [45]. These analyses were performed using the statistical program R<sup>®</sup> [47].

The processing of the data analysis for the preparation of the life tables was performed using the TWSEX-MSCHART program [48]. The means, variances and standard errors of the parameters were compared in pairs between treatments by the bootstrap method with 100,000 replicates [48]. The life table considers the means of survival parameters, life expectancy and fertility until age *x* and stage *j* are reached. Differences between treatments were analyzed using the paired bootstrap test with a significance level of 5%.

## 3. Results

### 3.1. Chemical Characterization of EOs

The chemical analysis of the EO of *E. staigeriana* indicated the presence of 25 constituents, and the main compounds were limonene, neral, and geranial, with amounts varying from 9.90% to 27.29%. The EO of *M. alternifolia* presented 19 chemical compounds; 4-terpineol, γ-terpinene and α-terpinene were the major compounds with areas of 42.23%, 22.10% and 10.44%, respectively (Table 1).

**Table 1.** Chemical composition of the essential oils of *Eucalyptus staigeriana* and *Melaleuca alternifolia*.

N	Compound	RI*	RI <sup>†</sup>	Area (≥0.1%±SD)	
				<i>E. staigeriana</i>	<i>M. alternifolia</i>
1	α-thujene	910	924	0.279 ± 0.003	0.710 ± 0.002
2	α-pinene	913	932	2.981 ± 0.050	3.074 ± 0.013
3	α-phelandrene	937	1002	Nd	0.635 ± 0.002
4	<b>α-terpinene</b>	950	1014	0.179 ± 0.023	<b>10.44 ± 0.091</b>
5	sylvestre	957	1025	Nd	2.109 ± 0.005

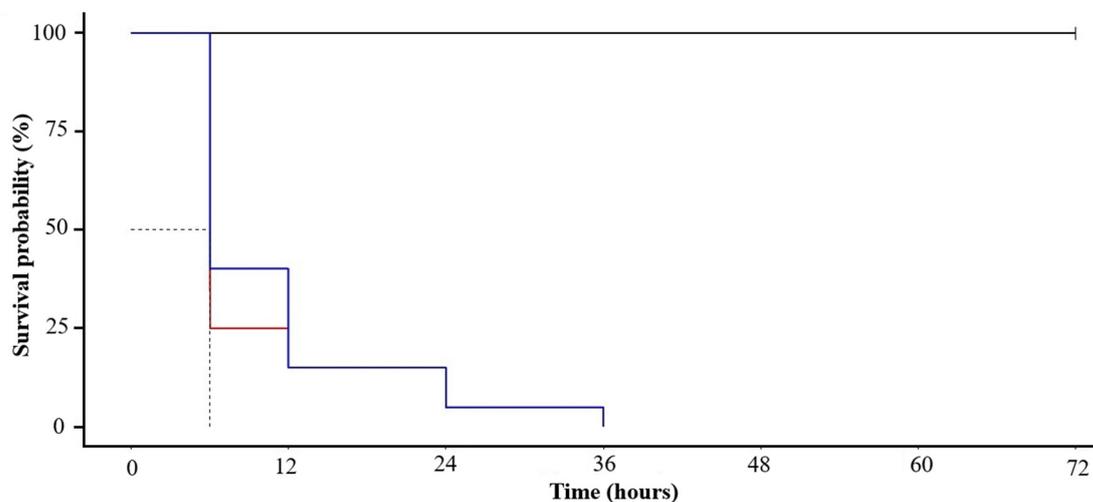
6	$\beta$ -pinene	974	974	1.454 $\pm$ 0.022	0.618 $\pm$ 0.002
7	$\gamma$ -terpinene	975	1054	Nd	<b>22.102 <math>\pm</math> 0.129</b>
8	NI	990	-	1.085 $\pm$ 0.003	Nd
9	$\alpha$ -phellandrene	1004	1002	2.257 $\pm$ 0.025	Nd
10	<i>o</i> -cymene	1022	1022	2.150 $\pm$ 0.001	3.378 $\pm$ 0.157
11	<b>limonene</b>	1027	1024	<b>27.298 <math>\pm</math> 0.330</b>	Nd
12	1.8-cineol	1030	1026	4.133 $\pm$ 0.055	2.241 $\pm$ 0.005
13	(Z)- $\beta$ -ocimene	1035	1032	0.227 $\pm$ 0.001	Nd
14	(E)- $\beta$ -ocimene	1045	1044	0.435 $\pm$ 0.002	Nd
15	$\gamma$ -terpinene	1055	1054	1.882 $\pm$ 0.016	Nd
16	<b>terpinolene</b>	1087	1086	<b>8.919 <math>\pm</math> 0.076</b>	3.353 $\pm$ 0.010
17	linalool	1100	1095	1.562 $\pm$ 0.009	Nd
18	NI	1170	-	0.847 $\pm$ 0.315	Nd
19	<b>terpinen-4-ol</b>	1176	1174	0.887 $\pm$ 0.316	<b>42.235 <math>\pm</math> 0.100</b>
20	$\alpha$ -terpineol	1190	1186	1.089 $\pm$ 0.016	3.386 $\pm$ 0.139
21	nerol	1228	1227	2.063 $\pm$ 0.019	Nd
22	<b>neral</b>	1241	1235	<b>9.905 <math>\pm</math> 0.024</b>	Nd
23	geraniol	1255	1249	6.386 $\pm$ 0.048	Nd
24	<b>geranial</b>	1272	1264	<b>13.825 <math>\pm</math> 0.079</b>	Nd
25	methyl geraniate	1324	1322	3.787 $\pm$ 0.021	Nd
26	neryl acetate	1366	1359	1.166 $\pm$ 0.012	Nd
27	geranyl acetate	1385	1379	3.246 $\pm$ 0.046	Nd
28	$\alpha$ -gurjunene	1406	1409	Nd	0.293 $\pm$ 0.001
29	E-caryophyllene	1415	1417	0.146 $\pm$ 0.001	0.224 $\pm$ 0.001
30	aromadendrene	1435	1439	Nd	1.238 $\pm$ 0.005
31	<i>allo</i> -aromadendrene <i>trans</i> -cadin-1(6),4-	1456	1458	Nd	0.364 $\pm$ 0.001
32	dieno	1470	1475	Nd	0.169 $\pm$ 0.001
33	viridiflorene	1492	1496	Nd	1.277 $\pm$ 0.004
34	$\delta$ -cadinene	1521	1522	Nd	0.900 $\pm$ 0.004
<b>Total</b>				<b>98.188</b>	<b>99.220</b>

\*Retention index relative to the n-alkane series (C8-C20) in the HP-5 MS column in elution order; <sup>1</sup> Retention rate according to the literature [49]; <sup>2</sup>Area ( $\geq 0.1\%$ ): mean of the relative area of the chromatographic peaks above de 0.1%. SD: standard deviation (n = 3); Nd: not detected or peaks with a relative area of less than 1%; NI: not identified; N = number of compounds.

### 3.2. Acute Toxicity of EOs in a Topical Application Against *P. absoluta*

The EOs of *M. alternifolia* and *E. staigeriana* caused 100% mortality of *P. absoluta* caterpillars at a dose of 120  $\mu$ L EO.caterpillar<sup>-1</sup> 36 h after application ( $\chi^2 = 183$ ; df = 2; p = < 0.001; Figure 1). Notably, after 6 h, both EOs caused mortality in 50% of the insects.

Treatments	Median Lethal Time (LT <sub>50</sub> )	Survival probability (%)
Acetone	> 72 h	100 a
<i>Eucalyptus staigeriana</i>	12 h	0 b
<i>Melaleuca alternifolia</i>	36 h	0 b



**Figure 1.** Mortality of *Phthorimaea absoluta* caterpillars, over time, treated topically with the essential oils of *Eucalyptus staigeriana* and *Melaleuca alternifolia* at a dose of 120  $\mu\text{g}$  of essential oil.caterpillar<sup>-1</sup>.

### 3.3. Determination of Dose–Response and Time-Response Curves of EOs

After the application of the EOs on second-instar *P. absoluta* caterpillars, it was found that the EOs of *E. staigeriana* and *M. alternifolia* presented similar LD<sub>50</sub> and LD<sub>90</sub> values (Table 2).

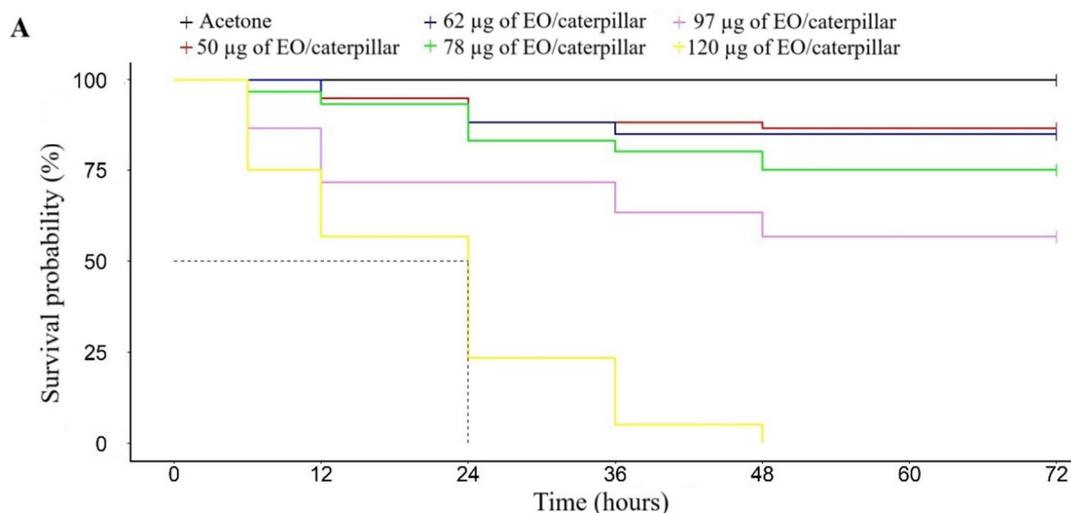
**Table 2.** Dose-resposta of *Melaleuca alternifolia* and *Eucalyptus staigeriana* for *Phthorimaea absoluta*.

Treatment	n	X <sup>2</sup>	P	*b	*e	DL <sub>50</sub> ( $\mu\text{g}\cdot\mu\text{L}^{-1}$ ) (LS –LI)	DL <sub>90</sub> ( $\mu\text{g}\cdot\mu\text{L}^{-1}$ ) (LS –LI)
<i>E. staigeriana</i>	60	186	0.56	-4.259	78.514	78.5 (72.0 – 85.0)	131.5 (111.2– 151.8)
<i>M. alternifolia</i>	60	285.9	0.68	-3.947	76.571	76.5 (70.3 – 82.8)	133.6 (110.0– 157.3)

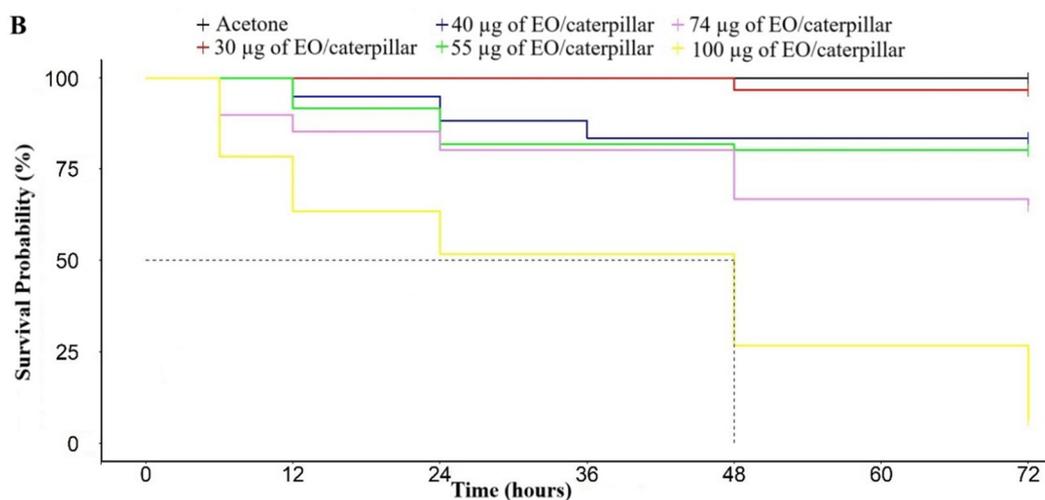
\*Doses in  $\mu\text{g}\cdot\mu\text{L}^{-1}$ . SL= upper limit; LI – lower limit. b is proportional to the slope at the LD<sub>50</sub>  $\chi^2$  value, and the p values correspond to the fit test \*\* “b” = coefficients of the equation  $f(x)=1/1+\exp(b(\log(x)-\log(e)))$ ). n = number of specimens at each developmental stage.

An increase in the mortality of *P. absoluta* caterpillars was observed with the increase in the tested doses of *E. staigeriana* EOs ( $\chi^2 = 242$ ; df= 5; p <0.001 and *M. alternifolia* ( $\chi^2 = 223$ ; df=5; p <0.001). The lowest dose needed to reduce the probability of survival was 50  $\mu\text{g}$  for *E. staigeriana* EO and 40  $\mu\text{g}$  *M. alternifolia* EO. For the *E. staigeriana* EO, the dose of 97  $\mu\text{g}\cdot\mu\text{L}^{-1}$  caused a probability of survival of 56.7% at the end of the evaluation period; while for *M. alternifolia* EO the dose of 100  $\mu\text{g}\cdot\mu\text{L}^{-1}$  was enough to cause a probability of survival of 6.67% (Figure 2).

Treatments	Median Lethal Time (LT <sub>50</sub> )	Survival probability (%)
Acetone	100.0 ± 0.0 a	> 72 h
50 µg of EO/caterpillar	87.7 ± 4.4 b	> 72 h
62 µg of EO/caterpillar	85.0 ± 4.6 b	> 72 h
78 µg of EO/caterpillar	75.0 ± 5.6 c	> 72 h
97 µg of EO/caterpillar	56.7 ± 6.4 d	> 72 h
120 µg of EO/caterpillar	23.3 ± 5.7 e	24 h



Treatments	Median Lethal Time (LT <sub>50</sub> )	Survival probability (%)
Acetone	100.0 ± 0.0 a	> 72 h
30 µg of EO/caterpillar	96.7 ± 2.3 a	> 72 h
40 µg of EO/caterpillar	83.3 ± 4.8 b	> 72 h
55 µg of EO/caterpillar	80.0 ± 5.2 b	> 72 h
74 µg of EO/caterpillar	65.0 ± 6.2 c	> 72 h
100 µg of EO/caterpillar	6.7 ± 3.2 d	48 h



**Figure 2.** Survival analysis, over time, of *Phthorimaea absoluta* caterpillars that were topically treated with different doses of essential oils of a) *Eucalyptus staigeriana* and b) *Melaleuca alternifolia*. EO: essential oil.

### 3.4. Effects of Sublethal Doses of EOs on the Life History Parameters of *P. absoluta*

It was observed that *M. alternifolia* EO reduced the development time of the third and fourth instars when compared to the other treatments and increased the duration of the pupal stage compared to the acetone control. The EOs of *E. staigeriana* and *M. alternifolia* reduced the longevity of adults. The EO of *M. alternifolia* decreased the life cycle of females, while that of *E. staigeriana* prolonged this biological parameter (Table 3).

**Table 3.** Mean ( $\pm$  SE) of the development time of the life stages and longevity (days) of *Phthorimaea absoluta* subjected to treatments with sublethal doses of essential oils of *Melaleuca alternifolia* (76.5  $\mu$ g of essential oil.caterpillar<sup>-1</sup>) and *Eucalyptus staigeriana* (78.5  $\mu$ g of essential oil.caterpillar<sup>-1</sup>).

Parameter	Stage	Acetone		<i>Melaleuca alternifolia</i>		<i>Eucalyptus staigeriana</i>	
		N	Mean $\pm$ SE	N	Mean $\pm$ SE	N	Mean $\pm$ SE
Development time (days)	Egg	100	3.96 $\pm$ 0.02 a	120	3.00 $\pm$ 0.00a	100	3.00 $\pm$ 0.00a
	L <sub>1</sub>	100	2.06 $\pm$ 0.03 a	120	2.00 $\pm$ 0.00a	100	2.00 $\pm$ 0.00a
	L <sub>2</sub>	96	3.83 $\pm$ 0.09 a	92	6.86 $\pm$ 0.71b	51	7.39 $\pm$ 0.38b
	L <sub>3</sub>	96	2.16 $\pm$ 0.04 a	84	8.41 $\pm$ 0.59b	48	4.33 $\pm$ 0.23c
	L <sub>4</sub>	92	2.36 $\pm$ 0.07 a	55	3.74 $\pm$ 0.27ab	48	4.12 $\pm$ 0.24b
Longevity (days)	Pupa	90	8.00 $\pm$ 0.12 b	49	5.63 $\pm$ 0.23a	46	5.63 $\pm$ 0.15a
	Egg - Pupa	90	22.44 $\pm$ 0.18 a	49	12.14 $\pm$ 0.47a	35	11.83 $\pm$ 0.37ab
	Adult	90	34.02 $\pm$ 0.79 a	49	39.00 $\pm$ 0.00a	35	37.63 $\pm$ 0.58b
Life cycle (days)*	Female	50	37.36 $\pm$ 0.47 a	9	32.22 $\pm$ 1.99 b	16	37.73 $\pm$ 1.03 a
	Male	40	35.00 $\pm$ 0.66 b	40	34.85 $\pm$ 0.82 b	23	38.48 $\pm$ 0.93 a
	Egg - Adult	90	36.31 $\pm$ 0.41 b	49	34.37 $\pm$ 0.78 c	39	38.2 $\pm$ 0.69 a

The means on the same line followed by different letters differed from each other ( $p < 0.05$ ). Differences between treatments were obtained using the paired bootstrap test with 100,000 replicates. N = number of specimens at each developmental stage. \* Mean total life cycle for males and females (days) only for insects that became adults; L<sub>1</sub> = 1st instar caterpillar, L<sub>2</sub> = 2nd instar caterpillar, L<sub>3</sub> = 3rd instar and L<sub>4</sub> = 4th instar caterpillar.

Except for the oviposition period that increased in the treatment with *E. staigeriana* EO, the reproductive parameters of *P. absoluta* were not affected whose second-instar caterpillars were treated with the EOs of *M. alternifolia* and *E. staigeriana*, as there was no difference compared with treatment with acetone (Table 4).

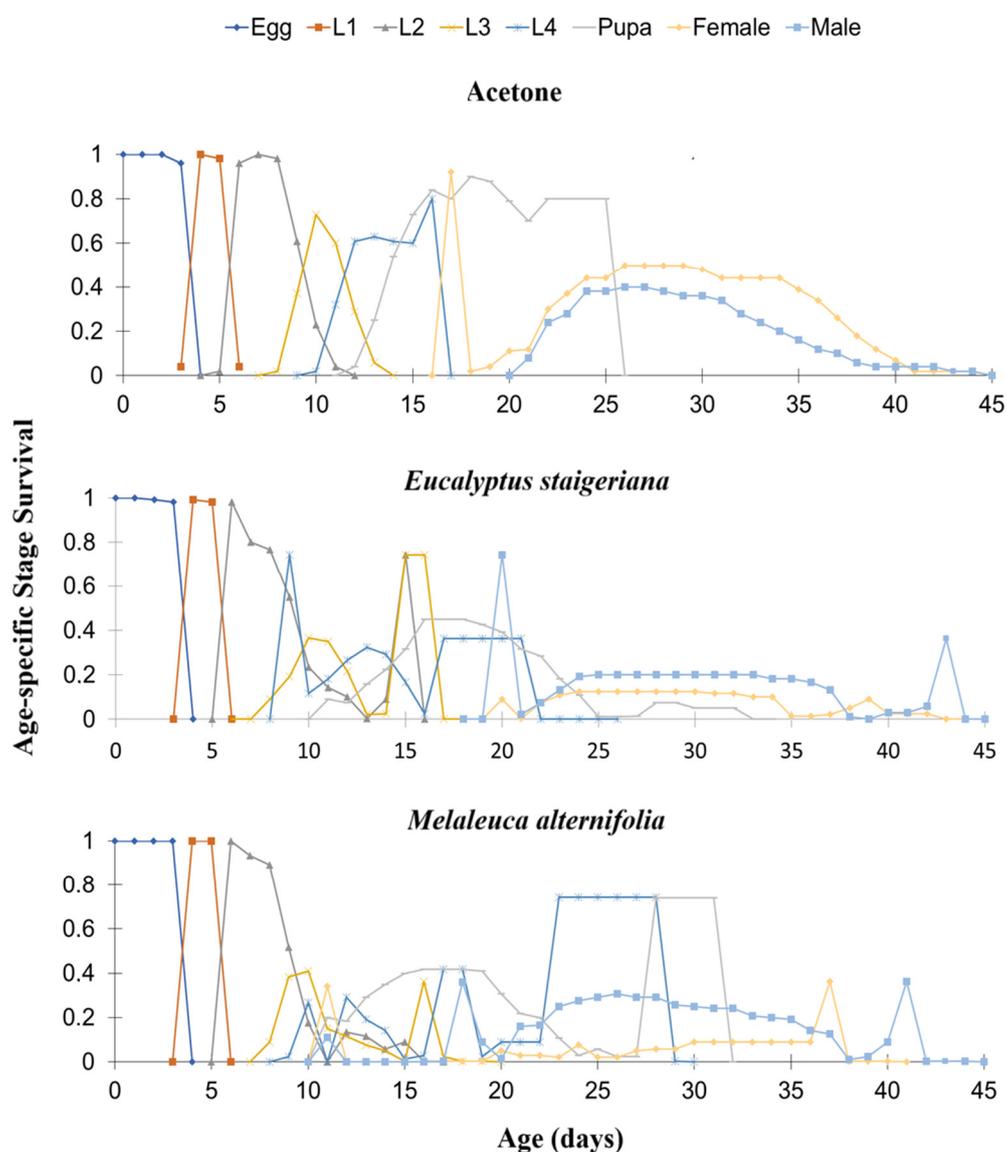
**Table 4.** Reproductive parameters of *Phthorimaea absoluta* adults derived from second-instar caterpillars that survived exposure to *Melaleuca alternifolia* (76.5  $\mu$ g of essential oil.caterpillar<sup>-1</sup>) and *Eucalyptus staigeriana* (78.5  $\mu$ g of essential oil.caterpillar<sup>-1</sup>).

Parameter	Acetone		<i>Melaleuca alternifolia</i>		<i>Eucalyptus staigeriana</i>	
	N	Mean $\pm$ SE	N	Mean $\pm$ SE	N	Mean $\pm$ SE
Total fecundity (O/F)	50	62.76 $\pm$ 4.28 a	9	46.78 $\pm$ 9.97 a	15	56.13 $\pm$ 6.23 a
Fertility (O/F)*	44	71.32 $\pm$ 3.08 a	8	60.14 $\pm$ 6.03 a	14	60.14 $\pm$ 5.12 a
Oviposition (days)	44	4.57 $\pm$ 0.26 b	8	4.00 $\pm$ 0.22 b	14	5.50 $\pm$ 0.36 a
PPOA (days)	44	1.73 $\pm$ 0.17 a	8	2.29 $\pm$ 0.64 a	14	1.71 $\pm$ 0.22 a
PPOT (days)	44	24.02 $\pm$ 0.39 a	8	22.86 $\pm$ 0.96 a	14	23.29 $\pm$ 0.42 a
TFM (O/F)	-	107	-	74	-	93
DFM (O/F)	-	70	-	50	-	39

Legend: Maximum daily fertility (DMF), maximum total fertility (FMT), adult preoviposition period (PPOA), (O/F) = eggs per female, N= number of specimens for each parameter, and total preoviposition period (PPOT). \* Total number of females that laid eggs. The means in the same row followed by different letters are significantly

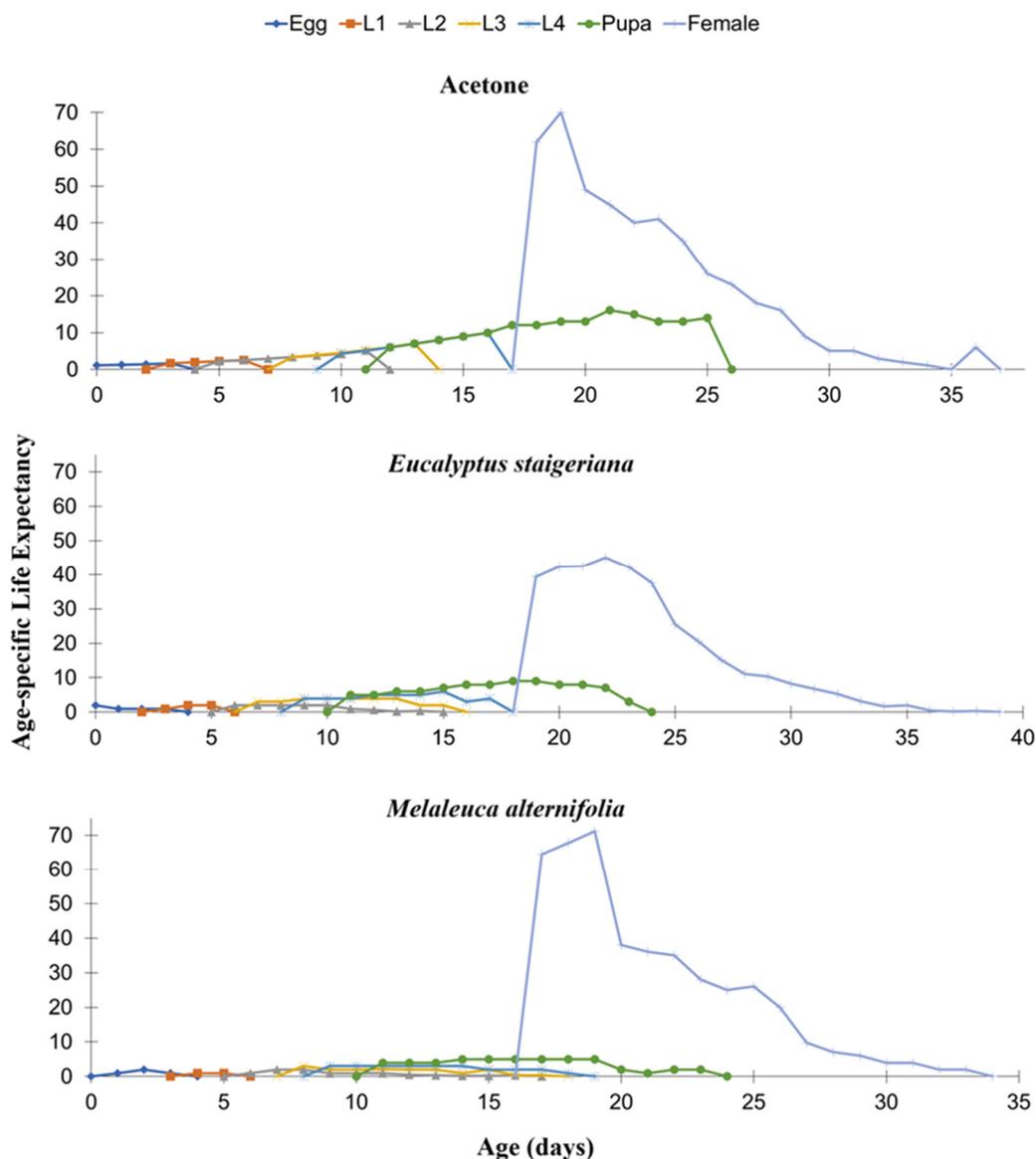
different at  $p < 0.05$ . Differences between treatments were obtained using the paired bootstrap test with 100,000 replicates.

The age-specific survival rate ( $s_{xj}$ ) was affected by the treatments. Both EOs prolonged the fourth instar and the pupal stage and reduced adult survival (males and females) (Figure 3).



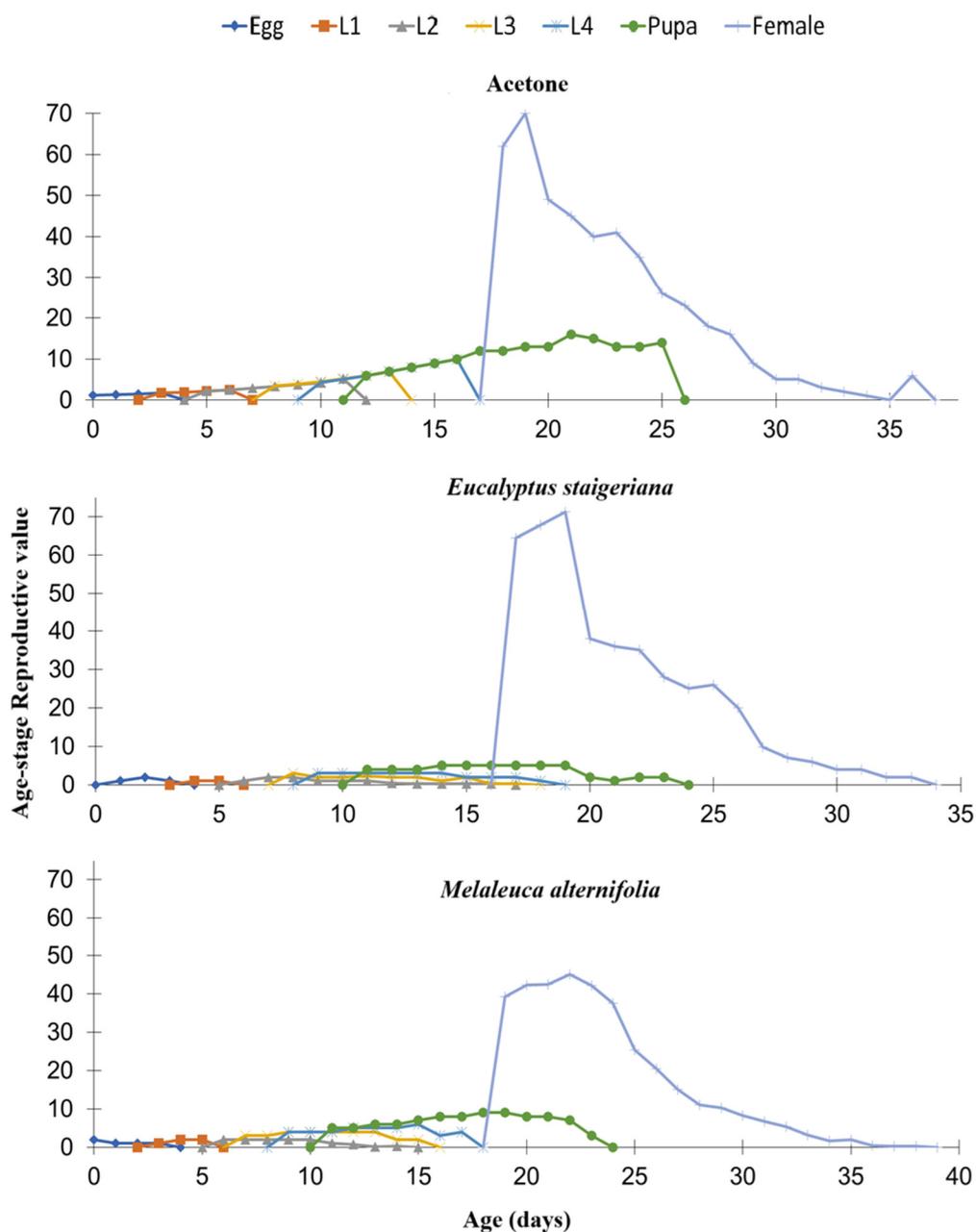
**Figure 3.** Age-specific survival rate ( $s_{xj}$ ) of *Phthorimaea absoluta* in the acetone treatments, *Melaleuca alternifolia* ( $76.5 \mu\text{g}$  of essential oil.caterpillar<sup>-1</sup>) and *Eucalyptus staigeriana* ( $78.5 \mu\text{g}$  of essential oil.caterpillar<sup>-1</sup>). L1 = 1st instar caterpillar, L2 = 2nd instar caterpillar, L3 = 3rd instar and L4 = 4th instar caterpillar.

It was observed that life expectancy by age ( $e_{xj}$ ) decreased consistently with time in the treatment with acetone. However, there were fluctuations throughout the cycle in the other treatments (Figure 4).



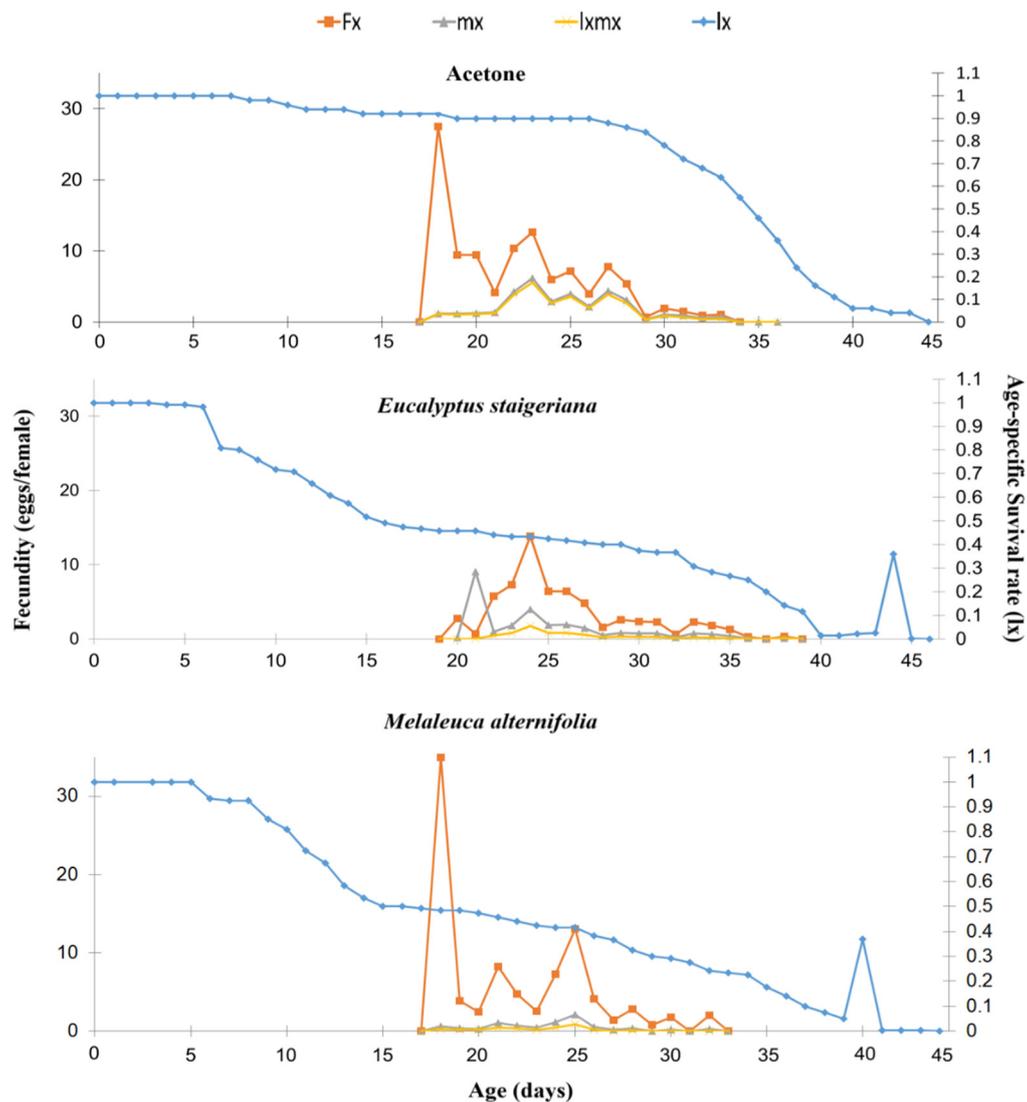
**Figure 4.** Age-specific life expectancy (exj) of *Phthorimaea absoluta* in the acetone, *Melaleuca alternifolia* (76.5  $\mu\text{g}$  of essential oil.caterpillar<sup>-1</sup>) and *Eucalyptus staigeriana* (78.5  $\mu\text{g}$  of essential oil.caterpillar<sup>-1</sup>) treatments. L<sub>1</sub> = 1st instar caterpillar, L<sub>2</sub> = 2nd instar caterpillar, L<sub>3</sub> = 3rd instar and L<sub>4</sub> = 4th instar caterpillar.

The age-specific survival rate (lx) decreased from the 6th to the 9th day of life of the insects in the treatments with EOs. However, in the treatment with acetone, this decrease was observed later on the 28th day of the life cycle. There was a delay and a lower age-specific fecundity peak (fxj) in the treatment with *E. staigeriana* EO, with approximately 15 eggs on the 24th day, while in the other treatments, the fecundity peak occurred on the 19th day of the gestation cycle, with approximately 30 eggs (Figure 5).



**Figure 5.** Age-specific survival rate ( $l_x$ ), age-specific fertility and stage of development ( $f_x$ ), age-specific fertility ( $m_x$ ) and age-specific maternity ( $l_x m_x$ ) of *Phthorimaea absoluta* in the acetone, *Melaleuca alternifolia* ( $76.5 \mu\text{g}$  of essential oil.caterpillar $^{-1}$ ) and *Eucalyptus staigeriana* ( $78.5 \mu\text{g}$  of essential oil.caterpillar $^{-1}$ ) treatments.

When analyzing the reproductive value by specific age ( $v_{xj}$ ), it was found that in the treatment with the EO of *M. alternifolia*, the females reached a lower peak, with approximately 40 eggs, while in the other treatments, the values reached approximately 70 eggs (Figure 6).



**Figure 6.** Reproductive value by age (vxd) of *Phthorimaea absoluta* in the acetone, *Eucalyptus staigeriana* (78.5  $\mu\text{g}$  of essential oil.caterpillar<sup>-1</sup>) and *Melaleuca alternifolia* (76.5  $\mu\text{g}$  essential oil.caterpillar<sup>-1</sup>) treatments. L<sub>1</sub> = 1st instar caterpillar, L<sub>2</sub> = 2nd instar caterpillar, L<sub>3</sub> = 3rd instar and L<sub>4</sub> = 4th instar caterpillar.

The intrinsic rate of increase ( $r$ ) was reduced by 50% when insects were treated with *M. alternifolia* EO. Both EOs caused lower values of the finite rate of increase ( $\lambda$ ) and net reproductive rate ( $R_0$ ). Regarding the crude reproductive rate (GRR), only the treatment with *M. alternifolia* EO differed from that with acetone (Table 5).

**Table 5.** Demographic parameters of *Phthorimaea absoluta* treated with the EO of *Melaleuca alternifolia* (76.5  $\mu\text{g}$  of essential oil.caterpillar<sup>-1</sup>) and *Eucalyptus staigeriana* (78.5  $\mu\text{g}$  of essential oil.caterpillar<sup>-1</sup>).

Demographic parameter	Acetone	<i>M. alternifolia</i>	<i>E. staigeriana</i>
	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
Intrinsic growth rate ( $r$ )	0.13 $\pm$ 0.005 a	0.05 $\pm$ 0.017 b	0.07 $\pm$ 0.010 b
Finite growth rate ( $\lambda$ )	1.14 $\pm$ 0.006 a	1.05 $\pm$ 0.018 b	1.08 $\pm$ 0.011 b
Net reproductive rate ( $R_0$ )	31.38 $\pm$ 3.800 a	3.51 $\pm$ 1.324 b	7.02 $\pm$ 1.847 b
Average generation time ( $T$ )	25.81 $\pm$ 0.422a	25.03 $\pm$ 1.011 a	26.66 $\pm$ 0.425 a
Gross reproductive rate (GRR)	35.80 $\pm$ 4.073 a	8.40 $\pm$ 3.056 b	17.42 $\pm$ 4.233 ab
Intrinsic growth rate ( $r$ )	0.13 $\pm$ 0.005 a	0.05 $\pm$ 0.017 b	0.07 $\pm$ 0.010 b

## 4. Discussion

The EOs of aromatic plants and their main constituents are considered an alternative to conventional pesticides for the population control of many arthropod pests [15,16]. The EOs of *M. alternifolia* and *E. staigeriana* showed insecticidal activity against important insect pests, such as *Lucilia cuprina* (Wiedemann, 1830) (Diptera: Calliphoridae), *C. maculatus*, *Paropsisterna tigrina* (Olivier, 1807) (Coleoptera: Chrysomelidae) and *Faex* sp. (Coleoptera: Chrysomelidae), *S. frugiperda* and *S. zeamais* [36,37,39,49,50]. Although, to the best of the authors' knowledge, this is the first report of the lethal and sublethal effects of these EOs on *P. absoluta* larvae, other studies have previously reported the toxicity of Myrtaceae-derived oils against this pest [30,31].

The estimated LD<sub>50</sub> for the EOs of *M. alternifolia* and *E. staigeriana* for *P. absoluta* was 76.5 µg of EO.caterpillar<sup>-1</sup> and 78.5 µg of EO.caterpillar<sup>-1</sup>, respectively, values higher than the LD<sub>50</sub> of 50.28 µg.caterpillar<sup>-1</sup> of the EO of *M. alternifolia* for *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae), which also caused food deterrence [41]. The achievement of high mortality rates in insect pest populations should not be the main objective of the use of botanical insecticides [17–19]. Generally, high mortality rates result from the use of high concentrations and large amounts of oil feedstock. Thus, sublethal effects on the life cycle, oviposition, reproduction and demographic parameters are also desirable in the management of insect pest populations.

The investigation of the chemical composition of natural products is very important during the process of developing new compounds, especially considering the variety of flora worldwide [17,51]. In this sense, the present study identified the constituents of the EOs of *M. alternifolia* and *E. staigeriana* using GC/MS, and it was found that compound terpinene-4-ol (42.23%) was the most abundant in the EO of *M. alternifolia*, revealing the chemotype. Several studies have shown that terpinene-4-ol is a monoterpene present in the EOs of many aromatic plants [52–54]. The principal components of the EO of *M. alternifolia* were similar to those reported by other authors, with small variations in the percentages of α-terpinene (11%), γ-terpinene (21%) and terpinene-4-ol (40%) [39,41].

The EOs of *Eucalyptus* spp. are complex mixtures of various volatile organic components whose composition and proportion vary with plant species [32,34–36,38,55–58]. The GC–MS results found for the EO of *E. staigeriana* corroborate those found in the literature [34]. There were small variations in the percentages of limonene (28.7%), geranial (15.2%) and neral (12.16%). However, qualitative and quantitative variations may occur in the chemical composition of the EO of *E. staigeriana*. For example, limonene, (E)-citral and (Z)-citral were present in the respective amounts of 28.82%, 14.16% and 10.77%, respectively [57], while in another study, chemical analysis showed that the EO of *E. staigeriana* was formed by limonene (72.9%), cineole (9.5%) and o-cymene (4.59%) [58]. Differences in the chemical composition of EOs are usually due to the location of the plant, climate, soil type, fertility regime, method used to dry the plant material and method of oil extraction [56–58].

The constituents of EOs may have different mechanisms of action in target arthropods. The EO of *M. alternifolia* inhibited the activities of the enzymes acetylcholinesterase (AChE) and glutathione-S-transferase (GST) in *H. armigera* [41]. On the other hand, terpinene-4-ol strongly inhibited Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in *Musca domestica* (Linnaeus, 1758) (Diptera: Muscidae) [59], whereas the same compound reduced the activity of the enzymes GST, catalase (CAT), and AChE and the sodium/potassium pump (Na<sup>+</sup>/K<sup>+</sup>-ATPase) in *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) [60].

Among the major constituents identified in the EO of *E. staigeriana*, limonene, a monoterpene, is known for its insecticidal activity via multiple routes of exposure, including cuticular penetration (contact toxicity), respiratory absorption (fumigant action), and ingestion [39]. Its toxic effects are frequently attributed to AChE inhibition, leading to the accumulation of acetylcholine (ACh) at synaptic junctions, which results in hyperexcitability, paralysis, and ultimately insect death [61–63]. The AChE plays a key role in the hydrolysis of ACh, thereby restoring the resting potential of the neuronal membrane and terminating nerve impulses [61–63]. The physiological consequences of this disruption include ataxia and neuromuscular failure [64,65]. In addition, Na<sup>+</sup>/K<sup>+</sup>-ATPase, an essential ion pump involved in the maintenance of electrochemical gradients in nerve cells - has also been

identified as a molecular target for botanical insecticides, due to its central role in neuronal signaling and homeostasis [65,66].

Caterpillars of *S. frugiperda*, when subjected to limonene, by topical contact and in high doses, exhibited a state of hyperexcitation, followed by paralysis and death [41], similar to the mode of action of synthetic insecticides belonging to the organophosphate and carbamate chemical groups, which are commonly used to control of *P. absoluta*. The limonene also causes adverse effects on the nutrition of *S. frugiperda*, with reduced amounts of lipids, proteins, carbohydrates and total sugars. This occurs in addition to the induction of apoptosis, which affects the reproduction of the insect and essential parameters for its survival and establishment in agricultural crops [41].

In the present study, it was observed that insects exposed to the LD<sub>50</sub> of the EOs of *M. alternifolia* and *E. staigeriana* had reduced parameters that indicate the population growth of *P. absoluta*, such as the intrinsic growth rate, finite growth rate and reproduction rate. Toxicological analyses that consider population parameters are more efficient in evaluating the impact of the compound on the insect over prolonged periods when compared to studies of the lethal effect alone [67]. Therefore, the use of life tables by developmental stage for both sexes makes it possible to more accurately determine the population changes of the arthropod pest, as this incorporates the dynamic rates of development over time and the differentiation of the individual growth stages [48,68]. Thus, the results provide evidence supporting the integration of EOs from *E. staigeriana* and *M. alternifolia* into IPM strategies targeting *P. absoluta*.

## 5. Conclusions

The EO of *M. alternifolia* has nineteen chemical compounds the majority ones being terpinene-4-ol,  $\gamma$ -terpinene and  $\alpha$ -terpinene, and that of *E. staigeriana* contains twenty-five constituents, the majority compounds being limonene, geranial and neral. The EOs of *M. alternifolia* and *E. staigeriana* are toxic to *P. absoluta* and negatively affected the duration of the pupal stage and longevity of the insects. The demographic parameters of the pest life table were also negatively affected, highlighting the intrinsic growth rate for the OE of *M. alternifolia*, which indicates a 50% reduction in population growth. Thus, the results found in the present study provide insights for the use of EOs from *E. staigeriana* and *M. alternifolia* in integrated management programs for *P. absoluta*.

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All authors read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

EOs	Essential Oils
EO	Essential Oil

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