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Effects of essential oils on egg mortality of *Neoleucinodes elegantalis* and on parasitism performance of *Trichogramma pretiosum*

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Abstract

The use of botanical products such as essential oils has gained prominence as a control method as they have insecticidal properties that can be used in the management of pests such as *Neoleucinodes elegantalis* (Guenee) (Lepidoptera Crambidae). Combining chemical and botanical products with natural enemies is a desirable strategy for managing this pest species. *Trichogramma pretiosum* Riley (Hymenoptera Trichogrammatidae) is a potential biological control agent for *N. elegantalis*. In this study, the objective was to evaluate the toxicity of *Pelargonium graveolens* L. and *Amyris balsamifera* L. oils on *N. elegantalis*, and the sublethal effects of the oils and the insecticides azadirachtin and deltamethrin on biological parameters of *T. pretiosum*, as well as to determine their selectivity and functional response. Chemical analysis reveals that the chemical profile of the *A. balsamifera* is dominated by sesquiterpenes, whereas for *P. graveolens* it is monoterpenes. The LC_{50} (0.078 µL/mL for *A. balsamifera* and 0.0081 µL/L for *P. graveolens*) and LC_{30} (0.032 µL/mL for *A. balsamifera* and 0.0034 µL/mL for *P. graveolens*) were estimated, indicating that they have a toxic effect on *N. elegantalis* eggs. *A. balsamifera* oil and the insecticide deltamethrin affected *T. pretiosum* parasitism. The type of functional response of *T. pretiosum* on host eggs treated with essential oils followed type II as in the control, whereas on host eggs treated with azadirachtin a type III functional response was exhibited. *P. graveolens* presented the best result for use in association with the parasitoid in pest management, being a promising alternative.

Key words: essential oils chemical composition, biological control, response curve, handling time, sublethal effect.

Introduction

Insect pests such as *Neoleucinodes elegantalis* (Guenee) (Lepidoptera Crambidae), are of great importance in solanaceous crops, such as tomatoes, since the damage caused by this pest can lead to the loss of 90% of production, and the costs with the use of synthetic chemical pesticides contribute to the high cost of cultivation (Gravena and Benvenga, 2003; CONAB, 2023). This makes it necessary to develop efficient methods to control this pest, in order to contribute to reducing production costs (Pavela, 2016).

In response to this need, the use of biopesticides, such as essential oils, has proven to be an interesting strategy (Pavela, 2016; Jankowska *et al*., 2017; Ferreira *et al*., 2019). Coming from the secondary metabolism of plants, essential oils have broad action on various arthropods through lethal and sublethal effects, which can cause repellence, affect fertility, cause effects on physiology, compromise development, alter behaviour and cause mortality (Li *et al*., 2014; Cruz *et al*., 2016; Pavela and Benelli, 2016; Bedini *et al*., 2019; Santana *et al*., 2022; Sombra *et al*., 2022).

Among the essential oils that have been the subject of studies to evaluate their use as bioinsecticide, geranium (*Pelargonium graveolens* L.) and sandalwood (*Amyris balsamifera* L.) can be mentioned, showing promising results against several agricultural and urban pests. This demonstrates the potential of these essential oils as an option to consider in pest control (Carroll *et al*., 2010; Park and Park, 2012; Niculau *et al*., 2013; Benelli *et al*., 2017; Rios *et al*., 2017; Saraiva *et al*., 2020).

Biological control, through the use of parasitoids of the genus *Trichogramma*, which acts by parasitizing the eggs of various insect pests such as *Tuta absoluta* (Meyrick) (Lepidoptera Gelechiidae) and *N. elegantalis*, which are important for tomato crops, is another alternative to be considered, as its action occurs through the suppression of pests before they cause damage to crops (Coelho Junior *et al*., 2016; Sigsgaard *et al*., 2017; Manohar *et al*., 2019; Oliveira *et al*., 2020).

The use of biological control associated with control with essential oils is interesting, meeting the assumptions of integrated pest management, since essential oils are considered more selective to natural enemies than conventional chemicals, which can result in increase for pest control (Pavela, 2016; Sombra *et al*., 2022).

In the literature, there are few studies that address the interaction of these control methods, which are fundamental to understanding how essential oils can influence non-target organisms, since these products contain different substances that act with multiple modes of action (Pavela, 2016; Sombra *et al*., 2022). The use of non-selective products can have a sublethal effect on *Trichogramma*, which compromises their development and affects their performance and action, which can

cause a reduction in parasitism and emergence (Parreira *et al.*, 2019; Sombra *et al.*, 2022; Ray *et al*., 2023).

These effects on natural enemies are harmful and demonstrate that the products used can interfere in the interaction of the parasitoid with its host, which reflects in a change in the behaviour of the parasitoid, and makes functional response studies essential to understand the behaviour of the natural enemy in the face of different factors (Ray *et al*., 2023).

However, studies reporting the association between the use of essential oils and *Trichogramma pretiosum* Riley (Hymenoptera Trichogrammatidae) in the control of *N. elegantalis* are scarce, such as the study by Santana *et al*. (2022), who evaluated the use of these methods together and concluded that the essential oil studied did not affect parasitism and parasitoid emergence. Aiming to fill this knowledge gap on the interaction of these control methods, this work aimed to analyse the chemical composition of essential oils from *A. balsamifera* and *P. graveolens* and their effects on *N. elegantalis* eggs, and to analyse the effects of these oils on the parasitoid *T. pretiosum*, as well as determine their selectivity and functional response.

Materials and methods

The present study was conducted at the Chemical Ecology Laboratory in the Department of Fundamental Chemistry of the Federal University of Pernambuco (DQF-UFPE), and in the Insect Histology and Physiology Laboratory in the Department of Morphology and Animal Physiology (DMFA) of the Federal Rural University of Pernambuco. Pernambuco (UFRPE), Recife, Pernambuco, Brazil.

Tomato planting

Tomato plants of the Yoshimatsu cultivar were cultivated to obtain fruits, which were used in bioassays. The seedlings were produced in polyethylene trays with 128 cells and transplanting was carried out 29 days after sowing, when the plants had four definitive leaves, on average. At that time, they were transplanted in the Garden of the Department of Agronomy at UFRPE, using a spacing of 1.0 m × 0.5 m, where they received the following cultural treatments: pruning, tying and fertilization.

Rearing of *N. elegantalis*

To begin rearing the pest in the laboratory, infested tomato fruits were collected from plantations in the cities of Camocim de São Félix (8°21'31"S 35°45'43"W) and Bezerros (8°14'00"S 35°47'49"W), in Pernambuco, Brazil. The tomato fruits were taken to the Insect Physiology Laboratory at UFRPE and were placed in plastic trays covered with paper towel and voile until the caterpillars reached the last larval instar, when they leave the fruit, passing into the pupa stage. Then, the pupae were placed in wooden and organza cages (60 × 60 × 60 cm) until the adults emerged, which were feed with a 10% sucrose solution. Green tomato fruits measuring approximately 3 cm in diameter were offered for oviposition in plastic containers (500 mL) containing water, changed daily, with tomato leaves were used to stimulate oviposition. The eggs were transferred to organic scarlet eggplant fruits (obtained from commercial establishments) approximately 7 cm long. The fruits were kept in plastic trays covered with paper towels for approximately 15 days, until the caterpillars reached the last larval instar, when they abandon the fruits and pass to the pupa stage on the paper towel. The breeding of *N. elegantalis* was maintained at 25 ± 2 °C and 70 ± 10% relative humidity, with a 12 hours photophase.

Obtaining *T. pretiosum*

The egg parasitoid *T. pretiosum* was obtained commercially from the company TopBio, Mossoró, Rio Grande do Norte, Brazil, reared on eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera Pyralidae). Obtained in the egg phase, where after emergence, the parasitoids were reared under laboratory conditions in B.O.D. chamber (Biochemical Oxygen Demand) regulated to 25 ± 2 °C, relative humidity of 70 ± 10% and photophase of 12 hours, on *N. elegantalis* eggs as host of laboratory for parasitism activities. The eggs were previously glued on to sky-blue cardboard sheets (1.5 × 7.0 cm) using a 20.0% dilution of gum arabic. The cards containing eggs were placed in glass tubes (2.5 × 8.5 cm) and were offered to the females of *T. pretiosum*, closing the tubes with PVC® plastic film, and allowing contact for 24 hours. A drop of honey was used to feed the adult parasitoids. The cards were then removed and placed in individual glass tubes until the adults emerged. The *T.pretiosum* progeny were used in the bioassays.

Obtaining essential oils and formulated insecticides

The essential oils of *A. balsamifera* and *P. graveolens*, used in the experiments, were obtained from the company Ferquima Ind. e Com. Ltda. (Vargem Grande Paulista, São Paulo, Brazil). The technical information on these products and their quality parameters (colour, purity, odour, density at 20 °C) are described in a technical report on the company's website (http://ferquima.com.br).

The insecticides azadirachtin (Azamax®) (produced by UPL do Brasil Indústria e Comércio de Insumos Agropecuários S.A., Ituverava, São Paulo, Brazil) and deltamethrin (Decis®) (produced by Bayer S.A., São Paulo, São Paulo, Brazil) were purchased from a pesticide distributor in Recife-PE. Azamax® is a natural insecticide from the group of tetranotriterpenoids with repellent and insecticidal action, by inhibiting insect feeding and growth (AGROFIT, 2024). In turn, Decis® is a contact and ingestion insecticide from the pyrethroid group, which acts on the nerve endings in the insect's body (AGROFIT, 2024).

Analysis of the chemical profile of essential oils

Analyses of the chemical composition of essential oils were carried out at the Chemical Ecology Laboratory in DQF-UFPE. The analyses used gas chromatography (GC) coupled to mass spectrometry (MS) on an Agilent 5975C Series GC/MS quadruple system (Agilent Technologies, Palo Alto, USA), equipped with a DB-5 apolar column (Agilent J&W; 60 mx 0.25 mm i.d., 0.25 µm film thickness). A 1.0 µL (2000 µg/mL) solution of each essential oil diluted in hexane was injected in a 1:50 split, as was

the hexane solution of the C8-C30 hydrocarbon standard mixture (Sigma-Aldrich®). The GC temperature was adjusted to 60 °C for 3 minutes, and then increased by 2.5 °C min^{-1} until reaching 240 °C, which was maintained for 10 minutes. The helium flow was maintained at a constant pressure of 100 kPa. The MS interface was set at 200 °C and mass spectra recorded at 70 eV (in EI mode), with a scanning speed of 0.5 scan-s from m/z 20-350 (Santos *et al*., 2014). The constituents of essential oils were quantified through GC on a Thermo Fisher Scientific (Waltham, MA, USA) Trace GC Ultra gas system equipped with a flame ionization detector (DIC), with an HB-5 column (30 mx 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was maintained at 40 °C for 2 minutes and then increased at 4 °C min^{-1} to 230 °C. The injector and detector were maintained at 250 °C. To perform quantification, 1 µL of the solution (2000 µg/mL) of each essential oil prepared in hexane was injected splitless. The composition of each component was expressed as percentages of the total peak area as recorded by GC-FID. The Retention Indices (RI) of each component of the essential oils were calculated according to the equation of van Den Dool and Kratz (1963), according to the retention times of the sample components of each essential oil, of the hydrocarbon standard (C8-C30) and the combination of each essential oil with the mixture of this standard. The components of each essential oil were previously identified by similarity in the retention index (RI) values and subsequently confirmed by comparing the respective mass spectra with those available in the GC/MS library (MassFinder 4, NIST08 and Wiley Registry™ 9th Edition) and with those described by Adams (2009).

Bioassay for determination of lethal concentrations

The treatments consisted of diluting *A. balsamifera* and *P. graveolens* oils in pure acetone with distilled water according to the methodology of Prajapati *et al*. (2005). Concentrations were obtained; 0.008; 0.015; 0.03; 0.06; 0.5; 2.0 µL/mL for *A. balsamifera* and 0.0005; 0.001; 0.0019; 0.0039; 0.0078; 0.125 µL/mL for *P. graveolens* through preliminary tests aiming to obtain mortality rates of around 5% and 95% to establish definitive concentrations. The bioassay consisted of spraying 100 eggs (0-24 hours old) using a manual sprayer, which were placed in Petri dishes. Five replications were used with 20 eggs for each treatment. Eggs from the control treatment received only pure acetone solution. The non-viability of the eggs was assessed by counting the caterpillars hatched after 72 hours (incubation period of *N. elegantalis* eggs) (Santana *et al*., 2022) after the experiment was set up, using Probit analysis using the SAS PROC PROBIT program (SAS Institute, 2002), which obtained the LC_{50} and the LC_{30} used in subsequent tests.

Effects of essential oils and formulated products on the biology and parasitism of *T. pretiosum*

In the bioassay, to evaluate the biological parameters of *T. pretiosum* and determine its selectivity regarding treatments, the LC_{50} of essential oils was used, according to a methodology adapted from Parreira *et al*. (2019) and Santana *et al*. (2022), where around 10 *N. elegantalis* eggs were collected and glued with gum arabic onto sky blue cardboard cards (1.5 × 7.0 cm). The cards were immersed for 5 seconds in solutions containing the LC_{50} of essential oils diluted in pure acetone and distilled water (0.07 µL/mL for *A. balsamifera* and 0.0081 µL/mL for *P. graveolens*) and in commercial concentrations of the formulated products practiced in Brazil available in the leaflet and authorized by MAPA (Ministério da Agricultura e Pecuária) (AGROFIT, 2024) (200 mL of azadirachtin in 100 L of water and 40 mL of deltamethrin in 100 L of water) in each treatment, plus the control (distilled water + acetone), then placed on paper towels to evaporate the solvent for 30 minutes. The cards were inserted into glass tubes (2.5 × 8.5 cm), containing a previously mated female *T. pretiosum* (0-24 hours old) and closed with plastic film, allowing parasitism for a period of 24 hours. A drop of honey was used to feed the females. The tubes were transferred to the BOD set at 25 ± 2 °C, relative humidity of 70 ± 10% and photophase of 12 hours. The females were discarded after 24 hours, and the cards were kept in the tubes until the parasitoids emerged. From the data obtained, the following were calculated: the percentage of parasitism [(number of parasitized eggs / total number of eggs) × 100]; percentage of emergence [number of black eggs detected / total number of parasitized eggs) × 100]; sex ratio [number of emerged females / (number of females + males)] and parasitoid/egg [(number of emerged parasitoids / total number of parasitized eggs)] were evaluated. The reduction in the parasitism rate (RP) of parasitoids, for each treatment, was determined by comparison with the control and calculated using the formula: RP = (1 – Rt / Rc) × 100, where: RP: average percentage of reduction parasitism (%); Rt: average parasitism value for each treatment and; Rc: average parasitism observed in the control (Hassan *et al*., 2000). Based on the reduction percentages, the formulated products and essential oils were classified according to the proposal of the "International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC)", where: 1 = harmless (< 30%), 2 = slightly harmful (30-79%), 3 = moderately harmful (80-99%) and 4 = harmful (> 99%) (Sterk *et al*., 1999). A completely randomized experimental design was used, totalling 20 replications per treatment, each replication consists of a card contain 10 eggs from the host. The results were submitted to the tests to verify the normality of their residuals and homogeneity of their variances, when the data did not present normal residuals and homogeneous variances the data were submitted to non-parametric tests (Kruskal-Wallis test). All analyses were conducted using the SAS statistical program (SAS Institute, 2002).

Functional response bioassay

To investigate the functional response of *T. pretiosum* subjected to treatment with sublethal doses of essential oils, their LC_{30} was used, according to the methodology adapted from Saber *et al*. (2020). For this, *N. elegantalis* eggs were collected and transferred to sky blue cardboard paper (1.5 × 7.0 cm) and glued with gum arabic. Subsequently, the cards with these eggs were immersed for 5 seconds in solutions containing the LC_{30} of essential oils diluted in pure acetone with distilled water (0.03 µL/mL for *A. balsamifera* and 0.0034 µL/mL for *P. graveolens*) and the recommended commercial concentration

for the insecticides (200 mL of azadirachtin in 100 L of water and 40 mL of deltamethrin in 100 L of water) in each treatment, plus the control (distilled water + acetone), were then placed on paper towels to evaporate the solvent for 30 minutes. The established egg densities were 4, 8, 16, 32 and 64 eggs/replication according to the methodology of Saber *et al*. (2020). The cards were placed in glass tubes (2.5 × 8.5 cm), containing a previously mated female *T. pretiosum* aged up to 24 hours, and closed with plastic film, allowing parasitism for a period of 24 hours. A drop of honey was used to feed the females. The tubes were transferred to the BOD set at 25 ± 2 °C, relative humidity of 70 ± 10% and photophase of 12 hours. After 24 hours, the females were removed and parasitism (black coloured eggs) was observed after 4 days with the aid of a stereomicroscope. Each treatment of essential oils, insecticides and control were bioassayed in twenty repetitions, with each repetition consisting of one female, totalling 100 females.

Holling's model (1959) served as the basis for other models to be determined to identify the type of functional response of different natural enemies. In this study, the cubic polynomial function was used to adjust the data that determine the type of functional response: $NaN_0 = \exp (P_0 + P_1 N_0 + P_2 N^2_0 + P_3 N^3_0) / [1 + \exp (P_0 + P_1 N_0 + P_2 N^2_0 + P_3 N^3_0)]$. Where: Na = number of hosts attacked by *T. pretiosum*, N_0 = different host densities (4, 8, 16, 32 and 64 eggs of *N. elegantalis*), and P_0; P_1; P_2 and P_3 are the intercept, linear, quadratic and cubic coefficients, respectively. If the linear coefficient P_1 does not differ significantly from zero, the response will be Type I (linear). If $P_1 < 0$ and differs significantly from zero, the response will be Type II (non-linear). If $P_1 > 0$ and $P_2 < 0$ the response will be Type III (non-linear) (Juliano, 2020).

To estimate the attack and manipulation rate values, an adaptation of the Rogers model (1972) was used, as explained below: $N_a = aT_t N_0 (1 + aT_h N_0)$. Where: N_a = number of hosts attacked by *T. pretiosum*, N_0 = different host densities (4, 8, 16, 32 and 64 *N. elegantalis* eggs), T_t = total experiment time (24 hours), a = attack rate (host discovery area) by *T. pretiosum*, T_h = handling time of *T. pretiosum* to its host.

Linear regression models were applied to determine the types of functional response and to estimate the parasitoid attack rate and handling time in the different essential oil treatments and control, respectively, using the SAS software (SAS Institute, 2002).

Results

Chemical profile of essential oils

The analysis generated by GC-MS identified 29 compounds for the essential oil of *A. balsamifera* (table 1)

Table 1. Chemical identification of essential oil of *A. balsamifera*.

No.	Compound	RI^L	RI^C	Compound %
1	Amorpha-4,11-diene	1449	1452	0.58
2	α-Acoradiene	1464	1457	0.15
3	β-Acoradiene	1469	1461	1.40
4	β-Chamigrene	1476	1476	0.26
5	y-curcumene	1481	1480	0.69
6	ar-Curcumene	1479	1483	2.36
7	α-Zingiberene	1493	1496	2.25
8	4-epi-Z-Dihydroagarofuran	1499	1501	1.21
9	β-Dihydro agarofuran	1503	1505	0.36
10	β-Bisabolene	1505	1509	1.08
11	7-epi-α-Selinene	1520	1518	0.57
12	β-Sesquiphellandrene	1521	1525	2.60
13	Seline-3,7(11)-diene	1545	1543	1.11
14	α-Agarofuran	1548	1547	0.83
15	Elemol	1548	1551	10.83
16	E-Nerolidol	1561	1564	0.42
17	Rosifoliol	1600	1603	0.81
18	Eudesmol <5-epi-7-epi-α->	1607	1607	1.18
19	10-epi-y-eudesmol	1622	1622	8.71
20	y-Eudesmol	1634	1634	7.36
21	Hinesol	1640	1641	0.40
22	β-Eudesmol	1649	1653	10.69
23	Valerianol	1656	1657	23.94
24	Eudesmol 7-epi-α	1662	1662	10.63
25	Eudesm-7(11)-in-4-ol	1700	1697	0.31
26	Caryophyllene acetate	1701	1702	1.42
27	Bisabolone (6R,7R)	1740	1741	0.24
28	Bisabolone (6S,7R)	1748	1748	0.77
29	Drimenol	1766	1764	1.71
	Total			94.87

RI^L- Kratz Retention Index from Literature (Adams, 2009); RI^C- Kratz Retention Index Calculated.

Table 2. Chemical identification of essential oil of *P. graveolens*.

No.	Compound	RI^L	RI^C	Compound %
1	α-Pinene	932	930	0.24
2	Limonene	1024	1027	0.17
3	Linalool	1095	1099	2.09
4	Z-Rose oxide	1106	1110	0.89
5	E-Rose oxide	1122	1127	0.35
6	Menthone	1148	1152	2.11
7	<iso->Menthone	1158	1163	4.48
8	α-Terpineol	1186	1190	0.16
9	Citronellol	1223	1228	22.07
10	Neral	1235	1241	1.18
11	Geraniol	1249	1254	12.69
12	Geranial	1264	1271	0.86
13	Citronellyl formate	1271	1275	11.63
14	Neryl format	1280	1281	1.93
15	Geranyl format	1298	1302	6.14
16	α-Cubebene	1351	1350	0.14
17	Citronellyl acetate	1350	1354	0.38
18	α-Copaene	1374	1376	0.46
19	β-Bourbonene	1387	1385	1.76
20	β-Elemene	1389	1392	0.19
21	E-Caryophyllene	1417	1419	2.73
22	β-Copaene	1430	1429	0.11
23	α-Guaiene	1437	1439	0.64
24	<6.9>Guaiadiene	1442	1444	9.93
25	Aromadendrene	1439	1449	0.80
26	α-Humulene	1452	1454	0.91
27	<allo->Aromadendrene	1458	1461	0.17
28	Geranyl propanoate	1476	1475	1.24
5	Germacrene D	1480	1482	0.89
6	β-Selinene	1489	1487	0.64
7	Valencene	1496	1495	0.19
8	α-Muurolene	1500	1500	0.28
9	δ-Amorphene	1511	1508	0.15
10	y-Cadinene	1513	1514	0.49
11	δ-Cadinene	1522	1524	1.46
12	Citronellyl butanoate	1530	1529	0.68
13	E-Cadina-1,4-diene	1533	1533	0.22
14	Furopelargone A	1538	1541	0.74
15	Geranyl butanoate	1562	1562	1.29
16	Caryophyllenoxide	1582	1584	0.81
17	Geranyl 2-methyl butanoate	1601	1603	0.34
18	Humulene epoxide II	1608	1610	0.23
19	<1,10-di-epi->-Cubenol	1618	1616	0.38
20	<1-epi->-Cubenol	1627	1630	0.23
21	Cubenol	1645	1644	0.23
	Total			95.70

RI^L- Kratz Retention Index from Literature (Adams, 2009); RI^C- Kratz Retention Index Calculated.

and 45 compounds for *P. graveolens* (table 2). The essential oil of *A. balsamifera* is predominantly formed by sesquiterpenes, 94.87% of the oil, and its main constituents are: Valerianol (23.94%), Elemol (10.83%), β-Eudesmol (10.69%), Eudesmol 7-epi-α (10.63%), 10-epi-y-eudesmol (8.71%) and y-Eudesmol (7.36%). On the other hand, the essential oil of *P. graveolens* has a majority of monoterpenes in its composition, in 95.70% of the oil, and its main compounds are: Citronellol (22.07%), Geraniol (12.69%), Citronellyl formate (11.63%), <6.9>Guaiadiene (9.93%) and Geranyl formate (6.14%).

Biotest for determination of lethal concentrations

Mortality data obtained through the concentration response curve showed χ^2 values (<7.96) and P value (>0.09), indicating that the Probit model is adequate. The LC_{50} was estimated and used in bioassays to demonstrate the toxicity of the oils to *N. elegantalis*. The LC_{50} of *A. balsamifera* was 0.07 µL/mL (0.04-0.11) and for *P. graveolens* 0.0081 µL/mL (0.006-0.010). The

LC_{30} was estimated to examine the effects of the oils on the performance of *T. pretiosum* parasitizing *N. elegantalis* eggs. The LC_{30} of *A. balsamifera* was 0.03 µL/mL (0.018-0.04) and for *P. graveolens* 0.0034 µL/mL (0.002-0.004) (table 3).

Effect of essential oils and formulated products on biology and parasitism of *T. pretiosum*

The values of emergence percentage were 87.9 ± 19.8; 73.3 ± 40.8; 49.5 ± 25.7; 100 ± 0.0 (χ^2 = 7.9119; DF = 3; P = 0.0579); For sex ratio the percentage were 0.81 ± 0.2; 0.57 ± 0.4; 0.79 ± 0.2; 1.00 ± 0.0 (χ^2 = 3.6836; DF = 3; P = 0.2977) and the values for parasitoid/egg 1.00 ± 0.0; 1.00 ± 0.0; 1.0 ± 0.0; 1.00 ± 0.0 (χ^2 = 2.1429; DF = 3; P = 0.5433); for control, *P. graveolens*, azadirachtin and deltamethrin treatments, respectively. These parameters were not affected by *P. graveolens* essential oil or formulated products, showing no significant difference by the Kruskal-Wallis Test (table 4). For the essential oil of *A. balsamifera* it was not possible to estimate this parameter.

For the parasitism parameter, the control, azadirachtin and *P. graveolens* treatments did not differ from each other. Deltamethrin and *A. balsamifera* treatments affected *T. pretiosum* parasitism (table 4). The average parasitism percentages were 21.5 ± 6.41; 17.5 ± 5.75; 8.5 ± 5.04; 0.5 ± 0.5 and 0 ± 0.0% for the control, azadirachtin, *P. graveolens*, deltamethrin and *A. balsamifera* treatments, respectively (χ^2 = 19.9654; DF = 4; P = 0.0005), by the Kruskal-Wallis Test.

The reduction in parasitism rate (RP) and toxicity classification according to IOBC standards are shown in table 4. The insecticide azadirachtin and *P. graveolens* essential oil were classified as harmless and slightly harmful (classes 1 and 2, respectively) for *T. pretiosum*. The insecticide deltamethrin and *A. balsamifera* essential oil showed a greater reduction in the parasitism rate (97.67 and 100%), being classified as moderately harmful and harmful (classes 3 and 4, respectively) for *T. pretiosum*.

Functional response bioassay

Based to the Holling's model (1959), the results obtained indicated the control, *P. graveolens* and *A. balsamifera* treatments provided a type II functional response to *T. pretiosum* parasitoids on *N. elegantalis*. The insecticide azadirachtin provided the parasitoids with a type III functional response. For the insecticide deltamethrin, it was not possible to estimate the type of functional response, as the average parasitism was below 2% in all treatments (table 5).

The average number of parasitized hosts increased with the availability of these individuals until it reached stability and showed a drop, evidencing the behaviour of each functional response curve (figure 1). The average number of parasitized individuals considering all host densities was 2.17; 1.8; 1.87; 2.69 and 0.19 for the control treatments, *A. balsamifera*, *P. graveolens*, azadirachtin and deltamethrin.

According to the estimated linear equation based to the Rogers model (1972), host handling time was estimated at 7.45 ± 2.59 hours and attack rate at 0.020 ± 0.02 for the control. For *A. balsamifera* essential oil provided a handling time of 11.31 ± 3.68 hours and an attack rate of 0.044 ± 0.08. For *P. graveolens* essential oil, the handling time was 11.38 ± 3.91 hours and the attack rate was estimated at 0.061 ± 0.06 (figure 2). As for the insecticides used, azadirachtin provided a handling time of 6.39 ± 1.83 hours and an attack rate of 0.032 ± 0.03. Since there was overlap in the confidence intervals, no difference was observed between the treatments analysed. For the insecticide deltamethrin, it was not possible to estimate, as the average parasitism was below 2% in all treatments (figure 2).

Table 3. The concentration-mortality curve of *N. elegantalis* eggs treated with the essential oils of *A. balsamifera* and *P. graveolens*. Temperature: 25 ± 2 °C, RH: 70 ± 10% and 12-hour photophase.

Treatments	N	DF	Slope (± SE)	LC_{50} (µL/mL) (CI 95%)	LC_{30} (µL/mL) (CI 95%)	(χ^2)	P
Amyris balsamifera	100	4	0.18 ± 0.13	0.07 (0.04-0.11)	0.03 (0.018-0.04)	7.96	0.09
Pelargonium graveolens	100	4	0.26 ± 0.10	0.0081 (0.006-0.010)	0.0034 (0.0027-0.0041)	4.069	0.39

N- number of eggs/treatment; DF- degrees of freedom.

Table 4. Biological parameters (mean ± SD) and percentage of reduction in parasitism of *T. pretiosum* treated with *P. graveolens*, *A. balsamifera*, azadirachtin, deltamethrin, and classification according to selectivity.

Treatments	Parasitism	Emergency	Sex Ratio	Parasitoid/egg	RP	Class
Control	21.5 ± 6.41 a	87.9 ± 19.8 a	0.81 ± 0.2 a	1.00 ± 0.0 a	-	-
P. graveolens	8.5 ± 5.04 a	73.3 ± 40.8 a	0.57 ± 0.4 a	1.00 ± 0.0 a	60.46	2
A. balsamifera	0 ± 0.0 c	-	-	-	100	4
Azadirachtin	17.5± 5.75 a	49.5 ± 25.7 a	0.79 ± 0.2 a	1.00 ± 0.0 a	18.6	1
Deltamethrin	0.5 ± 0.5 b	100 ± 0.0 a	1.00 ± 0.0 a	1.00 ± 0.0 a	97.67	3
	χ^2 = 19.965	χ^2 = 7.9119	χ^2 = 3.6836	χ^2 = 2.1429		
	DF = 4	DF = 3	DF = 3	DF = 3		
	P = 0.0005	P = 0.0579	P = 0.2977	P = 0.5433		

RP- reduced parasitism; Class- toxicity index established by IOBC/WPRS (Sterk *et al*., 1999), in with: class 1 = not harmful; class 2 = little harmful; class 3 = moderately harmful; class 4 = highly harmful.

Table 5. Coefficients estimated by a logistic regression of proportion of *N. elegantalis* eggs, exposed or not to pesticides, parasitized by *T. pretiosum*.

Treatments	Holling disc equation	χ^2	DF	*P*	Logistic Regression Coefficient				
					I (*P*)	L (*P*)	Q (*P*)	C (*P*)	Type
Control	$y = \frac{\exp[(0.00054x^2) - (0.07150x) - 0.70]}{1 + \exp[(0.00054x^2) - (0.07150x) - 0.70]}$	697.65	97	<.001	−0.70 (<.001)	−0.0715 (<.001)	0.00054 (<.001)		II
Deltamethrin	$y = 0.02$								
Azadirachtin	$y = \frac{\exp[(-0.0015x^2) + (0.051x) - 1.51]}{1 + \exp[(-0.0015x^2) + (0.051x) + 1.51]}$	611.0	97	<.001	−1.51 (<.001)	0.051 (<.001)	−0.0015 (<.001)		III
P. graveolens	$y = \frac{\exp[-(0.054x) - 0.71]}{1 + \exp[-(0.054x) - 0.71]}$	648.58	98	<.001	−0.71 (<.001)	−0.054 (<.001)			II
A. balsamifera	$y = \frac{\exp[(0.0001x^3) - (0.01x^2) + (0.18x) - 1.99]}{1 + \exp[(0.0001x^3) - (0.01x^2) + (0.18x) - 1.99]}$	621.98	96	<.001	−1.99 (<.001)	0.18 (0.003)	−0.01 (<.001)	0.0001 (<.001)	II

I- intercept; L- linear; Q- quadratic; C- cubic; Type- type of functional response.

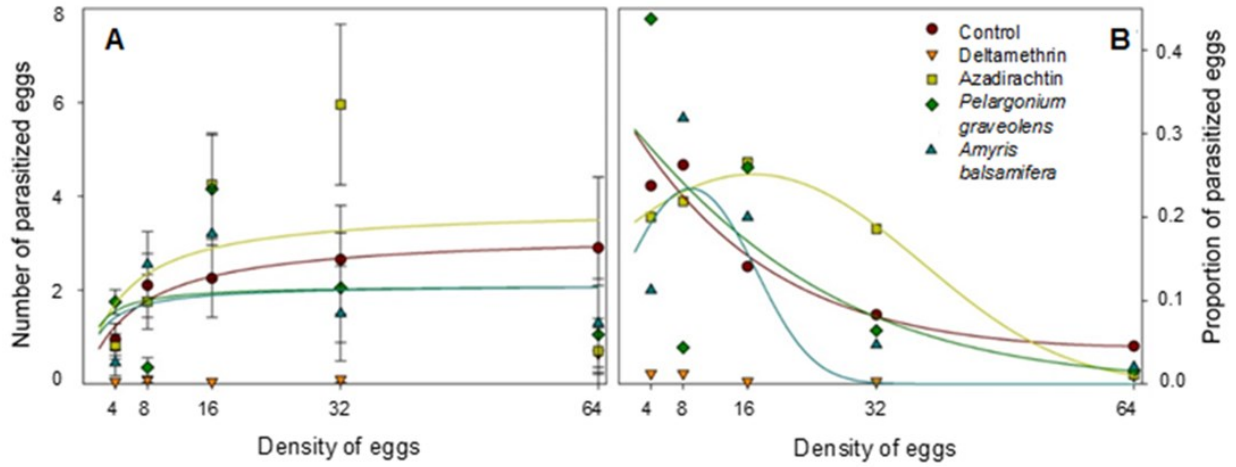

Figure 1. Functional response curves of *T. pretiosum* parasitizing increasing densities of *N. elegantalis* eggs after exposure to two essential oils and two insecticides (**A**) and proportions of parasitism of eggs by the parasitoid (**B**). The lines represent the values estimated by the functional response from the Holling equation.

Discussion and conclusions

The essential oils that are the subject of this study have in their composition the presence of terpenes, which are responsible for conferring their biological properties and characteristics capable of causing toxic action on insects (Bakkali *et al*., 2008; Pavela and Benelli, 2016). Each bioactive present in the constitution of essential oils has its own mode of action, acting in different sites, its properties can have neurotoxic action, such as inhibiting the action of acetylcholinesterase, which is one of the most important enzymes for insects, as well as acting at the cellular level, which gives it its power as a pest control agent (Bakkali *et al*., 2008; Pauliquevis and Favero, 2015; Mossa, 2016; Jankowska *et al*., 2017).

By evaluating the composition of *A. balsamifera* essential oil, 29 compounds were identified. Pino *et al*. (2006) identified the presence of 56 components for the same oil, with emphasis on Valerianol (43.8%) as the largest constituent in both studies. For *P. graveolens*, 45 compounds were identified, differing from other authors who found variations in the amounts of these substances, but maintaining the same major constituents, Citronellol and Geraniol (Ali *et al*., 2020; Niculau *et al*., 2020; Wei *et al*., 2022). The variation presented can be explained by the chemical variability found in plants of the same species, which can be influenced by several factors, including biotic and abiotic, as well as being altered by the extraction method adopted (Bakkali *et al*., 2008; Morais, 2009; Moraes *et al*., 2014; Niculau *et al*., 2020; Wei *et al*., 2022).

Different studies reinforce that the presence of sesquiterpenes and monoterpenes in oils from *A. balsamifera* and *P. graveolens*, confer their potential as a bioinsecticide, where repellence, sublethal effects and mortality were found in different insects, such as *Aedes aegypti* L., *Culex pipiens pallens* L., *Spodoptera frugiperda* (J.E. Smith) and *Bemisia tabaci* (Gennadius) biotype B (Paluch *et al*., 2009; Park and Park, 2012; Niculau *et al*., 2013; Fanela *et al*., 2016; Tabari *et al*., 2017), which corroborates its toxic potential presented regarding the treatments carried out in this study on *N. elegantalis*.

The compatibility observed between the essential oil of *P. graveolens*, the insecticide azadirachtin and the parasitoid *T. pretiosum*, through parasitism rates (17.5 and 8.5%, respectively) indicates that these products did not affect the search process and acceptance of the host carried out by the parasitoid female when compared to the

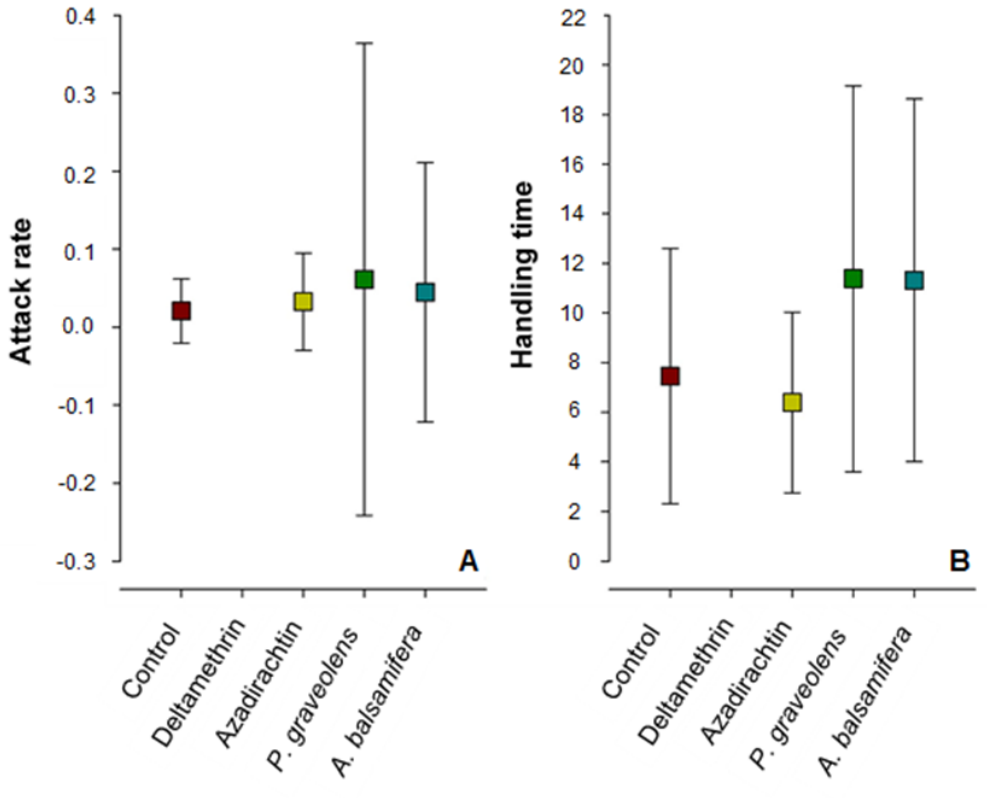

Figure 2. Attack rate (in units of hosts parasitized by the parasitoid per unit of search time) (**A**) and handling time (average time for parasitism of hosts) (**B**). Bars correspond to the confidence interval.

control (21.5%). These values indicate a performance close to what has already been found in the field by Oliveira *et al*. (2020) and under laboratory conditions by Santana *et al*. (2022) who found parasitism rates ranging from 3.8 to 19% of parasitized eggs.

The essential oil of *P. graveolens* and the insecticide azadirachtin were classified as slightly harmful and innocuous, respectively. The chemical properties present in these products, whose compounds derive from monoterpenes and tetraterpenoids, can cause toxic effects on the pest, as well as attracting natural enemies (Miresmailli and Isman, 2014). Similar results were observed in different studies with essential oils of *Lippia origanoides* Kunth, *Copaifera officinalis* L. and azadirachtin, where it was found that they did not compromise the parasitism and emergence rates of *T. pretiosum* and were considered selective for the parasitoid (Almeida *et al*., 2010; Santana *et al*., 2022; Sombra *et al*., 2022).

The reduction in parasitism caused by the essential oil of *A. balsamifera* and the insecticide deltamethrin, which were classified as harmful and moderately harmful, respectively, reinforces the importance of knowing the behaviour of the parasitoid, since, in order to guarantee its offspring, it evaluates the potential of the host, identifying the presence of harmful chemical substances, which results in egg rejection and, as a consequence, non-parasitism. This occurs due to residues that can cause both adult repellence, as it poses a risk to the development of immature animals, and their mortality (Thubru *et al*., 2016; Asma *et al*., 2018; Parreira *et al*., 2018).

The normality observed for the parameters of emergence, parasitoid/egg and sex ratio indicates that the development cycle of *T. pretiosum* was not affected by the tested products. Similar studies found that this fact may be associated with no change in the nutritional composition of the eggs, which maintained the availability of nutrients necessary for the embryo and represents an increase in the parasitism potential of *T. pretiosum* females (Parreira *et al*., 2019; Bibiano *et al*., 2022; Sombra *et al*., 2022).

The evaluation of the functional response appears as an alternative to contribute to the handling of the parasitoid as a control method, based on a suggestion of how the natural enemy behaves, and whether the association of the different methods adopted will be efficient (Rashidi *et al*., 2018; Oliveira and Reigada, 2023). The bioassay carried out in this study showed two predominant types of responses, type II for the essential oils of *P. graveolens* and *A. balsamifera* and for the control, and type III response for the insecticide azadirachtin. These types of curves are common to represent the behaviour of parasitoids from the Trichogrammatidae family and have also been observed in parasitoids subjected to treatments with different essential oils and insecticides (Paes *et al*., 2018; Asadi *et al*., 2018; Milanez *et al*., 2018; Saber *et al*., 2020; Heidarian *et al*., 2021; Oliveira and Reigada, 2023), which confirms that the presence of these products interferes with the action of the parasitoid.

Type II responses allow us to infer that this parasitoid will perform better in the presence of low host densities, indicating that there is a decreases stabilization in the parasitism rate with increase eggs density (Holling, 1959; Oliveira and Reigada, 2023; Ray *et al*., 2023). This behaviour reveals that to provide better results it would be appropriate the inundative releases of parasitoid aimed at immediate pest reduction (van Lenteren, 2012; Manohar *et al*., 2020).

For type III curves, it is possible to estimate that the natural enemy will be efficient regardless of host availability, represented by an increase in its activity as the density of available eggs increases followed by stability after satiety (Holling, 1959; Dong *et al*., 2017). Behaviours that were evidenced by the response curves observed in this study.

For the deltamethrin treatment, it was not possible to estimate a functional response curve, which indicates the influence of sublethal effects caused by the insecticide. Similar results obtained in other studies demonstrate the toxicity of this insecticide for the parasitoid, presenting a residual persistence that can influence host rejection and affect the development of the immature stages of this natural enemy (Delpuech and Delahaye, 2013; Thubru *et al*., 2016; Tabebordbar *et al*., 2020).

In the present study, there was no difference between the treatments regarding handling time and attack rate. These parameters influence parasitism rates, which for *N. elegantalis* tend to be low, as evidenced in different studies, indicating that the parasitoid takes longer time to parasite this host (Oliveira *et al*., 2020; Saber *et al*., 2020; Santana *et al*., 2022). Manohar *et al*. (2020) and Nikbin *et al*. (2014) observed low values for handling time and attack rate in *T. absoluta* and *E. kuehniella*, respectively, using the Trichogrammatidae family, which demonstrates the potential of the parasitoid.

The results presented here suggest that among the oils and insecticides tested, *P. graveolens*, in sublethal concentrations, and the insecticide azadirachtin were promising. The oil stood out for presenting toxicity to the pest and not affecting the performance of *T. pretiosum*, showing no adverse effects, and can be recommended for simultaneous use with the parasitoid, being a starting point for field studies to attest their viability.

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