Journal of Plant Protection Research eISSN 1899-007X

ORIGINAL ARTICLE

Effects of essential oils from the Brazilian pepper tree, eucalyptus and citronella on brassica aphids *Brevicoryne brassicae* and *Myzus persicae* (Hemiptera: Aphididae) and their parasitoid *Diaeretiella rapae* (Hymenoptera: Braconidae)

Suellen Godoy da Silva, Josué Sant'Ana, Simone Mundstock Jahnke, Carlos Diego Ribeiro dos Santos*

Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil

Vol. 63, No. 3: 286–296, 2023

DOI: 10.24425/jppr.2023.146879

Received: December 08, 2022
Accepted: April 10, 2023
Online publication: September 11, 2023

*Corresponding address:
carlosdiegoribeirodossantos@gmail.com

Responsible Editor:
Natasza Borodynko-Filas

Abstract

Essential oils (EOs) are alternatives to synthetic insecticides used to control aphids that attack brassica species. However, the effects of species such as the Brazilian pepper tree (BPEO) *Schinus terebinthifolius* (Raddi), lemon eucalyptus tree (LEEO) *Eucalyptus citriodora* (Hook), and citronella grass (CGEO) *Cymbopogon winterianus* (Jowitt) on these organisms, as well as on beneficial insects, has been poorly studied. This work was aimed to evaluate the activity of BPEO, LEEO, and CGEO, at concentrations of 0.5% and 1%, on aphids *Brevicoryne brassicae* (Linnaeus) and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) applied on leaf discs and/or cabbages, as well as the chemotaxic effects on its natural enemy *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae). The results showed that the essential oil of *C. winterianus* had a higher mortality rate for *B. brassicae* (100%) (0.5%, 48 h) and *M. persicae* (98.99%) (1%, 48 h). The average number of aphids (both species) found on cabbage leaf discs treated with 0.5% and 1% of the three essential oils (separately) was always lower than those found on leaf discs treated with water. Essential oils at 1% presented significantly higher mortality rates for *B. brassicae* and *M. persicae* than the control treatment. Females of *D. rapae* were attracted to plants of green cabbage with essential oil (0.5%) of *S. terebinthifolius*, but did not respond to *E. citriodora* and were significantly responsive to plants sprayed with water when contrasted with those in the presence of *C. winterianus* oil.

Keywords: aphids, chemotaxis, *Cymbopogom winterianus*, *Eucalyptus citriodora*, natural enemies, *Schinus terebinthifolius*

Introduction

Cabbage, *Brassica oleracea* (L.) var. *acephala* (Brassicaceae), is a commercial species that is often attacked by aphids that cause severe damage and productivity losses (Munthali and Tshegofatso 2014). *Brevicoryne brassicae* (L.) and *Myzus persicae* (Sulz.) (Hemiptera: Aphididae) are known as brassica pests that can cause chlorotic stains, holes, and shriveling of leaves, leading to a decrease in the photosynthetic area, as well as the injection of toxic substances and/or transmission of viruses (Holtz *et al*. 2015; Mpumi *et al.* 2020). The natural occurrence of the parasitoid species *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae) has already been observed in *B. brassicae* and *M. persicae* on cabbages (Souza *et al*. 2017).

There is a growing demand for products without synthetic insecticides as well as for more organically grown food. This has brought much attention to sustainable alternatives for pest control (Isman 2020), such as the use of essential oils (EOs) which are promising for pest control (Pavela and Benelli 2016).

These substances are made up of complex mixtures of chemical compounds, mainly mono- and sesquiterpenes derived from the secondary metabolism of plants (Pavela 2008). Since they are natural, biodegradable, and have little impact on mammals, botanical insecticides are recommended for organic food production and can be an important tool for integrated pest management (Marrone 2019). It has already been used on aphid species such as *B. brassicae* (Motazedian *et al.* 2014) and *M. persicae* (Toledo 2019).

The Brazilian pepper tree *Schinus terebinthifolius* (Raddi) (Anacardiaceae), lemon eucalyptus tree *Eucalyptus citriodora* (Hook) (Myrtaceae), and citronella grass *Cymbopogon winterianus* (Jovitt) (Poaceae) (Hussein *et al.* 2017) are plants that produce EOs and have insecticidal and/or repellent action. The insecticidal effect of *S. terebinthifolius* has been reported for two species of whitefly, *Bemisia tabaci* (Gennadius) and *Trialeurodes ricini* (Misra) (Hemiptera: Aleyrodidae) (Hussein *et al.* 2017) and for the cotton aphid, *Aphis gossypii* (Glover) (Hemiptera: Aphididae) (Andrade *et al.* 2013). The insecticidal activity of EOs from *E. citriodora* and/or its major compound, citronellal, has already been observed in nymphs of *M. persicae* and *Frankliniella schultzei* (Trybon) (Thysanoptera: Thripidae) (Costa *et al.* 2015). *Cymbopogon winterianus* is also reported to have a deadly effect on *M. persicae* (Pinheiro *et al.* 2013). Little is known about the impact that EOs have on natural enemies of aphids that attack cabbages. Pavela (2018) found that the oil of *Foeniculum vulgare* (Miller) (Apiaceae) was selective for adults and larvae of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). For *D. rapae*, the ethanol extract from *Acmella oleracea* (Murr.) (Asteraceae) caused no mortality (Gouvêa *et al.* 2019).

Schinus terebinthifolius, *E. citriodora,* and *C. winterianus* are abundant plants in Brazil which produce essential oils. However, their essential oils have been poorly studied, especially against aphids, as well as natural enemies associated with cabbage cultivars. Therefore, this work was aimed to evaluate how the essential oils from the Brazilian pepper tree, lemon eucalyptus tree, and citronella grass (all in two concentrations), affect *B. brassicae* and *M. persicae*, as well as their parasitoid, *D. rapae*.

Materials and Methods

Essential oils

Brazilian pepper tree ripe fruits of *S. terebinthifolius* were collected from a tree in an urban area in Porto Alegre, RS (30°02'48"S and 51°08'17"O). Fresh leaves of *C. winterianus* and *E. citriodora* were collected from plants cultivated without fertilizer and pesticides, in Ivoti, RS (29°36' 00"S and 51°09'13.68"O). The Brazilian pepper tree fruits were removed from the panicles and dried at room temperature for 72 h, and oil extraction occurred immediately after this period. Citronella grass and lemon eucalyptus tree leaves were collected and kept under refrigeration (4°C) for approximately 1 week, until extraction. Only entire leaves were used.

The essential oils were extracted by hydrodistillation through "steam dragging", using the Êxodo Tecnologia® model 3000 Clevenger device, coupled to a 3 l round bottom volumetric flask. To increase the surface area of plant materials, the Brazilian pepper tree fruits (600 g) and the fresh lemon eucalyptus tree leaves (180 g) were crushed in a blender, and the fresh citronella grass leaves (180 g) were cut with scissors. Each material was placed separately inside the flask and 1.5 l of distilled water was added. After 3 h of hydrodistillation (Oliveira Junior *et al.* 2013), the essential oils of each species were separated from the liquid phase (hydrolate) with a 10 ml graduated glass pipette and suction pump. The EOs were stored in glass containers wrapped with aluminum foil at 4°C for further examination and use.

Chemical analysis

The chromatographic profiles and chemical compositions of essential oils were analyzed by the researcher Márcia Ortiz M. Marques at the Agronomic Institute of Campinas/(IAC) in São Paulo, Brazil (Tables 1–3). In order to analyze the EOs, 1µL of solution (1 µl of the essential oil in 1ml of ethyl acetate-chromatographic grade) was injected for each oil into a GC-DIC (Shimadzu CG-2010, DB-5 column, i.d. 30 m × 0.25 mm, 0.25 µm film; Jand & W Scientific, Folsom, CA, USA) through an injector (AOS-Shimadzu), split mode (1/20) and helium as carrier gas. The initial ramp temperature was 60°C for 1 min with a gradual increase of 3°C/min to 240°C. The detector temperature was 300°C. Data were collected using Class-CG software and processed with Origin 5.0 software (Originlab Coorporation, Northamptom, MD, USA). For qualitative analysis of the compounds, selected samples were injected into a gas chromatograph coupled to mass spectrometry (Shimadzu QP-5000 GC-MS) with electron impact ionization (ionization energy 70 eV) with a quadrupole analyzer. The extracts were injected in split mode, 1/20 and helium was used as carrier gas. A fused silica capillary column was used: OV-5 (Ohio Valley Specialty Chemical, Inc. 30.0 m × 0.25 mm × 0.25 µm). The temperature program and column were identical to those used in GC analysis. The substances were identified by comparing their mass spectra with the database of the GC-MS system (Nist62.LIB,

Wiley139.LIB) and literature and by comparing their calculated linear retention indices (LRIs). A mixture of n-alkanes (C9-C24 Sigma Aldrich 99%), analyzed under the same operational conditions as the essential oils, was used to calculate the linear retention indices (LRIs), applying the equation of Van den Dool & Kratz (1963). Whenever possible, the synthetic standard was injected. All bioassays were performed with the same oils analyzed (Tables 1–3).

Cabbage cultivation

The seedlings of *B. oleraceae* var. *acephala* were transplanted into 1.5 l pots containing a 2 : 1 ratio of black soil and Carolina Soil® commercial substrate and irrigated according to individual plant needs. Cabbage seedlings were also planted in the field, in a 3 × 1.5 m flowerbed with 0.80 m spacing between rows and 0.5 m between plants, in the experimental garden of the Agronomy College of Federal University of Rio Grande do Sul in Porto Alegre, Brazil (30°4'28.04"S and 51°8'6.87"O). Weeds were removed from the crop during establishment. Phytosanitary and fertilization treatments were not performed on plants grown in greenhouses or in fields.

Aphid rearing

The aphids (*B. brassicae* and *M. persicae*) were also collected from an experimental garden of the Agronomy College of Federal University of Rio Grande do Sul in July 2018 and later reared on cabbage seedlings and leaf discs in a laboratory until December 2019. Aphids were transferred to the substrates with a fine bristle brush (n° 00). Nymphs were moved to new substrates and left on it for approximately 7 days until they became adults (1.5–2 mm). The infested plants and discs were kept under controlled environmental conditions (25 ± 1°C, 65 ± 10% relative humidity, 14 h photophase). Leaf discs were obtained from field-grown plants 50 days after planting. The cabbage discs (9 cm Ø) were packed in Petri dishes lined with 80 g (9 cm Ø) filter paper, which was moistened daily with distilled water. Fifteen apterous adult aphids were placed on each disc, according to the methodology proposed by Costa *et al.* (2013). The discs were replaced when they started to turn yellow, every 3 days.

Parasitoid rearing

The parasitoids were obtained by collecting mummies from *B. brassicae* and *M. persicae* on cabbage from the experimental garden of the Faculty of Agronomy. The mummies were stored in individual glass tubes (2 cm Ø × 6 cm) and kept in a climatic chamber (25 ± 1°C, 65 ± 10% relative humidity, 14 h photophase) until they emerged. The specimens were identified as *D. rapae* according to the dichotomous key of Rakhshani *et al.* (2015), deposited in the collection of the Museum of Natural Sciences of Secretariat for Environment and Infrastructure (SEMA), Porto Alegre, RS, Brazil. (Voucher specimen numbers: *D. rapae* female (MCN96835) and male (MCN96836)).

Table 1. Chemical composition (relative %) of the essential oil of *Schinus terebinthifolius*

Peak	Retention time [min]	LRI	LLRI	Compound	Relative %
1	6.688	931	932	α-pinene	5.38
2	7.800	967	–	un	0.27
3	7.888	970	969	sabinene	0.67
4	8.021	974	974	β-pinene	0.77
5	8.412	988	988	myrcene	42.64
6	8.926	1002	1002	α-phellandrene	4.06
7	9.148	1009	–	un	33.36
8	9.638	1020	1020	*p*-cymene	1.81
9	9.821	1025	1025	β-phellandrene	5.61
10	12.149	1084	1086	terpinolene	0.63
11	26.438	1411	1417	*E*-caryophyllene	2.78
12	28.968	1472	1478	γ-murolene	1.57
13	30.649	1514	1522	δ-cadinene	0.46
Total identified					66.38
Total unidentified					33.67

LRI – linear retention index (27); LLRI – Literature linear retention index (Adams 2007); un – unidentified

Table 2. Chemical composition (relative %) of the essential oil of *Eucalyptus citriodora*

Peak	Retention time [min]	LRI	LLRI	Compound	Relative %
1	6.018	911	908	isobutyl isobutyrate	0.43
2	6.689	932	932	α-pinene	0.15
3	8.022	974	974	β-pinene	0.62
4	9.914	1027	1026	1.8-cineol	0.40
5	10.954	1054	1054	γ -terpineol	0.18
6	12.555	1095	1095	linalool	0.83
7	14.529	1140	1145	isopulegol	6.14
8	14.851	1150	1148	citronellal	79.92
9	15.011	1154	–	un	3.41
10	15.536	1163	1167	*neo-iso*-isopulegol	0.37
11	18.076	1223	1223	citronellol	7.17
12	26.436	1412	1417	*E*-cariofileno	0.37
Total identified					96.58
Total unidentified					3.41

LRI – linear retention index (27); LLRI – Literature linear retention index (Adams 2007); un – unidentified

Table 3. Chemical composition (relative %) of the essential oil of *Cymbopogon winterianus*

Peak	Retention time [min]	LRI	LLRI	Compound	Relative %
1	9.808	1025	1024	limonene	2.36
2	12.556	1095	1095	linalool	0.70
3	14.530	1139	1145	isopulegol	0.96
4	14.823	1148	1148	citronellal	48.04
5	15.004	1153	–	un	0.51
6	18.601	1222	1223	citronellol	11.14
7	18.641	1233	1235	neral	0.37
8	19.218	1248	1249	geraniol	17.15
9	19.929	1263	1264	geranial	0.38
10	23.520	1346	1350	citronellil acetate	1.28
11	24.819	1376	1379	geranyl acetate	0.95
12	25.262	1385	1389	β-elemene	1.04
13	28.962	1472	1478	γ-murolene	0.93
14	30.639	1513	1513	δ-cadinene	0.9
15	31.639	1538	1548	elemol	9.55
16	32.693	1564	–	un	1.21
17	35.221	1624	1638	un	0.85
18	35.724	1636	1644	α-muurolol	1.68
Total identified					97.43
Total unidentified					2.67

LRI – linear retention index (27); LLRI – Literature linear retention index (Adams 2007); un – unidentified

Parasitoids were reared in PVC cages (3 l) with side and top openings covered with voile fabric, containing a cabbage seedling (7–8 completely expanded leaves) infested with *B. brassicae*. Drops of honey were placed at the top of the cage to feed the parasitoids. Virgin females, 24 h old, were used in bioassays.

Bioassays

Chemotaxis tests, with double-choice olfactometer and mortality, were performed in climatic chambers at 25 ± 1°C, 65 ± 10% relative humidity and 14 h photophase. The olfactometer chemotaxis bioassay was conducted in a climatized room (25 ± 1°C, 65 ± 10% relative humidity) during the photophase, with an incident light source (60 W, approximate luminance of 300 lux).

Chemotaxis of aphids in leaf discs with EOs

Cabbage discs (3.5 cm, diameter) were immersed for 30 s in the tested EO's aqueous solutions at concentrations of 0.5 and 1% (Lima *et al*. 2008). To dilute the oil Emulsifier Tween 80® (10 µL/mL) was added. Water plus Tween 80® was used as a control. Discs were dried on filter paper at room temperature for approximately 10 min. A pair of discs (always containing one with oil vs. control) were placed in Petri dishes (9 cm Ø) containing 6 ml of agar (100 ml of distilled water + 2.5 g of agar). Each plate contained a rectangular opening in the lid (2 × 3.5 cm) and was covered with voile fabric. The discs were equidistantly separated by a piece of paper (2 × 1.3 cm), with ten wingless viviparous females of the same age (24–48 h) of *B. brassicae* or *M. persicae* per plate, deposited in the middle of the paper. The chemotactic responses of aphids were evaluated after 1, 3, 6, and 24 h, quantifying the number of adult insects on each leaf disc. Ten repetitions were performed for each test (type of essential oil vs. water), totaling 100 females.

Insecticidal effects of EOs on aphids

Leaf discs (9 cm Ø) of cabbages treated according to the experimental protocol already described were placed on filter paper moistened with distilled water in Petri dishes (9 cm Ø). Each disc was infested with ten wingless viviparous females of *B. brassicae* or *M. persicae* (25 ± 1°C, 65 ± 10% relative humidity). Ten repetitions per treatment were performed. The number of dead aphids per plate was counted 24 and 48 h after exposure to EOs. Aphids that did not respond when touched with a fine bristle brush (n° 00) were considered dead.

Chemotaxis of *D. rapae* to plants with EOs

The chemotaxis response of *D. rapae* virgin females up to 24 hours old, was tested in a double-choice glass "Y" olfactometer, with 2 cm of diameter, the initial arena of 20 cm, and forked with two arms of 8 cm each. Airflow was put into the system with a propellant connected to a flowmeter, at a rate of 0.1 l/min. At the end of each olfactometer arm, a glass chamber (29.5 × 12.5 cm Ø) was attached, where a fully expanded, 7–8 leaf cabbage plant (40 days) was placed. The treatments contained healthy plants (without infestation) sprayed with one of the aqueous solutions with essential oils of the Brazilian pepper tree, lemon eucalyptus tree, or citronella grass (all at 0.5%) contrasted with plants sprayed with water (control). Tween 80® (10 µl/ml) emulsifier was added to aqueous solutions in all treatments. The insects tested were placed individually in glass tubes and acclimated for 1 h in the test room before the experiments began. Thus, the parasitoid choice between the plants sprayed with each of the essential oils versus the control was evaluated.

A positive response (first choice) occurred when a single insect reached 3 cm into the olfactometer arms and remained there for, at least, 30 s. Insects that did not move within the first 5 min or did not reach either arm were considered non-responsive. The arena was inverted (180° rotation) every 5 repetitions and was washed with neutral soap and alcohol 70% and it was dried in a sterilization oven at 150°C after every ten repetitions. A total of 40 repetitions (40 individuals) was performed for each contrast.

Statistical analysis

The chemotaxis choice percentages were compared using the Chi-square test (independence test) and the numbers of aphids on the leaf discs were compared with a G test (adherence test), at a 5% significance level, using BioEstat 5.0® software. Mortality data was analyzed for normality with the Shapiro-Wilk test and compared by Tukey and Kruskal-Wallis test, according to homoscedasticity data and at 5% probability level, using the R Studio® program (version 3.4.4). Mortality was corrected using Abbott formula (Abbott 1925).

Results

Chemotaxis of aphids to leaf discs with EOs

The average number of *B. brassicae* (Fig. 1) and *M. persicae* (Fig. 2) found on cabbage leaf discs treated with both concentrations of the three essential oils and during the four sampling periods was always lower than those found on leaf discs treated with water ($p < 0.001$) (Figs 1 and 2).

Insecticidal effects of EOs on aphids

The three essential oils (1% concentration) presented significantly higher mortality rates for *B. brassicae* and

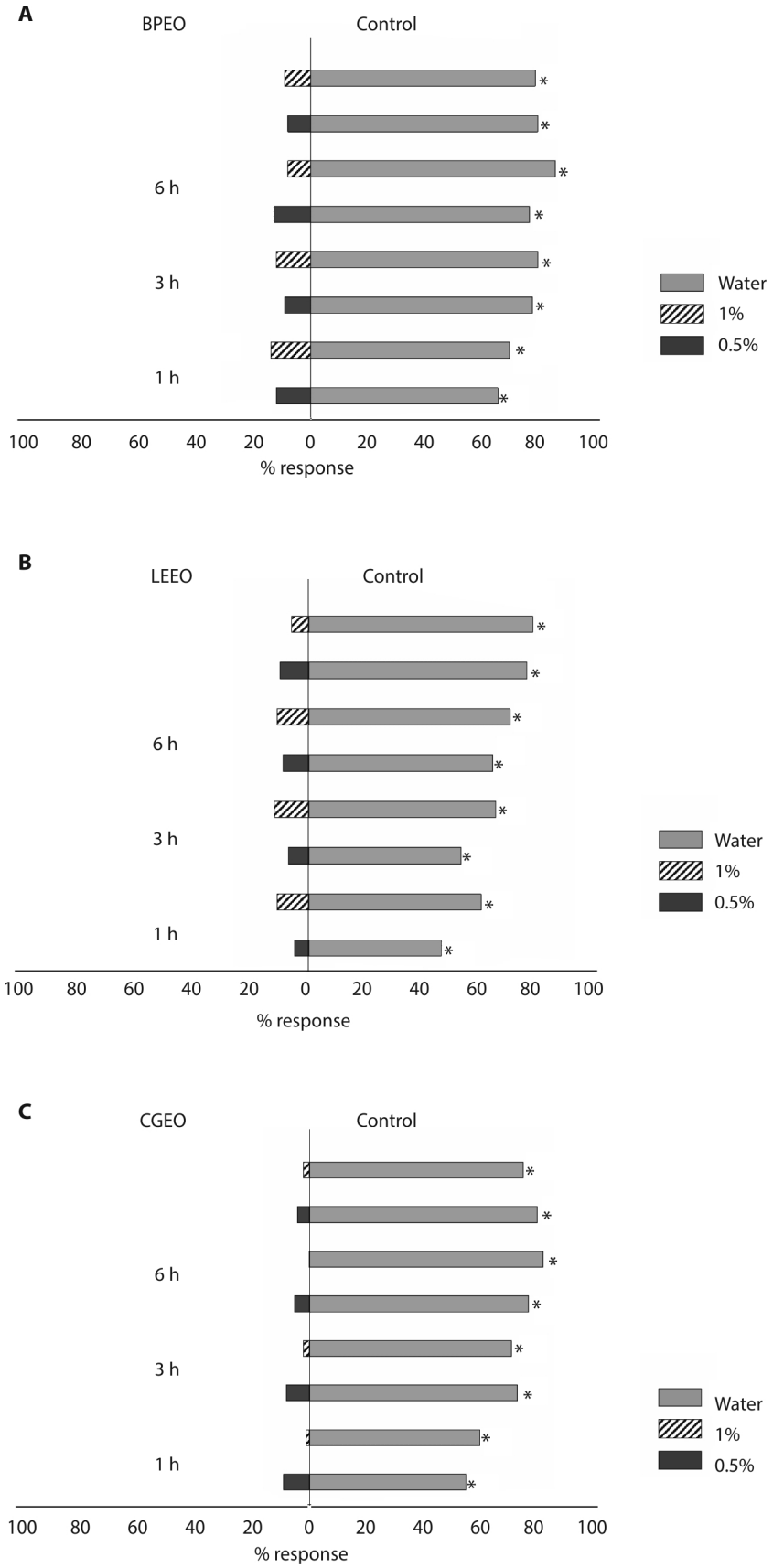

Fig. 1. Chemotactic response (%) of *Brevicoryne brassicae* to cabbage leaf discs (*B. oleracea* var. *acephala*) packaged in Petri dishes and treated with 0.5% and 1% of: (A) BPEO – Brazilian pepper tree essential oil (*Schinus terebinthifolius*); (B) LEEO – lemon eucalyptus tree essential oil (*Eucalyptus citriodora*); and (C) CGEO – citronella grass essential oil (*Cymbopogon winterianus*). Bars followed by asterisk indicate significant difference (χ^2, $p < 0.001$). Total number of insects evaluated in each contrast = 100 (10 repetitions)

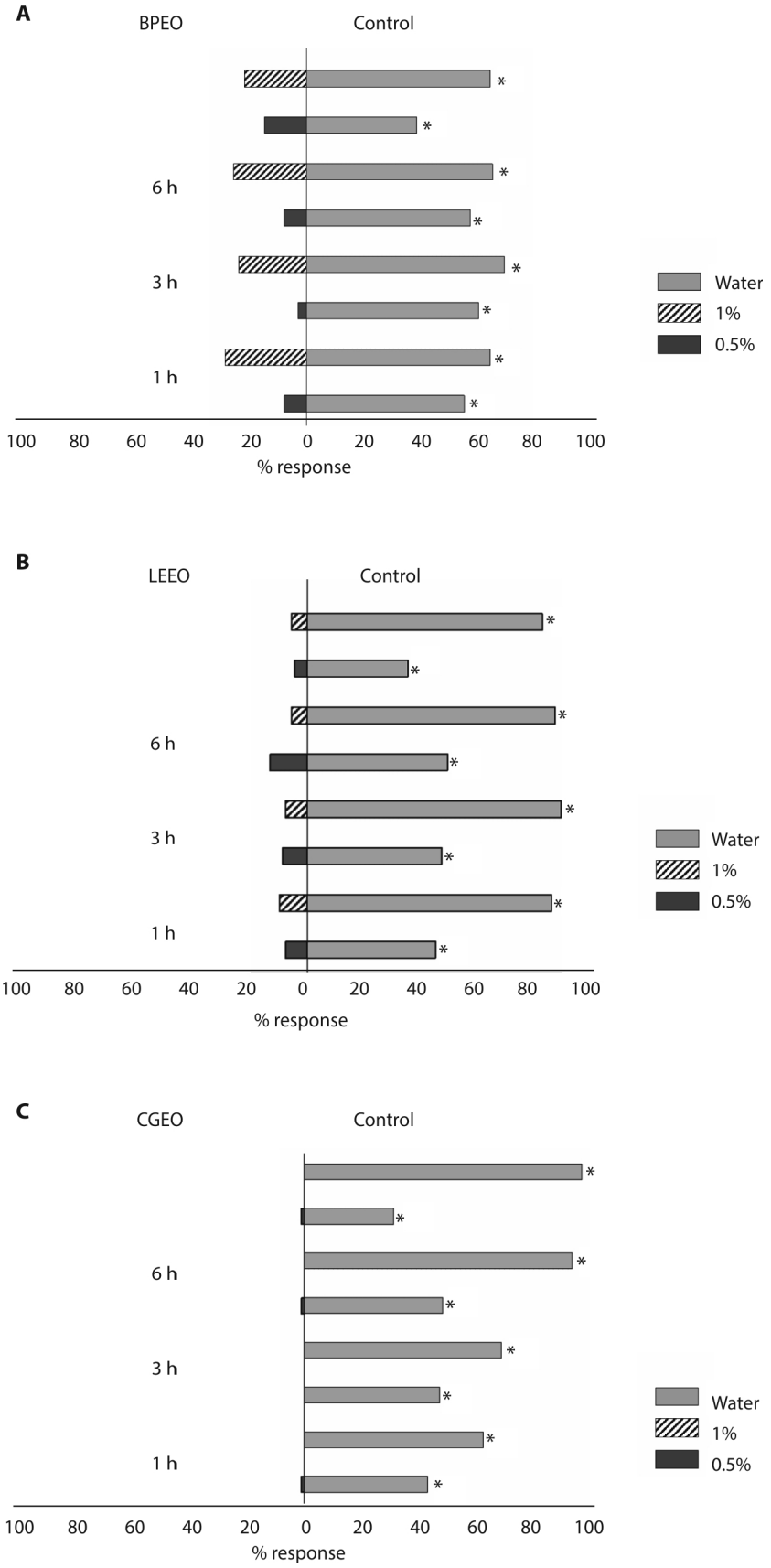

Fig. 2. Chemotactic response (%) of *Myzus persicae* to cabbage leaf discs (*B. oleracea* var. *acephala*) packaged in Petri dishes and treated with 0.5% and 1% of: (A) BPEO – Brazilian pepper tree essential oil (*Schinus terebinthifolius*); (B) LEEO – lemon eucalyptus tree essential oil (*Eucalyptus citriodora);* (C) CGEO – citronella grass essential oil (*Cymbopogon winterianus*). Bars followed by an asterisk indicate significant difference (χ^2, $p < 0.001$). Total number of insects evaluated in each contrast = 100 (10 repetitions)

Table 4. Average number (± SD) of *Brevicoryne brassicae* and *Myzus persicae* which died after 24 and 48 h on cabbage (*B. oleracea* var. *acephala*) leaf discs treated with essential oil of the Brazilian pepper tree (BPEO) (*Schinus terebinthifolius*), lemon eucalyptus tree (LEEO) (*Eucalyptus citriodora*), or citronella grass (*Cymbopogon winterianus*) (CGEO) essential oils or water (control)

Treatments		*Brevicoryne brassicae*				*Myzus persicae*			
		24 h	Abbott [%]	48 h	Abbott [%]	24 h	Abbott [%]	48 h	Abbott [%]
0.5%	BPEO	1.0 ± 1.15 cB	3.23	2.0 ± 2.05 cA	11.11	2.1 ± 3.64 bA	20.20	4.2 ± 2.08 bB	38.95
	LEEO	3.0 ± 3.57 bA	25.81	4.0 ± 3.40 bA	33.33	1.3 ± 1.70 bcB	12.12	4.6 ± 2.59 bA	43.16
	CGEO	9.5 ± 0.71 aB	94.62	10.0 ± 0.00 aA	100.00	5.8 ± 3.58 aB	57.58	7.3 ± 3.94 aA	71.58
1%	BPEO	6.5 ± 1.96 bB	62.37	8.8 ± 1.47 aA	87.10	7.4 ± 2.91 bB	73.74	8.4 ± 2.32 bA	83.84
	LEEO	3.8 ± 2.20 cB	33.33	6.5 ± 2.32 bA	62.37	6.3 ± 3.02 bB	62.63	7.7 ± 2.16 bA	76.77
	CGEO	9.0 ± 1.63 aA	89.25	9.6 ± 1.26 aA	95.70	9.2 ± 1.13 aA	91.92	9.9 ± 0.32 aA	98.99
Control		0.7 ± 0.82 dB		1.0 ± 1.05 cA		0.1 ± 0.32 cB		0.5 ± 0.71 cA	

Total number of insects evaluated in each contrast = 10 (10 repetitions). Means followed by distinct uppercase letters in the row (for each species) and distinct lowercase letters in the column are significantly different by Tukey and Kruskal-Wallis tests ($p < 0.05$)

M. persicae than the control treatment (Table 4). At 0.5%, *S. terebinthifolius* and *E. citriodora* did not differ statistically from water after 48 h of exposure to *B. brassicae* and after 24 h to *M. persicae* (Table 4). There was variation in mortality calculated by Abbott (1925) for species in response to each oil. However, *C. winterianus* OE was responsible for the highest mortality rates for both species (Table 4).

Chemotaxis of *D. rapae* to plants with EOs

Diaeretiella rapae females were more attracted to cabbages sprayed with the essential oil of *S. terebinthifolius* ($\chi^2 = 5$; gl = 1; $p = 0.0253$) than those with water (control) (Fig. 3). On the other hand, in the treatment with EO of *C. winterianus*, a change of behavior was observed, namely, females responded significantly to

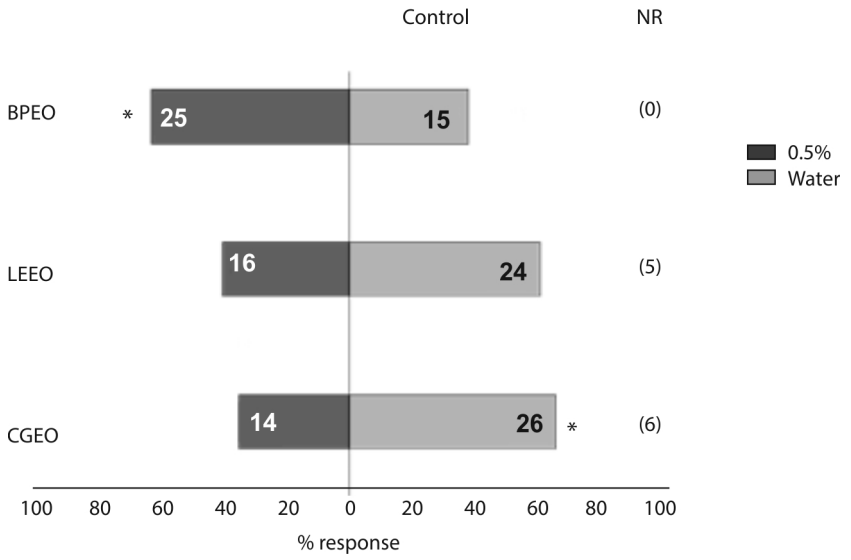

Fig. 3. Chemotactic response of *Diaeretiella rapae* females (up to 48 h old) from *Brevicoryne brassicae* or *Myzus persicae* tested in double "Y" olfactometer submitted to cabbage plants (*B. oleracea* var. *acephala*) sprayed with 0.5% essential oil of the Brazilian pepper tree (BPEO) (*Schinus terebinthifolius*), lemon eucalyptus tree (LEEO) (*Eucalyptus citriodora*) or citronella grass (CGEO) (*Cymbopogon winterianus*) contrasted with plants treated with water (control). Values in parentheses indicate the number of responsive insects. Bars followed by an asterisk differ (χ^2, $p < 0.05$); NR – number of non-responsive insects

the water (control) ($\chi^2 = 7.20$; gl = 1; $p = 0.0073$). There was no statistical difference between plants sprayed with essential oil of *E. citriodora* and plants sprayed with water ($\chi^2 = 3.20$; gl = 1; $p = 0.1175$) (Fig. 3).

Discussion

When *B. brassicae* and *M. persicae* had a choice between leaf discs treated with the essential oils of *S. terebinthifolius*, *E. citriodora* and *C. winterianus*, both preferred the discs treated with water, regardless of the concentration tested. Similarly, tomato leaf discs treated with EO from *Callistemon viminalis* (G.) (Myrtaceae) flowers (0.5% concentration) were less preferred by *M. persicae* adults (Sales *et al.* 2017). The results presented in this study corroborate those observed for *B. brassicae* and *M. persicae* to other essential oils, e.g., Lima *et al.* (2008), who verified a reduction in the number of *B. brassicae* on cabbage leaf discs treated with *Illicium verum* (L.) (Illiciaceae) at 0.5% concentration after 48 h, compared to those treated with water. The repellent effects of EOs from the same plant species evaluated in our study have already been found in other systems. *Schinus terebinthifolius* and *C. winterianus* (0.05% oil's concentration) also repelled *A. gossypii* when applied to cotton leaf discs (Andrade *et al.* 2013). Also, the oil of *E. citriodora* reduced the number of adult *B. tabaci* that landed on tomato plants treated with this product (0.5% concentration) after 24 h of exposure (Yang *et al.* 2010).

Among the major components of the EOs tested in this study were myrcene, β-phellandrene, and α-pinene (*S. terebinthifolius*), citronellal and citronellol (*E. citriodora*), and citronellal and geraniol (*C. winterianus*) which may have an effect on aphids' behavior, triggering positive or negative chemotaxis. Sales *et al.* (2017) reported that α-pinene and the minor compounds (β-pinene, terpinen-4-ol, p-cymene, y-terpinene, myrcene and linalool) present in the EO of *Callistemon viminalis* G. (Myrtaceae), were likely be responsible for repelling *M. persicae* from tomato leaf discs treated with this oil. Another species of the Myrtaceae family, *Eucalyptus urograndis* also showed repellent activity in its essential oil when tested against nymphs of *Rhodnius neglectus* (Hemiptera: Redu viidae) with 80% average repellency (Gomes and Favero 2013). The authors also observed a decrease in repellency after 24 h, inversely proportional to the time, due to oil evaporation. As a result, α-pinene and myrcene were identified in the composition of *S. terebinthifolius* oil and may also be associated with the lack of preference by aphids for discs treated with these oils.

We found in the bioassays that the EOs of the Brazilian pepper tree, lemon eucalyptus tree and citronella grass had high mortality rates for *B. brassicae* and *M. persicae* compared to the control treatment. Among the EOs tested, *C. winterianus* showed superior efficiency in causing the death of brassicas aphids. According to Graham-Bryce (1983), various species may have differences in susceptibility to essential oils, since these characteristics are attributed to physiological or biochemical differences, such as cuticle permeability, detoxifying enzymatic activity and sensitivity related to toxic damage at the target site.

Our study corroborates the results of Costa *et al.* (2015) who observed high mortality of *M. persicae* nymphs on leaf discs of cabbages with the essential oil of *E. citriodora* at 1% concentration. According to these authors, the toxicity presented by *E. citriodora* against *M. persicae* is related to the major compounds found in this study, e.g., citronellal, geraniol and β-citronellol. These same components were reported by Pinheiro *et al.* (2013) in the essential oil of *C. winterianus*, which caused mortality for *M. persicae* nymphs. The mixture of citronellal and isopulegol was highly toxic to larvae of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), and the citronellal insecticide activity was enhanced by isopulegol (Filomeno *et al.* 2017). Based on these results, it is possible to assume that substances such as citronellal and citronellol found in the EO of *E. citriodora*, as well as the geraniol found in *C. winterianus*, may have caused aphid death. Limonene observed that the chemical composition of *C. winterianus* is abundant in *Citrus* spp. species and has already shown insecticidal activity for insects such as *B. tabaci* (Zarrad *et al.* 2015).

The aphid mortality observed in this study can be attributed to the neurotoxic action of compounds present in the tested essential oils. Monoterpenes such as citronellol and geraniol, observed in the composition of *E. citriodora* and/or *C. winterianus,* were neurotoxic for *M. persicae* (Costa *et al.* 2015).

There are few studies evaluating the interaction of essential oils with non-target organisms, especially with aphid parasitoids. In our study we observed the attractiveness of *D. rapae* females to cabbage plants treated with EO of *S. terebinthifolius*. Similarly, when the aqueous extract of *Schinus molle* (L.) (Anacardiaceae), a species from the same genus used herein, was sprayed on *Brassica oleracea* (L.) var. *capitata* (Brassicaceae) the number of *B. brassicae* parasitized by *D. rapae* on this plant increased (Michael and Raja 2012). Thus, it is possible to assume that the oils of both species have similar compounds that trigger parasitoid search behavior. Brassicas have accumulated glucosinolates, i.e., a group of secondary metabolites derived from amino acids that play a central role in plant defense against herbivores and pathogens (Kissen *et al.* 2009). According to these authors, *D. rapae* is able to use glucosinolate

hydrolysis products as olfactory cues to host foraging behavior.

Furthermore, EOs can also induce plant defense, as evidenced by Pereira *et al*. (2008) who observed that essential oil of thyme *Thymus vulgaris* (L.) (Lamiaceae) induced the resistance of coffee *Coffea arabica* (L.) (Rubiaceae) through the production of phytoalexins and increased the synthesis of structural and biochemical defense compounds. Thus, it is possible that the oil of *S. terebinthifolius* may have been activated by the indirect defense mechanisms of the cabbages, attracting aphid parasitoids. In contrast the essential oil of *C. winterianus* repelled females of *D. rapae*. Abramson *et al*. (2007) also observed that adults of predator *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) were not attracted to fennel flowers (*Foeniculum vulgare* Mill) treated with 5% of *C. winterianus* oil. Castilhos *et al*. (2018) observed that botanical compounds and essential oils may have selectivity relative to non-target organisms, as in their study with *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) in which the monoterpenes (carvacrol and timol) were more acutely toxic and R-(+)-limonene had sublethal effects related to predatory fertility and fecundity, while oregano oil only affected fecundity.

On the other hand, we did not find any statistical differences in the responses of *D. rapae* between plants sprayed with the essential oil of *E. citriodora* and water. It is important to consider that essential oils generally have low persistence in the field (Isman *et al*. 2010). Thus, these oils would have a higher probability of influencing the chemotaxis of natural enemies immediately after application and would have minimal effects in a short time due to the high volatility of most of their compounds (Koul *et al.* 2008).

The results of our experiments showed that essential oils from *S. terebinthifolius*, *E. citriodora* and *C. winterianus* are promising alternatives for controlling aphids on cabbages for integrated pest management. We observed that essential oil of the Brazilian pepper tree (*S. terebinthifolius*) showed the highest mortality rates and repellence for both aphid species, and also attracted the parasitoid associated with these species, *D. rapae*. In contrast, *C. winterianus* oil (citronella grass) repelled this natural enemy. However, this treatment generally presented the best results in relation to repellency and toxic action against both aphids, even at the lowest concentration tested. Thus, future research might be conducted in the field in order to evaluate more carefully the trophic interactions associated with these oils and organisms.

Acknowledgements

The authors would like to thank the National Council for Scientific and Technological Development (CNPq) for Master Degree scholarships granted to the first author, CNPq for a doctorate fellowship (CNPq 141011/2020-3) to the fourth author and for fellowships awarded to the second author (CNPq 303758/2018-0) as well as research grants from funding agencies (Biological supplies for horticulture by Dr. Rosana Matos de Morais, UNIVERSAL CALL MCTI/CNPq).

References

Abbott W.S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18 (2): 265–267. DOI: https://doi.org/10.1093/JEE/18.2.265A

Abramson C., Wanderley P., Wanderley M.J.A., Souza O.B., Mina A.J.S. 2007. Effect of essential oil from citronella and alfazema on fennel aphids *Hyadaphis foeniculi* Passerini (Hemiptera: Aphididae) and its predator *Cycloneda sanguinea* L. (Coleoptera: Coccinelidae). American Journal of Environmental Sciences 3 (1): 9–10. DOI: https://doi.org/10.3844/AJESSP.2007.9.10

Adams R.P. 2007. Identification of essential oil components by gas chromatography/mass spectrometry. 4th ed. Allured Publishing Corporation, Carol Stream.

Andrade L.H., Oliveira J.V., Lima I.M.M., Santana M.F., Breda M.O. 2013. Efeito repelente de azadiractina e óleos essenciais sobre *Aphis gossypii* Glover (Hemiptera: Aphididae) em algodoeiro. Revista Ciência Agronômica 44 (3): 628–634. DOI: https://doi.org/10.1590/S1806-66902013000300002

Castilhos R.V., Grützmacher A.D., Coats J.R. 2018. Acute toxicity and sublethal effects of terpenoids and essential oils on the predator *Chrysoperla externa* (Neuroptera: Chrysopidae). Neotropical Entomology 47 (2): 311–317. DOI: https://doi.org/10.1007/S13744-017-0547-6

Costa A., Pinheiro P.F., Rondelli V.M., Queiroz V.T., Tuler A., Britto K. B., Stinguel P., Pratissoli D. 2013. *Cymbopogon citratus* (Poaceae) essential oil on *Frankliniella schultzei* (Thysanoptera: Thripidae) and *Myzus persicae* (Hemiptera: Aphididae). Bioscience Journal 29 (6): 1840–1847.

Costa A., Pinheiro P.F., Queiroz V.T., Rondelli V.M., Kulitz A., Valbon W.R., Pratissoli D. 2015. Chemical composition of essential oil from *Eucalyptus citriodora* leaves and insecticidal activity against *Myzus persicae* and *Frankliniella schultzei*. Journal of Essential Oil Bearing Plants 18 (2): 374–381. DOI: https://doi.org/10.1080/0972060X.2014.1001200

Filomeno C.A., Barbosa L.C.A., Teixeira R., Pinheiro A.C., Sá Farias E., Silva E.M.P., Picanço M. 2017. *Corymbia* spp. and *Eucalyptus* spp. essential oils have insecticidal activity against *Plutella xylostella*. Industrial Crops and Products 109: 374–383. DOI: https://doi.org/10.1016/J.INDCROP.2017.08.033

Gomes S. P., Favero S. 2013. Assessment of the insecticidal potential of *Eucalyptus urograndis* essential oil against *Rhodnius neglectus* Lent (Hemiptera: Reduviidae). Neotropical Entomology 42 (4): 431–435. DOI: https://doi.org/10.1007/S13744-013-0144-2

Gouvêa S.M., Carvalho G.A., Fidelis E.G., Vieira Ribeiro A., Sá Farias E., Picanço M. 2019. Effects of paracress (*Acmella oleracea*) extracts on the aphids *Myzus persicae* and *Lipaphis erysimi* and two natural enemies. Industrial Crops and Products 128: 399–404. DOI: https://doi.org/10.1016/J.INDCROP.2018.11.040

Graham-Bryce I.J. 1983. Novel chemical approaches to crop protection: Needs and solutions. Pesticide Science 14: 261–271.

Holtz A.M., Rondelli V.M., Celestino F.N., Bestete L.R., Carvalho J.R. 2015. Pragas das brássicas. 1° edição. Instituto Federal Espírito Santo. Colatina-ES, BR, 230 pp.

Hussein H., Salem M., Soliman A. 2017. Repellent, attractive, and insecticidal effects of essential oils from *Schinus terebinthifolius* fruits and *Corymbia citriodora* leaves on two whitefly species, *Bemisia tabaci*, and *Trialeurodes ricini*. Scientia Horticulturae 216: 111–119. DOI: https://doi.org/10.1016/J.SCIENTA.2017.01.004

Isman M.B., Miresmailli S., Machial C. 2010. Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. Phytochemistry Reviews 10 (2): 197–204. DOI: https://doi.org/10.1007/S11101-010-9170-4

Isman M.B. 2020. Botanical insecticides in the twenty-first century – fulfilling their promise? Annual Review of Entomology 65: 233–249. DOI: https://doi.org/10.1146/ANNUREV-ENTO-011019-025010

Kissen R., Pope T.W., Grant M., Pickett J.A., Rossiter J.T., Powell G. 2009. Modifying the alkylglucosinolate profile in *Arabidopsis thaliana* alters the tritrophic interaction with the herbivore *Brevicoryne brassicae* and parasitoid *Diaeretiella rapae*. Journal of Chemical Ecology 35 (8): 958–969. DOI: https://doi.org/10.1007/S10886-009-9677-6

Koul O., Walia S., Dhaliwal G.S. 2008. Essential oils as green pesticides: potential and constraints. Semantic Scholar 4: 63–84.

Lima R.K., Cardoso M.G., Moraes J.C., Vieira S.S., Melo B.A., Filgueiras C.C. 2008. Composição dos óleos essenciais de anis-estrelado *Illicium verum* L. e de capim-limão *Cymbopogon citratus* (DC.) Stapf: avaliação do efeito repelente sobre *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae). BioAssay 3 (0): 1–6. DOI: https://doi.org/10.14295/BA.V3.0.56

Marrone P.G. 2019. Pesticidal natural products – status and future potential. Pesticide Management Science 75 (9): 2325–2340. DOI: https://doi.org/10.1002/PS.5433

Michael H., Raja N. 2012. Evaluation of *Melia azedarach* Linn *Croton macrostachys* Hochst and *Schinus molle* linn plant extracts against cabbage aphid *Brevicoryne brassicae* Linn and their natural enemies *Diaeretiella rapae* (Mintosh) and *Hippodamia tredecimpunctata* Linn. Asian Journal of Agricultural Sciences 4 (6): 411–418.

Motazedian N., Aleosfoor M., Davoodi A., Bandani A.R. 2014. Insecticidal activity of five medicinal plant essential oils against the cabbage aphid, *Brevicoryne brassicae*. Journal of Crop Protection 2: 137–146.

Munthali D., Tshegofatso A. 2014. Factors affecting abundance and damage caused by cabbage aphid, *Brevicoryne brassicae* on four brassica leafy vegetables: *Brassica oleracea* var. *acephala*, *B. chinense*, *B. napus* and *B. carinata*. The Open Entomology Journal 8 (1): 1–9. DOI: https://doi.org/10.2174/1874407901408010001

Oliveira Junior L.F.G., Santos R.B., Reis F.O., Matsumoto S.T., Bispo W.M.S., Machado L.P., Oliveira L.F.M. 2013. Efeito fungitóxico do óleo essencial de aroeira da praia (*Schinus terebinthifolius* RADDI) sobre *Colletotrichum gloeosporioides*. Revista Brasileira de Plantas Medicinais 15 (1): 150–157. DOI: https://doi.org/10.1590/S1516-05722013000100021

Pavela R. 2008. Insecticidal properties of several essential oils on the house fly (*Musca domestica* L.). Phytotherapy Research 22 (2): 274–278. DOI: https://doi.org/10.1002/PTR.2300

Pavela R. 2018. Essential oils from *Foeniculum vulgare* Miller as a safe environmental insecticide against the aphid *Myzus persicae* Sulzer. Environmental Science and Pollution Research International. 25 (11): 10904–10910 DOI: https://doi.org/10.1007/S11356-018-1398-3

Pavela R., Benelli G. 2016. EOs as eco-friendly biopesticides? Challenges and constraints. Trends in Plant Science 21 (12): 1000–1007. DOI: https://doi.org/10.1016/J.TPLANTS.2016.10.005

Pereira R.B., Alves E., Ribeiro Júnior P.M., Resende M.L.V., Lucas G.C., Ferreira J.B. 2008. Extrato de casca de café, óleo essencial de tomilho e acibenzolar-S-metil no manejo da cercosporiose-do-cafeeiro. Pesquisa Agropecuária Brasileira 43: 1287–1296.

Pinheiro P.F., Queiroz V.T., Rondelli V.M., Costa A.V., Marcelino T.P., Pratissoli D. 2013. Insecticidal activity of citronella grass essential oil on *Frankliniella schultzei* and *Myzus persicae*. Ciência e Agrotecnologia 37 (2): 138–144. DOI: https://doi.org/10.1590/S1413-70542013000200004

Rakhshani E., Starý P., Tomanović Ž. 2015. Aphidiinae (Hymenoptera, Braconidae) aphid parasitoids of Malta: review and key to species: review and key to species. Bulletin of the Entomological Society of Malta 7: 121–137. DOI: https://doi.org/10.17387/BULLENTSOCMALTA.2015.10

Sales T., Cardoso M.G., Guimarães L.G.L., Camargo K.C., Rezende D.A.C.S., Brandão R.M., Souza R.V., Ferreira V.R.F., Marques A.E., Magalhães M.L., Nelson D.L. 2017. Essential oils from the leaves and flowers of *Callistemon viminalis*: chemical characterization and evaluation of the insecticide and antifungal activities. American Journal of Plant Sciences 8 (10): 2516–2529. DOI: https://doi.org/10.4236/AJPS.2017.810171

Souza M.F., Veloso L.F.A., Sampaio M.V., Davis J.A. 2017. Influence of host quality and temperature on the biology of *Diaeretiella rapae* (Hymenoptera: Braconidae, Aphidiinae). Environmental Entomology 46 (4): 995–1004. DOI: https://doi.org/10.1093/ee/nvx108

Toledo P.F., Ferreira T.P., Bastos I.M.A.S., Rezende S.M., Jumbo L.O.V., Didonet J., Andrade B.S., Melo T.S., Smagghe G., Oliveira E.E., Aguiar R.W.S. 2019. Essential oil from Negramina (*Siparuna guianensis*) plants controls aphids without impairing survival and predatory abilities of non-target ladybeetles. Environmental Pollution 255: 113–153. DOI: https://doi.org/10.1016/J.ENVPOL.2019.113153

Yang N.W., Li A.L., Wan F.H., Liu W.X., Johnson D. 2010. Effects of plant essential oils on immature and adult sweetpotato whitefly, *Bemisia tabaci* biotype B. Crop Protection 29 (10): 1200–1207. DOI: https://doi.org/10.1016/J.CROPRO.2010.05.006

Yi C.G., Kwon M., Hieu T.T., Jang Y.S., Ahn Y.J. 2007. Fumigant toxicity of plant essential oils to *Plutella xylostella* (Lepidoptera: Yponomeutidae) and *Cotesia glomerata* (Hymenoptera: Braconidae). Journal of Asia-Pacific Entomology 10 (2): 157–163. DOI: https://doi.org/10.1016/S1226-8615(08)60347-7

Zarrad K., Hamouda A.B., Chaieb I., Laarif A., Jemăa J.M. 2015. Chemical composition, fumigant and anti-acetylcholinesterase activity of the Tunisian *Citrus aurantium* L. essential oils. Industrial Crops and Production 76: 121–127. DOI: http://dx.doi.org/10.1016/j.indcrop.2015.06.039