Research Article

Received: 31 May 2010 Revised: 18 November 2010 Accepted: 21 November 2010 Published online in Wiley Online Library: 10 March 2011

(wileyonlinelibrary.com) DOI 10.1002/ps.2138

Composition and biological activity of essential oils from Labiatae against *Nezara viridula* (Hemiptera: Pentatomidae) soybean pest

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Abstract

BACKGROUND: Plant essential oils have been recognised as an important natural source of insecticide. This study analysed the chemical constituents and bioactivity of essential oils that were isolated via hydrodistillation from *Origanum vulgare* L. (oregano) and *Thymus vulgaris* L. (thyme) against eggs, second instar and adults of *Nezara viridula* (L.).

RESULTS: The major component of oregano was *p*-cymene, and, for thyme, thymol. The ovicidal activity was tested by topical application; the essential oil from thyme was more effective. The fumigant activity was evaluated in an enclosed chamber; the LC_{50} values for oregano were 26.8 and 285.6 μg mL^{-1} for nymphs and adults respectively; for thyme they were 8.9 μg mL^{-1} for nymphs and 219.2 μg mL^{-1} for adults. To evaluate contact activity, a glass vial bioassay was used; the LC_{50} values for oregano were 1.7 and 169.2 μg cm^{-2} for nymphs and adults respectively; for thyme they were 3.5 and 48.8 μg cm^{-2} respectively. The LT_{50} analyses for contact and fumigant bioassays indicated that thyme was more toxic for nymphs and adults than oregano. Both oils produced repellency on nymphs and adults.

CONCLUSION: These results showed that the essential oils from *O. vulgare* and *T. vulgaris* could be applicable to the management of *N. viridula*.

Keywords: essential oils; *Origanum vulgare*; *Thymus vulgaris*; southern green stink bug; *Nezara viridula*

1 INTRODUCTION

The southern green stink bug, *Nezara viridula* (L.), is one of the most economically important soybean pests. It has a worldwide distribution, occurring throughout the tropical and subtropical regions of Europa, Asia, Africa and America. This pest is in constant expansion as a consequence of the increased acreage for soybean production, particularly in South America. This pentatomid is highly polyphagous, attacking more than 145 species of plant (including cultivated and uncultivated species) within 32 families. In Argentina, this pentatomid causes important economic damage to soybean crops, from second instar to adults. Consequently, there can be premature fruit drop, delay in crop maturity and reduced seed quality and quantity. The pest also transmits plant pathogens.[1–5]

Generally, the southern green stink bug is controlled with non-selective insecticides, which belong to carbamates, the organophosphate group, such as monocrotophos, metamidophos and chlorpyrifos, or the cyclodiene group, such as endosulfan.[6] In Argentina, the most used insecticide is either endosulfan or mixtures of this product with pyrethroids and, in a minimal proportion, organophosphates. In the last few years, failures have been detected with these treatments in some productive areas.[7] Traditionally, in these cases the solution has involved an increase in the doses used; and it is known that the massive use of insecticides of this kind not only increases production costs but may also generate pentatomid-resistant populations by selective pressure.[7–9] Besides, as a result of increasing restrictions on the use of endosulfan in some countries, the most commonly used insecticide in soybean crops is the synthetic pyrethroid deltamethrin, which is harmful to natural enemies, making it incompatible with integrated pest management (IPM) strategies for soybean or pulse crops.[10] Therefore, the use of natural insecticides based on plant essential oils has been investigated as an alternative tool of control.

Essential oils (EOs) are volatile, natural, complex compounds characterised by a strong odour and are formed by aromatic plants as secondary metabolites;[11] they are an excellent alternative to traditional insecticides because of their low toxicity to humans and wildlife and short residual period.[12,13] Compared

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with other botanical insecticides, such as neem and pyrethrum, the active ingredients of many EOs are reasonably priced and are commonly used as flavours and fragrances. Minimum-risk pesticides that contain one or more EOs are currently exempt from US Environmental Protection Agency registration requirements and can reach the market faster than conventional insecticides.[12,13] Recent studies have shown that EOs possess insecticidal and repellent properties, the capacity to delay development, adult emergence and fertility and deterrent effects on oviposition. Because of the multiple sites of action through which the essential oil can act, the probability of developing a resistant population is very low.[14–19]

Origanun vulgare L. (oregano) and *Thymus vulgaris* L. (thyme) (Labiatae) have a widespread distribution, and their EOs have been shown to exhibit a range of biological activities: antibacterial, antiviral, antifungal, spasmolytic, antioxidant, antiparasitic, nematicidal and insecticidal; both are commonly used in foods, mainly for their flavour, aroma and preservation properties.[20–23]

There is a lack of information on the effectiveness of EOs against *N. viridula*. Therefore, the objective of this work was to determine the composition of the essential oils from *O. vulgare* and *T. vulgaris*, analyse their toxicity against eggs, second instar and adults and evaluate their repellent effects against second instar and adults of *N. viridula*.

2 MATERIALS AND METHODS

2.1 Essential oil extraction and gas chromatography–mass spectometry

Leaves of *O. vulgare* and *T. vulgaris* were collected during the summer season from the Capital Department in the Province of Salta, Argentina, located at 24° 47′21″ S, 65° 24′38″ W. The essential oils were provided by Universidad Nacional de Salta. They were extracted from leaves subjected to hydrodistillation using a modified Clevenger apparatus durfor a period of 3–4 h. The EOs obtained were dried over anhydrous sodium sulfate and refrigerated at 4 °C. The oils were analysed in the authors' laboratory using gas chromatography–mass spectrometry (GC-MS) with an HP 6890 chromatograph connected to an HP 5972A mass spectrometer equipped with an HP-5 capillary column (25 m × 0.25 mm). The CG oven temperature was held at 50 °C for 2 min, programmed at 5 °C min^{-1} ramp to 200 °C, and then held at this temperature for 15 min. The injector and detector temperature was 250 °C. The carrier gas was He (1 mL min^{-1}, split ratio 1 : 50), and the samples were diluted in diethyl ether (injection of 2 µL). Mass spectra were recorded at 70 eV, and the mass range was *m/z* 35–350 amu. The compounds were identified by comparing their retention indices (Kovats indices) with those of known compounds, and also by comparing their mass spectra with those stored in the MS database (NBS75K.L MS DATA). Relative percentage amounts were obtained directly from GC peak areas.

2.2 Insect cultures

A *Nezara viridula* colony was started from specimens field collected in Rivera (27° 09′38′ S, 63° 14′48″ N), Provincia de Buenos Aires, Argentina, without an insecticide history. Insects were reared at 27 ± 1 °C, 60 ± 10% HR and a 14 : 10 h light : dark photoperiod. The nymphs and adults were fed on a diet that was replaced twice a week, consisting of fresh green beans, raw peanuts and corn and sunflower and soybean seeds. Adults were maintained in rearing boxes (transparent cylindrical plastic containers, 13 cm diameter × 20 cm height) with the bottom covered with filter paper, which was changed periodically, and with the top perforated and covered with mosquito net. A 3 × 17 cm strip of glossy paper, on which the females deposited their eggs, was hung diagonally in the interior of the boxes. Egg masses were collected daily and placed in 9 cm diameter petri dishes, lined with filter paper and maintained under the conditions previously described. As first instar do not feed, they were maintained in the petri dishes until the beginning of the second instar, when they were transferred to rearing boxes with the respective diet until they were used for the bioassays.

2.3 Bioassays

All bioassays were performed at 27 ± 1 °C, 60 ± 10% HR and a 14 : 10 h light : dark photoperiod.

2.3.1 Ovicidal activity

Egg masses (>80 eggs, 5–6 days old) were topically treated on opercule with 10 µL EO solutions in hexane or with hexane alone (control) using a Hamilton microsyringe. The dosages evaluated were 0.625, 1.25, 6.25 and 12.5 µg egg^{-1}. After treatment, the egg masses were placed at the bottom of a petri dish and covered with gauze cloth. Percentage of inhibition of hatch (PIH) was calculated from the formula

$$\text{PIH (\%)} = \frac{C - T}{C} \times 100$$

where C is the control percentage hatch and T is the treated percentage hatch. Treated and control egg masses were held under the same conditions as those used for colony maintenance. The toxicity of the EOs was based on the number of unhatched eggs 7 days after treatment. All treatments were replicated independently 5 times. Data were analysed by ANOVA and DMS.

2.3.2 Fumigant toxicity

The fumigant toxicity of EOs against second instar and adults (3–4 days old) of *N. viridula* was evaluated in an enclosed chamber. The EOs were dissolved in hexane. Filter papers (8.5 diameter, Whatman No. 1) were impregnated with 1 mL of the test compound solutions to provide dosages ranging from 5.5 to 352 µg mL^{-1} using a pipette, and were allowed to dry for 10 min before being placed on the bottom of a petri dish (8.5 cm diameter × 2 cm high). This was then covered with a lid with a fine wire sieve attached over the central hole, where ten nymphs or ten adults were released. Finally, each petri dish was covered with another one, and all of them were fitted together with an adhesive film. Each concentration and control were replicated independently 5 times. Insect mortalities were determined every 15 min during the first 6 h, and every 2 h during the next 18 h. When no leg or antennal movements were observed, insects were considered dead. These symptoms were demonstrated to ensure ultimate irrecoverable death in each period of exposure. Lethal time 50% (LT_{50}) values were calculated with their respective 95% confidence intervals (CI95%) using a statistical software for correlated data.[24] Lethal concentration 50% (LC_{50}) values were calculated with their respective 95% confidence intervals (CI95%) using SPSS 15.0 statistical software. The LC_{50} or LT_{50} values were considered significantly different if the 95% confidence intervals did not overlap. To compare the LT_{50} responses, a toxicity index was used, the categories of which were as follows:[25,26] type 1: 0 < LT_{50} < 2 h = highly toxic; type 2: 2 < LT_{50} < 5 h = toxic; type 3: 5 < LT_{50} < 24 h = moderately toxic; type 4: LT_{50} > 24 h = slightly toxic.

2.3.3 Contact toxicity

To evaluate the contact activity of EOs against second instar and adults, two different glass vials were used according to the size of the insects: one of 14 mL for nymphs and the other of 53 mL for adults; the interior surface of the vials was coated with 0.5 mL or 1 mL of hexane EO solution or hexane alone (controls). These vials were then rotated on a modified hot-dog roller (heating element disconnected) until all of the hexane had evaporated. Ten nymphs or three adults were introduced in each vial. Five independent replicates were preformed for nymphs, and ten for adults. The dosages evaluated ranged from 0.7 to 360 µg cm^{-2}. Insect mortalities were determined every 15 min during the first 6 h and every 2 h during the next 18 h. When no leg or antennal movements were observed, insects were considered dead. LT_{50} values were calculated with their respective CI95% values using statistical software for correlated data.[24] LC_{50} values were calculated with their respective CI95% values using SPSS 15.0 statistical software. The LC_{50} or LT_{50} values were considered significant if CI95% values did not overlap. The LT_{50} responses were compared using the toxicity index described in Section 2.3.2.

2.3.4 Repellence bioassays

Because of the different behaviours of nymphs and adults, two bioassays were performed.

For second-instar nymphs, a simple olfactometer was used. The experimental area was formed by two hexagonal 190 mL glass vials (A and B). Each vial had a metal lid with a central hole connected to a glass tube (9 cm × 0.7 cm diameter) fitted together to prevent vapours from escaping. Both glass tubes were connected by a central tube (3 cm × 0.7 cm diameter). Filter papers (5.5 cm diameter, Whatman No. 1) were treated with 0.5 mL of hexane EO solution or solvent alone. The concentrations were 5, 10, 20 and 40 µg cm^{-2}. After solvent evaporation (10 min), treated papers were placed on the bottom of vial A, and untreated papers on vial B. In all cases, vapour stabilisation was allowed for 1 h. Ten insects were put in the central tube, and after 24 h the number of insects in each glass vial was recorded. Each concentration and control were replicated 5 times.

To evaluate the repellent effect on adults, a 9 cm round filter paper (Whatman No. 1) was cut in half. One of the halves was treated with 0.5 mL of hexane (control); the other half was treated with 0.5 mL of hexane EO solutions. The concentrations were 20, 40, 80 and 160 µg cm^{-2}. After solvent evaporation (10 min), the filter papers were fitted together to make a single layer and used to cover the floor of a petri dish. Ten insects were released in the centre of each petri dish, and their distribution was recorded 24 h later. Each experiment was repeated independently 5 times.

A distribution index was calculated as

$$DI = \frac{C - T}{C + T}$$

where C is the number of insects in the control vial or zone, and T is the number of insects in the treated vial or zone. Significant positive values expressed repellency; significant negative values expressed attractancy. Data were analysed by ANOVA and DMS.

3 RESULTS

3.1 Chemical constituents of essential oils

The composition of the EOs from *O. vulgare* L. (oregano) and *T. vulgaris* L. (thyme) used in the present experiments was determined by comparing their relative retention times and the mass spectra of the EO components from a data library. This information is summarised in Table 1. The main components of oregano were *p*-cymene and γ-terpinene, and, for thyme, thymol and *p*-cymene.

Table 1. Chemical constituents of the essential oils from *Origanum vulgare* and *Thymus vulgaris*

Retention time (minutes)	Compound	*O. vulgare* content (%)	*T. vulgaris* content (%)
6.84	α-Thujene		1.54
7.0	α-Pinene		1.42
8.61	β-Myrcene		1.93
9.34	α-Terpinene	7.74	
9.57	*p*-Cymene	26.00	28.37
9.70	β-Terpinene	4.26	
10.58	γ-Terpinene	21.89	8.06
11.45	δ-Terpinene	3.30	
14.02	1-Terpinen-4-ol	16.29	
17.21	Thymol	4.92	47.19
17.47	Carvacrol	3.11	3.17
20.60	β-Caryophyllene	8.25	2.84
24.56	β-Caryophyllene oxide	4.15	5.48

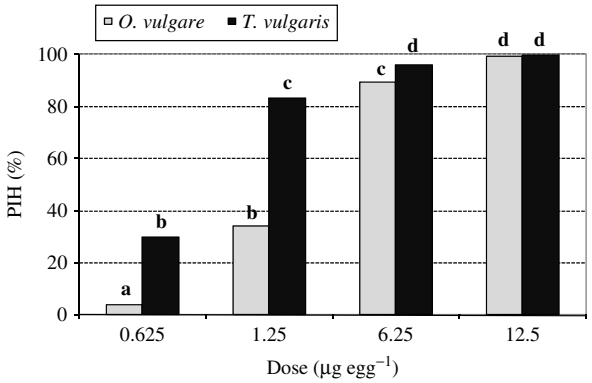

Figure 1. Percentage of inhibition of hatch (PIH) of essential oils from *Origanum vulgare* and *Thymus vulgaris* against *Nezara viridula*. Means with different letters are significantly different (DMS, $P < 0.05$).

3.2 Ovicidal activity

Ovicidal activity as measured by percentage of inhibition of hatch (PIH) was concentration dependent. The EO from *O. vulgare* inhibited hatching to 4% at 0.625 µg egg^{-1}, to 34% at 1.25 µg egg^{-1}, to 89.3% at 6.25 µg egg^{-1} and to 99.2% at 12.5 µg egg^{-1}.

With *T. vulgaris*, the PIH was 30% at 0.625 µg egg^{-1}, 83.2% at 1.25 µg egg^{-1}, 96.2% at 6.25 µg egg^{-1} and 99.6 at 12.5 µg egg^{-1}. Significant differences were observed between both oils at the lower concentrations ($P < 0.05$) (Fig. 1).

3.3 Fumigant toxicity

Both EOs showed strong fumigant activity against second instar and adults of *N. viridula*. The concentration of fumigant applied and the times after treatment significantly influenced percentage insect mortality. Generally, LT_{50} values decreased with increasing concentrations of EO. In second instar, according to the toxicity index, the EO from oregano at the lowest concentration

Table 2. LT_{50} values from fumigant activity of *Origanum vulgare* and *Thymus vulgaris* essential oils (EOs) to second instar and adults of *Nezara viridula*

Stage	EO	Dose (μg mL^{-1})	LT_{50} (h)[a,b,c]	Slope (± SE)
Second Instar	*O. vulgare*	22	NC	–
		44	11.5 (10.2–13.2) a	1.6 (±0.2)
		88	13.0 (12.0–17.6) a	1.1 (±0.2)
		176	2.9 (1.9–4.0) b	1.4 (±0.3)
	T. vulgaris	11	NC	–
		22	11.6 (9.7–12.8) a	2.2 (±0.3)
		44	1.0 (0.9–1.2) b	1.8 (±0.3)
		88	1.0 (0.9–1.1) b	1.2 (±0.2)
		176	0.8 (0.6–1.0) b	2.0 (±0.3)
Adults	*O. vulgare*	264	NC	–
		352	14.7 (11.4–19.4)	2.7 (±0.4)
	T. vulgaris	176	NC	–
		264	10.5 (5.6–20.5) a	3.4 (±0.4)
		352	4.5 (3.5–4.7) b	1.1 (±0.2)

[a] 95% lower and upper confidence intervals are shown in parentheses.
[b] Within each insect stage and each essential oil, LC_{50} values in the same column followed by different letters are significantly different ($P < 0.05$).
[c] NC: LT_{50} values not calculated (mortality <50% at 24 h).

Table 3. LC_{50} values from fumigant activity of *Origanum vulgare* and *Thymus vulgaris* essential oils (EOs) to second instar and adults of *Nezara viridula*

Stage	EO	LC_{50} (μg mL^{-1})[a,b]	Slope (± SE)
Second instar	*O. vulgare*	26.8 (19.3–34.2) b	3.29 (±0.54)
	T. vulgaris	8.9 (6.5–11.0) a	1.99 (±0.32)
Adults	*O. vulgare*	285.6 (262.8–308.3) a	1.75 (±0.33)
	T. vulgaris	219.0 (196.7–241.0) a	1.85 (±0.29)

[a] 95% lower and upper confidence intervals are shown in parentheses.
[b] Within each insect stage, LC_{50} values in the same column followed by different letters are significantly different ($P < 0.05$).

(≤22 μg mL^{-1}) was slightly toxic, at 88 and 44 μg mL^{-1} it was moderately toxic and at the highest concentration (176 μg mL^{-1}) it was toxic. For thyme, at the smaller concentrations (≤11 μg mL^{-1}) the EO was slightly toxic, at 22 μg mL^{-1} it was moderately toxic and between 176 and 44 μg mL^{-1} it was highly toxic (Table 2). In adults, EO from oregano at 264 μg mL^{-1} was slightly toxic, and at the highest concentration (352 μg mL^{-1}) it was moderately toxic. The EO from thyme at 176 μg mL^{-1} was slightly toxic, at 264 μg mL^{-1} it was moderately toxicity and at 352 μg mL^{-1} it was toxic (Table 2).

The LC_{50} values were determined at 24 h after treatment, and they are summarised in Table 3. For second instar, the LC_{50} value from thyme (8.9 μg mL^{-1}) was significantly lower than the LC_{50} value from oregano (26.8 μg mL^{-1}) ($P < 0.05$). For adults, the LC_{50} value for thyme was 219.0 μg mL^{-1} and for oregano it was 285.6 μg mL^{-1}, and no differences were found ($P > 0.05$).

3.4 Contact toxicity

A considerable percentage mortality of nymphs and adults of *N. viridula* was observed as oil concentration and exposure time

Table 4. LT_{50} values from contact activity of *Origanum vulgare* and *Thymus vulgaris* essential oils (EOs) to second instar and adults of *Nezara viridula*

Stage	EO	Dose (μg cm^{-2})	LT_{50} (h)[a,b,c]	Slope (±SE)
Second instar	*O. vulgare*	1.4	NC	–
		2.8	19.3 (14.5–27.1) a	2.5 (±0.4)
		5.6	13.9 (6.4–31.2) a	2.8 (±0.3)
		11.2	9.6 (7.8–11.2) a	1.4 (±0.2)
		22.5	1.47 (1.4–1.5) b	5.3 (±0.7)
		45	0.8 (0.6–0.9) b	1.1 (±0.2)
	T. vulgaris	2.8	NC	–
		5.6	7.7 (6.2–10.1) a	1.2 (±0.2)
		11.2	4.5 (2.5–7.0) a	1.3 (±0.2)
		22.5	0.6 (0.4–0.8) b	2.3 (±0.3)
		45	0.6 (0.4–0.8) b	1.8 (±0.3)
Adults	*O. vulgare*	180	NC	–
		270	13.3 (9.9–17.9)	3.2 (±0.6)
	T. vulgaris	45	NC	–
		90	13.5 (10.5–18.1) a	3.5 (±0.6)
		180	3.0 (2.2–3.6) b	1.8 (±0.3)
		270	1.4 (1.0–1.9) c	3.3 (±0.6)

[a] 95% lower and upper confidence intervals are shown in parentheses.
[b] Within each insect stage and each essential oil, LC_{50} values in the same column followed by different letters are significantly different ($P < 0.05$).
[c] NC: LT_{50} values not calculated (mortality <50% at 24 h).

Table 5. LC_{50} values from contact activity of *Oroganum vulgare* and *Thymus vulgaris* essential oils (EOs) to second instar and adults of *Nezara viridula*

Activity against	EO	LC_{50} (μg cm^{-2})[a,b]	Slope (± SE)
Second instar	*O. vulgare*	1.7 (0.9–2.4) a	3.07 (±0.36)
	T. vulgaris	3.5 (2.3–4.3) a	2.94 (±0.35)
Adults	*O. vulgare*	169.2 (157.3–178.1) b	2.29 (±0.85)
	T. vulgaris	48.8 (35.2–61.8) a	1.39 (±0.26)

[a] 95% lower and upper confidence intervals are shown in parentheses.
[b] Within each insect stage, LC_{50} values in the same column followed by different letters are significantly different ($P < 0.05$).

were increased. In second instar, at 2.8 μg cm^{-2}, only the EO from *O. vulgare* was moderately toxic; at 11.2 μg cm^{-2} the EO from thyme was toxic while the oil from oregano was moderately toxic, and at 5.6 μg cm^{-2} both oils were moderately toxic; finally, at higher concentrations (45 and 22.5 μg cm^{-2}), both oils were highly toxic (Table 4). In adults, the EO from oregano was slightly toxic at 180 μg cm^{-2} and moderately toxic at 270 μg cm^{-2}, while the EO from thyme was toxic at the first concentration and highly toxic at the second. At 90 μg cm^{-2}, thyme produced moderate toxicity, and at 45 μg cm^{-2} it produced slight toxicity (Table 4).

The LC_{50} values were determined at 24 h after treatment, and the values are summarised in Table 5. For second instar, the LC_{50} value for *O. vulgare* was 1.7 μg cm^{-2}, and for *T. vulgaris* it was 3.5 μg cm^{-2}, but no significant differences were observed between these oils ($P > 0.05$). For adults, the LC_{50} value for thyme

Table 6. Repellent effect of essential oils (EOs) from *Origanun vulgare* and *Thymus vulgaris* against second instars and adults of *Nezara viridula*

Stage	EO	Dose (μg cm^{-2})	DI (± SE)[a,b]	Biological activity
Second Instar	*O. vulgare*	0	0.04 (±0.01) a	Neutral
		5	0.08 (±0.01) a	Neutral
		10	0.48 (±0.03) b	Repellent
		20	0.52 (±0.03) b	Repellent
		40	0.72 (±0.01) b	Repellent
	T. vulgaris	0	−0.04(±0.01) a	Neutral
		5	0.2 (±0.02) ab	Neutral
		10	0.36 (±0.02) bc	Repellent
		20	0.56 (±0.04) c	Repellent
		40	0.92 (±0.01) d	Repellent
Adults	*O. vulgare*	0	0.08 (±0.02) a	Neutral
		20	0.16 (±0.04) ab	Neutral
		40	0.32 (±0.04) bc	Repellent
		80	0.60 (±0.02) c	Repellent
		160	0.92 (±0.02) d	Repellent
	T. vulgaris	0	0.04 (±0.01) a	Neutral
		20	0.28 (±0.01) b	Repellent
		40	0.60 (±0.02) c	Repellent
		80	0.68 (±0.03) c	Repellent
		160	0.96 (±0.01) d	Repellent

[a] Distribution index (± standard error).
[b] Within each insect stage and each essential oil, means in the same column followed by different letters are significantly different (DMS, $P < 0.05$).

(48.8 μg cm^{-2}) was significantly lower than the LC_{50} value from oregano (169.2 cm^{-2}) ($P < 0.05$).

3.5 Repellent activity

Both oils induced a repellent effect in second instar at 40, 20 and 10 μg cm^{-2} ($P < 0.05$); no effects were observed at 5 μg cm^{-2} ($P > 0.05$). In adults, at 160, 80 and 40 μg cm^{-2} the oils showed repellent activity ($P < 0.05$). At the lowest concentration (20 μg cm^{-2}), thyme showed a repellent effect ($P < 0.05$) while oregano had neutral activity ($P > 0.05$) (Table 6).

4 DISCUSSION

The present study is the first report on the ovicidal and insecticidal activities and repellent effect of EOs from *O. vulgare* L. (oregano) and *T. vulgaris* L. (thyme) (Labiatae) against *N. viridula* L., a major soybean pest in the world.

In this study, the insecticidal activity of both EOs varied according to the development stages of *N. viridula* and the method of application (topical, contact or exposure to vapours). Differential susceptibility of development stages of insects to chemicals has been described previously.[27,28]

In topical toxicity bioassay, both oils exhibited potent ovicidal activity against *N. viridula*, but the EO from thyme was more effective at the lower concentrations. Many phytochemicals possess ovicidal activity against various insect species.[12,29] The lipophilicity of the EOs may allow the diffusion of the active metabolites through the corion, affecting the insect's embryos. In previous work, the present authors demonstrated that the EOs from *Aloysia citriodora* Ort. and *Aloysia polystachya* (Griseb.) Moldenke (Verbenaceae) produced the same response as oregano and thyme at the highest concentration, but at the lowest the EOs evaluated in this work had more ovicidal activity.[30] It has been reported that EOs from other Labiatae species produced ovicidal activity against *Triatoma infestans* Klug.[31] The EOs from *O. vulgare* and *T. vulgaris* also showed toxic effects against eggs of *Acanthoscelides obtectus* (Say), *Tribolium confusum* DuVal, *Ephestia cautella* Walker, *Ephestia kuehniella* Zeller and *Trialeurodes vaporium* (Westw.).[28,32–35]

Both EOs produced contact and fumigant toxicity against nymphs and adults of *N. viridula*, and this could indicate that the penetration of the biocide compounds is via tegument and the respiratory system. However, second instars were found to be more susceptible than adults to the EOs evaluated. This could be attributable to an increase in body size, a decrease in penetration or biochemical and physiological changes in the insect itself.[27]

In the space fumigation study, both oils showed an insecticidal activity against nymphs and adults that was highly dependent on the dosage and the time after treatment. In exposure to vapours, the main access to the organism is airborne: the volatile substance enters through the spiracles as part of the respiratory process.[36] The substances are transported to different tissues through the network of tracheas and tracheoles, thus reaching their site of action. The toxic effect of a substance depends on different toxicokinetic steps, but also on its physicochemical properties. In the case of volatile substances entering through the respiratory system, their toxic effect is strongly associated with their volatility rate.[37]

The EOs from oregano and thyme at 176 μg mL^{-1} were toxic and highly toxic, respectively, to the second instar; however, at the same concentration, the EOs from fruits and leaves of *S. molle* were moderately toxic.[26] In adults, only *T. vulgaris* was toxic. In recent work it was demonstrated that the EO from *Thymus persicus* L. showed toxicity against adults of *Tribolium castaneum* (Herbst.) and *Sitophilus oryzae* L.[38] The rapid action of the EOs from oregano and thyme against *N. viridula* could suggest a neurotoxic mode of action, and there is evidence for interference with the neuromodulator octopamine by some EOs and with GABA-gated chloride channels by thymol, the main component of thyme.[39,40]

The values analysed from LC_{50} fumigant bioassay showed that the EO from thyme was more potent than the EO from oregano against nymphs and adults. In a previous study using second instar, similar values of LC_{50} were obtained between *A. polystachya* and *O. vulgare*, and between *A. citriodora* and *T. vulgaris*.[30] In other works, EOs from oregano and thyme produced little or no fumigant toxicity against *E. cautella* larvae, *Plutella xylostella* (L.) larvae and adults of *Thrips palmi* Karny.[41–43] On the other hand, the EOs from oregano and thyme exhibited fumigant activity against adults of *A. obtectus* and *Sitophilus zeamais* (Motsch.) and nymphs and adults of *T. vaporariorum*.[28,44] *Origanum vulgare* also had fumigant activity against *E. kuehnielia, Lasioderma serricone* (F.) and *Sitophilus granarius* (L.).[45]

In contact toxicity bioassay, the EOs, which are complex mixtures of non-polar or minimally polar substances, cross the cuticle and diffuse horizontally and vertically. By diffusing horizontally, they reach the tracheae system, where they continue diffusing to the rest of the tissues in the organism and therefore reach their site or sites of action.[36] By diffusing vertically, the substances cross from the tegument to the epidermis, enter the organism and are distributed by the haemolymph either dissolved in lipids or bound to proteins.[46]

The values analysed from LT_{50} contact bioassay showed that both EOs at the highest concentration were highly toxic against second instar, and only thyme at the higher dose was highly toxic against adults. At 45 μg cm^{-2}, only the EO from fruits of *S. molle* had similar LT_{50} values to those reported in this work for oregano and thyme.[26] Using a greater concentration, similar LT_{50} values were obtained in adults of *Pediculus humanus capitis* Deg. in relation to second instars of *N. viridula*.[47]

When LC_{50} values from contact toxicity were analysed, the EOs from oregano and thyme had the same effect on nymphs, while the EO from thyme had a higher insecticidal activity against adults. For second instar, the present authors previously reported LC_{50} values of 3.4 μg cm^{-2} for *A. polystachya* and 8.1 μg cm^{-2} for *A. citriodora* under the same conditions as this work.[30] It was recently reported that oregano produced contact toxicity on adults of *A. obtectus* and thyme produced contact toxicity on fourth-instar larvae of *Spodoptera litura* (F.), larvae of *Culex quinquefasciatus* Say, workers of *Apis mellifera* L. and adults of *Varroa destructor* Anderson & Trueman.[35,47–50]

The EO from *T. vulgaris* was more effective than the EO from *O. vulgare* against *N. viridula*. This finding could be related to the structural and biological activity of the EO constituents. For *O. vulgaris*, the main compounds were *p*-cymene and the related γ-terpinene. For *T. vulgaris*, the main compounds were thymol (a *p*-cymene derivative with a hydroxyl group located at the *meta* position) and *p*-cymene. As aromatic compounds such as thymol and *p*-cymene are not easily detoxified, they are more toxic than aliphatic and cyclic compounds such as γ-terpinene.[51,52] In addition, thymol contains both a hydroxyl group and a system of delocalised electrons, and it was described earlier that the hydroxyl group (bound to a benzene ring) is important for the biological activities of this product, for example, as an antimicrobial compound.[53,54] Thymol has also been reported to be highly toxic to adults of *Lipaphis pseudobrasicae* Dav., the larvae of *S. litura*, the tracheal mite *Acarapis woodi* Rennie, to adults of *S. oryzae, T. castaneum* and *Rhyzopertha dominica* F., to the malarial vector *Anopheles stephensi* Liston and to nymphs and adult males and females of *Blatella germanica* (L.).[14,55–59]

The essential oils contain volatile compounds that can modify the insect's behaviour. A repellent is a chemical or mixture of chemicals that, acting in the vapour phase, cause the insect to behave in ways that result in its movement away from the source of the material.[60] The repellency of some EOs from Labiatea has previously been evaluated against some insects, but no studies have been done using Pentatomidae bugs.[61] The results presented here indicate that EOs from *O. vulgare* and *T. vulgaris* contain volatile compounds that generate an olfactory response on *N. viridula*. The EOs from fruits of *S. molle, A. polystachya* and *A. citriodora* also produced repellent effects on second instar of *N. viridula*.[26,30] EO from *T. vulgaris* proved to be an efficient repellent against different mosquito species.[17,62,63] Thymol also produced repellency against other pests.[64,65] On the other hand, the EO from *O. vulgare* did not produce a repellent effect on *Culicoides imicola* (Kieffer), the vector of the African horse sickness virus.[66,67] No repellent activity was observed against *S. oryzae* and *T. castaneum* using either EO.[68]

In summary, the present results indicate that the essential oils from *Origanum vulgare* and *Thymus vulgaris* are good options to control *Nezara viridula*. Furthermore, the EOs evaluated in this study are used in folk medicine and in foods, and are thus considered to be less harmful to humans and the environment than the majority of conventional insecticides.[12] Consequently, the possibility of employing these natural products to control *Nezara viridula* may warrant further investigation.

ACKNOWLEDGEMENTS

Financial assistance was provided by SECYT-UNS. CONICET is thanked for a research fellowship to Jorge O Werdin González. The authors also wish to thank Associate Professor NA Bonini of the Facultad de Ciencias Exactas, Universidad Nacional de Salta, who provided the essential oils used in this study.

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