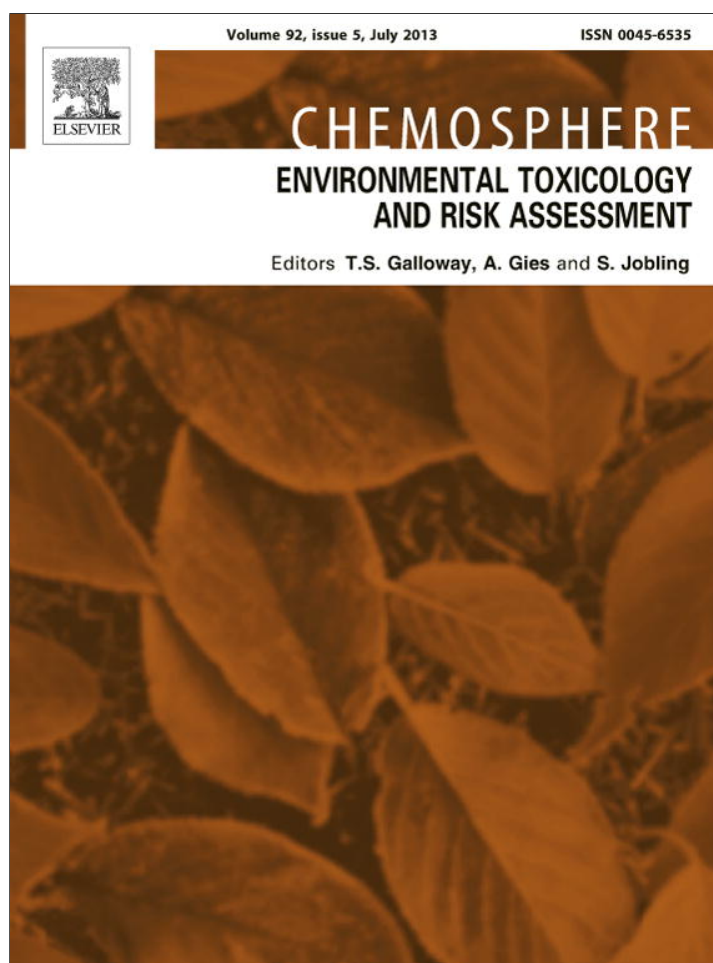


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Lethal and sublethal effects of four essential oils on the egg parasitoids *Trissolcus basalis*



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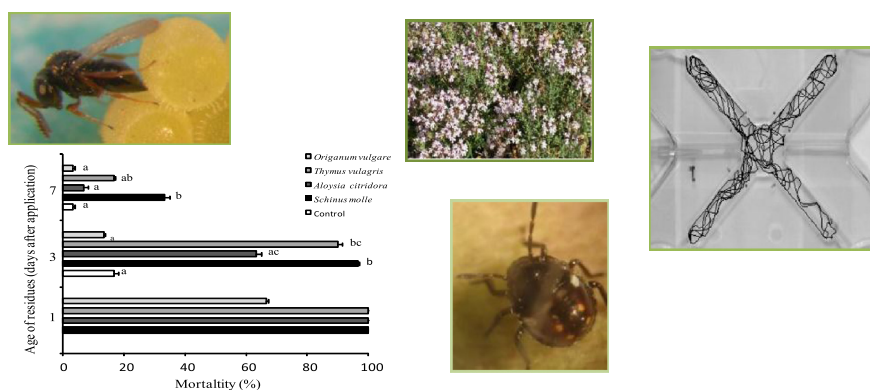
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HIGHLIGHTS

- Essential oils from *Origanum vulgare* and *Thymus vulgaris* are selective for *Trissolcus basalis* females.
- These EO do not affect parasitoid behavior.
- The EO residues do not produce sublethal effects after 1 week.

GRAPHICAL ABSTRACT



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ABSTRACT

The essential oils from leaves of *Schinus molle* var. *areira*, *Aloysia citriodora*, *Origanum vulgare* and *Thymus vulgaris* have showed potential as phytoinsecticides against the green stink bug, *Nezara viridula*. In this work we evaluated their toxicological and behavioral effects on the parasitoid *Trissolcus basalis*, a biological control agent of this pest insect. Essential oils were obtained via hydrodistillation from fresh leaves. Insecticide activity in *T. basalis* females was evaluated in direct contact and fumigation bioassays. Behavioral effects were evaluated in olfactometer bioassays. To evaluate the residual toxicity, females of the parasitoids were exposed to oil residues; in these insects, the sublethal effects were evaluated (potential parasitism and survivorship of immature stages). The essential oils from *O. vulgare* and *T. vulgaris* proved to be highly selective when used as fumigant and did not change parasitoid behavior. After one week, the residues of these oils were harmless and did not show sublethal effects against *T. basalis*. According with these results, essential oils have potential applications for the integrated management of *N. viridula*.

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1. Introduction

Stink bugs (Hemiptera: Pentatomidae) are major pest crops in several countries around the world (Panizzi and Slansky, 1985;

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Panizzi, 1997; Panizzi et al., 2000). *Nezara viridula* L. is a common pest in Europe, Asia, Africa, Australia and North and South Americas, and this wide geographic distribution is expanding in response to the global warming (Kiritani, 2011). It is a highly polyphagous insect that feeds on more than 150 species from over 30 families of plants, although with a strong preference for certain legumes (Panizzi, 1997; Panizzi et al., 2000; Musolin, 2012). For this reason, it is considered a key pest in several crops like soybean, rice, cotton,

tomato and many other (Panizzi et al., 2000; Kiritani, 2011). Control of stink bugs populations has been principally achieved through the use of conventional synthetic insecticides which belong to carbamates, organophosphates group, such as monocrotophos, metamidophos, chlorpyrifos, or cyclodiene group, such as endosulfan (Vandekerckhove and De Clercq, 2004). In Argentina, the most used insecticide is either endosulfan or mixtures of this product with pyrethroids and, in a minimal proportion, organophosphates (Sosa and Gamundi, 2008). Their massive use increases production costs and usually results in reduction of beneficial insects, pest resurgence and leads to other environmental and human health damages (Peres and Corrêa-Ferreira, 2004). In order to avoid this problem, alternative control methods are proposed, as the phytochemical insecticides based on essential oils (phytoinsecticides) and the biological control.

Essential oils (EOs) have been suggested as alternative sources for insect control products because some are selective, biodegrade to nontoxic products, and have few effects on the environment (Isman, 2000, 2006). They are volatile complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites. Because the multiple sites of action through which the EO can act, the probability of developing a resistant population is very low (Regnault-Roger et al., 2012). However, Papa-christos and Stamopoulos (2003) demonstrated that *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) developed resistance to lavender essential oil vapors even if exposed to them for only a few generations.

Biological control is other alternative to use chemical pesticide. Egg parasitoids (Scelionidae) are important natural enemies of stink bugs (Panizzi and Slansky, 1985; Corrêa-Ferreira and Moscardi, 1995 and references therein). Some species of this family have been considered and used in many countries for biological control of stink bugs, especially, *Trissolcus basalus* Wollaston (Hymenoptera: Scelionidae) (Caltagirone, 1981; Clarke, 1990; Corrêa-Ferreira, 2002). This idiobiont parasitoid present a field preferences for *N. viridula*; even though, at laboratory conditions it could parasitize eggs of *Piezodorus guildinii* Westwood, *Dichelops furcatus* Fabricius, *Dichelops melacanthus* Dallas and *Euchistus heros* Fabricius (Hemiptera: Pentatomidae) (Corrêa-Ferreira, 2002; Sujii et al., 2002; Molinari et al., 2008; Laumann et al., 2008, 2010).

The integration of phytoinsecticides and biological control in an integrated pest management program (IPM) for *N. viridula* demand the concerning studies about the possible toxic effects of these compounds in the natural enemies. We have already reported the efficiency (mortality) and sublethal effects (i.e., those effects observed on individual that survive the exposure to a pesticide) of different EOs on the biology of *N. viridula* (Werdin González et al., 2008, 2010, 2011a, 2011b).

It is known that insecticides can cause lethal and sublethal effects on natural enemies (Desneux et al., 2007). Although a high volume of information has been published on the effects of EOs on pest species little information is available on its effects on parasitoids. Phytoinsecticides have shown to have not or little impact on natural enemies (de Souza Tavares et al., 2009). The studies focused mainly in crude plants extract activities (Al-mazráawi and Ateyyat, 2009; Iannaccone and Alvarino, 2010) or commercial derivatives, especially those from *Azadirachta indica* Juss. (Meliaceae) (Charleston et al., 2005; Almeida et al., 2010). Similarly, Yi et al. (2007) show that 14 EOs were more selective to adults of the caterpillar parasitoid *Cotesia glomerata* L. (Hymenoptera: Braconidae) than the synthetic insecticide dichlorvos.

This study aimed to reveal the lethal and sublethal effects of EOs of *S. molle* var. *areira* (Anacardiaceae), *Aloysia citriodora* (Verbenaceae), *Origanum vulgare* and *Thymus vulgaris* (Lamiaceae) on *T. basalus* females. Essential oils of these species have previously showed insecticidal properties on *N. viridula* (Werdin González

et al., 2008, 2010, 2011a, 2011b). The study focus on fumigant and contact acute toxicity, behavioral responses and effects on parasitoid life-history traits in order to test the compatibility of their use to control this pest.

2. Material and methods

2.1. Insects

Colonies of *T. basalus* were started from insects collected on soybean crops and natural areas of Distrito Federal, near Brasília, Brazil (15°47' S, 47°55' W). The parasitoids were maintained in an environmental chamber in plastic cages (tissue culture 25 cm² flask, angled neck – ICN Biomedicals, Irvine, CA). Host eggs (*E. heros*), glued with arabic glue on card strips (5 × 1 cm) were exposed to parasitoids for 24 h. The cages with the parasitized eggs were maintained at 26 ± 1 °C; 65 ± 10% HR and a 14: 10 h light : dark photoperiod. Droplets of honey were offered to adult parasitoids as food. Females and males obtained from parasitized eggs were maintained in plastic cages during 24 h for copulation. All the insects used in bioassays were mated females with 24–48 h in adult stage.

2.2. Essential oils

Leaves of *Schinus molle* var. *areira* and *A. citriodora* were collected during summer season in Bahía Blanca, Argentina (38°43' 2"S, 62°15' 54"W) and leaves of *Origanum vulgare* and *Thymus vulgaris*, from Capital Department in the Province of Salta, Argentina (24°47' 21"S, 65°24' 38"W). All specimens were authenticated at the Herbarium of Department of Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (BBB). Essential oils were extracted from fresh foliar material subjected to hydrodistillation using a modified Clevenger apparatus during 3–4 h, on a laboratory scale. With this method secondary metabolites (mainly terpenes and phenolic compounds) are obtained in a relatively pure fraction excluding most of primary metabolites (Regnault-Roger et al., 2012). The EOs obtained were dried over anhydrous sodium sulfate and stored in airtight containers in a refrigerator at 4 °C. The chemical composition was already determined in previous works (Werdin González et al., 2008, 2010, 2011b).

2.3. Bioassays

2.3.1. Fumigant toxicity

The fumigant toxicity of EOs against females of *T. basalus* was evaluated in an enclosed chamber. The EOs were dissolved in *n*-hexane. Filter papers (3.5 diameter, Whatman N°1) were impregnated with 1 mL of a serial dilution of the test compound (between 0.5 and 20 mg mL⁻¹), to provided dosages ranging from 5.5 to 176 µg mL⁻¹ and were allowed to dry for 10 min before being placed on the bottom of Petri dishes (3.5 cm diameter × 2 cm high). The Petri dishes were covered with a lid of a fine wire sieve attached over the central hole where the insects 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 was replicated independently five times. Insect mortalities were determined after 24 h.

2.3.2. Contact toxicity

To evaluate the contact activity of EOs against females of *T. basalus*, 14 mL glass vials were used, their interior surface was coated with 0.5 mL of the hexanic oil solutions of EOs or hexane alone (controls). These vials were then rotated until completely evaporation of the solvent. Ten insects were introduced in each

vial. Five independent replicates were preformed. The dosages evaluated of EOs range from 0.56 to 45 $\mu\text{g cm}^{-2}$. Insect mortalities were determined after 24 h.

2.3.3. Olfactometer bioassays

Bioassays were carried out using a X-shaped olfactometer consisting of an acrylic block (26 × 26 × 1 cm) with a X-shaped (7.5 × 1 cm branches and a central circular area of 4 cm diameter) cavity (1.5 cm thickness). The olfactometer block was placed on the top of a translucent glass plate, and the upper surface was covered with a transparent glass, the glass plates were held in place with large paper clips.

Filtered (activated charcoal) and humidified air was pushed through the system at 0.8 L min⁻¹ to push, and pulled through at 0.4 L min⁻¹, in a push–pull system. Before reach the olfactometer the air flowed through two chambers, treatment and control. The treatment consists of filter papers impregnated with 10 μL of the pure EOs and control, filter paper alone. Then, the air was conducted to the appropriate arms of the olfactometer (two treatment and two control arms). A single female was introduced into the central chamber of the olfactometer; the behavior of the insect was monitored during 10 min by a camera (CCD Sony SPT M324CE fitted with a 4–50 mm/F1:1.6 zoom lens with an infrared filter) coupled to SACAM software (Jorge et al., 2005) for registering behavioral parameters. The olfactometer was illuminated from above by two fluorescent lamps (40 W) and from below by two infrared lamps (homogenous emission of wavelengths at 950 nm provided by 108 LEDs). For each EOs, 20 replicates were made. To avoid any bias in the insects responses, after every four replicates, the olfactometer was cleaned and the positions of the arms were inverted, between controls and treatments.

It is known that semiochemicals can provoke taxis (directed motion in response to a stimulus) and/or kinesis (undirected motion). Likewise, the kinetics reactions can be classified as orthokinesis responses (changes on the speed or activity frequencies of the insects) or klinokinesis (changes on the tortuosity or direction ratio). For each bioassay, the first choice and residence time were recorded. The first choice was considered to have been made when the insect entered in more than a half of the arm length and remained there for at least 30 s. Additionally, the effects of the EO on the walking pattern parameters were investigated; i.e., linear velocity, angular deviation, tortuosity and direction ratio (Borges et al., 2003). Table 1 summarizes the variables evaluated.

2.3.4. Residual toxicity and sublethal effects

In order to establish the residual toxicity of the EOs against females of *T. basalis*, glass vials were treated according 2.3.2. The dosage evaluated was 45 $\mu\text{g EO cm}^{-2}$, the higher dose used for contact toxicity bioassay. After the evaporation of the hexanic oil solutions, the vials were maintained on darkness at 26 ± 1 °C; 65 ± 10% HR for 1, 3, or 7 d. Next, 10 females of *T. basalis* (24 h old and previously maintained with male to mate) were introduced in each vial. Five independent replicates were performed. Insect mortalities were determined after 48 h.

Table 1

Variables evaluated in the olfactometer bioassay.

Variable (units)	Definition	Behavioral category
First choice	First olfactometer arms chosen by the insects	Taxis (attraction or repellency)
Residence time (s)	Time spent in each olfactometer area	Taxis (attraction or repellency)
Linear velocity (mm/s)	Speed insect on linear tracking in each olfactometer area.	Orthokinesis
Angular deviation (°/s)	Speed insect on nonlinear tracking.	Orthokinesis, Klinokinesis
Tortuosity index	The value can range from 0 to 1, with 0 indicating a completely linear tracking and 1 the maximum of tortuosity.	Klinokinesis
Direction ratio (n°/s)	Numbers of time that the insects change its linear tracking.	Orthokinesis, Klinokinesis

To evaluate the possible sub-lethal effects of the exposition to EOs residues, after the 48 h exposure, in those treatments were 10 or more females survived, the reproductive potential and the survivorship of immature stages of *T. basalis* were estimated. Females ($n = 10$ for each treatment) were individually maintained in glass vials (3 × 0.8 cm) for 3 d. Every day, 20 *E. heros* eggs were offered, glued on card strips, to parasitoids for 24 h, these experimental set up was based in previous work that show that the oviposition of *T. basalis*, with constant offer of eggs, is concentrated in the firsts 3–4 d of adult life span (Laumann et al., 2008). After this period the egg masses were placed into glass tubes (7.5 cm long × 1 cm diameter) and maintained under rearing conditions until adult emergence. Every morning, parasitized egg masses were observed in order to evaluate the adult emergence and mortality of immature stages, calculated using the ratio of adults emerging to eggs originally parasitized. To estimate the number of parasitized eggs the color change was considered (Medeiros et al., 1997). When egg color did not offer a clear indication and the parasitoid did not complete its development, the eggs were dissected under stereoscopic microscopy in order to confirm parasitism (Laumann et al., 2008).

2.4. Statistical analysis

Mortality data from fugimant and contact toxicity bioassays were submitted to probit analysis using the statistical software SPSS 15.0; Lethal Concentration 50% (LC₅₀) and 95% confidence intervals were estimated. The LC₅₀ values were considered significantly different if their 95% confidence intervals did not overlap.

A Selectivity Toxicity Index (STI) was calculated as the ratio of *T. basalis* LC₅₀/second instar or adults of *N. viridula* LC₅₀. A STI > 1 means that the EO is selective; i.e., the product is more toxic to the pest than to its natural enemy; whereas a STI < 1 means that it is not selective, i.e., the EO is more toxic to the parasitoid than to the insect pest. The LC₅₀ values for *N. viridula* were obtained from previous data of the authors following the same methodology, in order to standardize the results (Werdin González et al., 2008, 2010, 2011b).

In the olfactometer bioassays the first choice were analyzed by a χ^2 test, the residence time by Wilcoxon's matched-pairs test and the others variables by Mann–Whitney U test.

The significance of differences in mortality when the insects were exposed to residues of different ages were tested using a GLM and Deviance Analyses, binomial distribution was adjusted to the response variable and a quasi-likelihood method to the heterogeneity factor estimation was applied to correct for over dispersion of the data, logit was used as link function. When the effect of treatment was significant mean mortality were compared by contrast analyses ($P = 0.05$). The data of residues from 1 d do not show variability in most of the cases (control mortality = 0% in all repetitions *S. molle*, *A. citriodora* and *T. vulgaris* 100% mortality in all treatments) (Fig. 3), so to these period no statistical test was applied. According with the mean mortality observed, the EOs were

classified in four categories: 1 = harmless (<30%), 2 = slightly harmful (30–79%), 3 = moderately harmful (80–99%), 4 = harmful (>99%) (Sterk et al., 1999).

The mean proportion of parasitized eggs by females that survived to the contact with EOs residues of different ages and the survivorship of immature stages (mean proportion) were analyzed by GLM and Deviance Analyses, a binomial distribution was adjusted to the response variable and a quasi-likelihood method to the heterogeneity factor estimation was applied to correct for over dispersion of the data, logit was used as link function. When more than two treatments were compared (7 d residues), mean comparisons were conducted using contrast analysis ($P = 0.05$).

3. Results

3.1. Fumigant toxicity

The toxicity order for *T. basalis* females was *T. vulgaris* > *S. molle* var. *areira* > *O. vulgare* = *A. citriodora*. The selectivity toxicity index was dependent on oil and insect stage studied. Selectivity was observed related with second instars of *N. viridula*; high STI was observed in *A. citriodora* followed by *T. vulgaris* and *O. vulgare*. *S. molle* was slightly selective. No adult selectivity was observed (Table 2).

3.2. Contact toxicity

For the EOs from *A. citriodora*, *O. vulgare* y *T. vulgaris*, the LC_{50} values ranged between, 1.53 and 1.97 $\mu\text{g cm}^{-2}$, and on this range of amount no significant differences were observed on the effect of EOs. The EOs from *S. molle* var. *areira* produced 100% mortality at all concentration so the LC_{50} values could not be calculated. In contact bioassays, no selectivity was found (Table 2).

3.3. Olfactometer bioassays

In X-shape olfactometer bioassays, no differences were found between the insect proportion which chose the treatment or the control areas ($P > 0.05$) (Fig. 1A). Additionally, no significance differences were found between the residence time in treatment and control areas ($P > 0.05$) (Fig. 1B).

Table 3 shows the effects of EOs from *S. molle* var. *areira*, *T. vulgaris*, *O. vulgare* and *A. citriodora* on tortuosity index, linear velocity, angular deviation and direction ratio. The EOs did not show significant effects on *T. basalis* walking pattern parameters.

3.4. Residual toxicity and sublethal effects

In residual toxicity bioassays, mortality differences were found according treatment (different EOs) (Deviance Analyses, Residual Deviance = 83.88, $df = 4.38$, $P < 0.01$) and age of residues (1, 3 or 7 d after application) (Deviance Analyses, Residual Deviance = 251.18, $df = 2.42$, $P < 0.01$) (Fig. 2). The *O. vulgare* residues showed lower toxicity than the others EOs. 1 d old residues from *S. molle* var. *areira*, *T. vulgaris* and *A. citriodora* caused 100% mortality (category 4 = harmful) and *O. vulgare* residues, 66% (category 2 = slightly harmful). With 3 d old residues, a similar tendency was observed. A high decrease in *O. vulgare* and *A. citriodora* toxicity was observed (category 1: harmless and 2, respectively) (Fig. 2). Differences were found between the different EO residues and between these and control (Contrast analyses $P < 0.05$). No differences were found in mortality for *S. molle* var. *areira* and *T. vulgaris* (category 3).

After 7 d application, all EOs showed a significant decrease in their toxicological effects against *T. basalis* females. The percentage of mortality caused by *O. vulgare*, *A. citriodora* and *T. vulgaris* were similar as control (category 1) (Fig. 2).

Only 1 d old residues from *O. vulgare* allow studying the sublethal effects. *T. basalis* females in contact with this residues showed a lower potential of parasitism than control (GLM, $t = 3.39$, $P = 0.004$); while no significances differences were observed for the survivorship of immature stages (Fig. 3A and B).

Sublethal effects on *T. basalis* females disappear after 3 d of application of *O. vulgare* EOs. The potential of parasitism and the survivorship of immature stages were not altered after the contact with the residues (Fig. 3C and D).

Residues of all EOs showed a lower toxicity and insects that survived to EOs residues showed no significant sublethal effects. Just *A. citriodora* and *T. vulgaris* residues reduced significantly the potential parasitism in relation to control (GLM and contrast analyses, control vs. *A. citriodora* $t = 2.57$, $df = 38$, $P = 0.014$ and control vs. *T. vulgaris* $t = 2.40$, $df = 38$, $P = 0.021$). The survivorship of immature stages was not affected by the residues (Fig. 3E and F).

4. Discussion

In the present study we analyzed the lethal, sublethal and behavioral effects of essential oils from *S. molle* var. *areira*, *A. citriodora*, *O. vulgare* and *T. vulgaris* on *T. basalis* females.

To our knowledge no effects of EOs on *Trissolcus* spp are been previously reported. Only biological effects of synthetic insecticides on immature and adults stage have been reported. Smilanick et al. (1996) reported that methamidophos residues affected survivorship of immature stages and adults females from *T. basalis* and

Table 2

LC_{50} values from fumigant and contact activity of EO against *T. basalis* females at 24 h exposure and selectivity toxicity index (STI).

Biological activity	Essential oil	LC_{50} ^a	CI95% ^b	STI _{vsadults} ^c	STI _{vsnymphs} ^d
Fumigant activity	<i>S. molle</i> var. <i>areira</i>	75.69 $\mu\text{g mL}^{-1}$ B	73.55–77.83	Not calculated	1.03
	<i>A. citriodora</i>	94.23 $\mu\text{g mL}^{-1}$ C	92.11–96.37	0.35	6.98
	<i>T. vulgaris</i>	50.55 $\mu\text{g mL}^{-1}$ A	48.33–52.69	0.23	5.68
	<i>O. vulgare</i>	92.40 $\mu\text{g mL}^{-1}$ C	90.28–94.54	0.32	3.44
Contact activity	<i>S. molle</i> var. <i>areira</i>	0.56 $\mu\text{g cm}^{-2}$ (100%)	Not calculated	Not calculated	Not calculated
	<i>A. citriodora</i>	1.53 $\mu\text{g cm}^{-2}$ A	0.001–2.33	0.02	0.19
	<i>T. vulgaris</i>	1.97 $\mu\text{g cm}^{-2}$ A	0.01–3.93	0.04	0.56
	<i>O. vulgare</i>	1.54 $\mu\text{g cm}^{-2}$ A	0.07–3.01	0.01	0.91

^a LC_{50} values in the same column followed by different letters are significantly different ($P < 0.05$).

^b 95% lower and upper confidence intervals.

^c STI_{vsadults} = calculated using LC_{50} for *Nezara viridula* adults previously informed.

^d STI_{vsnymphs} = calculated using LC_{50} for *Nezara viridula* second instar previously informed (Werdin González et al., 2008, 2010, 2011b). A STI > 1 means that the EO is selective and a STI < 1 means that it is not selective.

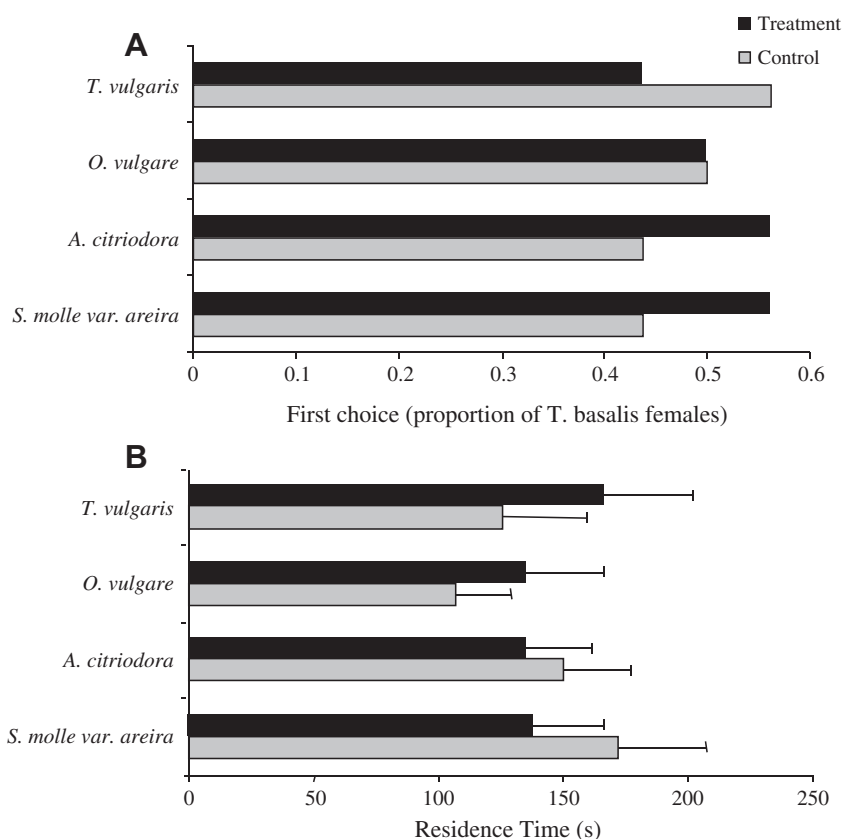


Fig. 1. (A) First choice of *T. basalis* and (B) residence time of *T. basalis* females for treated areas with 10 µL of different essential oils (Treatment) and control areas (n-hexane) in a X-tube olfactometer. No significant differences were found for first choice and residence time between treatment and control areas. (χ^2 test, $P > 0.05$; Wilcoxon's matched-pairs test, $P > 0.05$, respectively).

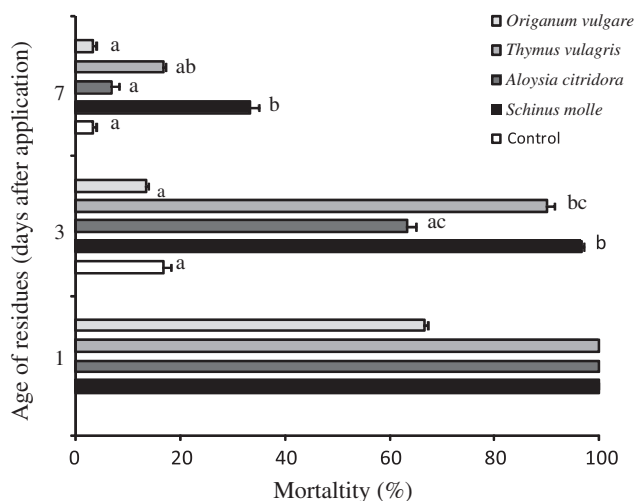


Fig. 2. Mortality (mean% ± SD) of *T. basalis* females after 48 h exposure to residues of different age (1, 3 and 7 d after treatments) of essential oils. Residues were applied in glass surfaces at 45 µg essential oil/cm². Bars followed by same lower case letter in each age of residues indicate nonsignificant differences between the mean mortality of each treatment (deviance analysis and contrast analysis $P > 0.05$).

adults from *T. utahensis* Ashmead; the potential parasitism was not affected on both species. In the other hand, adults from *T. grandis* Thompson exposed to field recommended concentrations of fenitrothion and deltamethrin suffered 100% mortality within 24 h; in this case the survival of immature stages decreased but the potential parasitism was not affected (Saber et al., 2005). When

fenitrothion residues were evaluated on adults *T. nigripedius* Nakagawa at field concentration, 100% mortality was observed at 24 h; moreover the survival of immature stages and the potential parasitism were affected (Lim and Mahmoud, 2008).

In this work, when acute fumigant toxicity was evaluated, the EOs from *A. citriodora*, *T. vulgaris* and *O. vulgare* were selective to *T. basalis* in relation to second instar of *N. viridula*. However, these EOs were not selective when contact toxicity was evaluated. This could be related to some parasitoids characteristics like as their minor size, higher mobility (resulting in more contact with toxics located on surfaces) and the slight development of detoxifying metabolic routes and excretion or parasitoids for phytochemical products (Morales Ramos and Rojas, 2003).

On the other hand, taxis and kinesis reaction were not observed when the females were exposed to the different EOs in olfactometer bioassays. It is known that *T. basalis* depends largely on olfactory cues released from its adult host, *N. viridula*, such as the male sex pheromone or defensive compounds (Colazza et al., 1999; Laumann et al., 2009); however, for long-range attraction, the parasitoid uses plant volatile chemicals induced by host feeding and oviposition (Colazza et al., 2004; Reddy, 2012). Different pentatomids influence in the volatiles blend emitted by the host plants. For example, in soybean plants, *E. herois* feeding behavior provoke the released of α -farnesene, methyl salicylate, 3-hexenyl acetate, and 2-octen-1-ol (Michereff et al., 2011). In maize plants injured by *N. viridula* the most important volatiles released are linalool, β -caryophyllene, α -trans bergamotene, and β -farnesene (Williams et al., 2005). *N. viridula* also affect volatile release in bean plants (*Vicia faba* L. and *Phaseolus vulgaris* L.) where feeding and oviposition increase β -caryophyllene, linalool, and 4,8,12-trimethyl-1,3,7,11-tridecatetraene (Colazza et al., 2004). This author

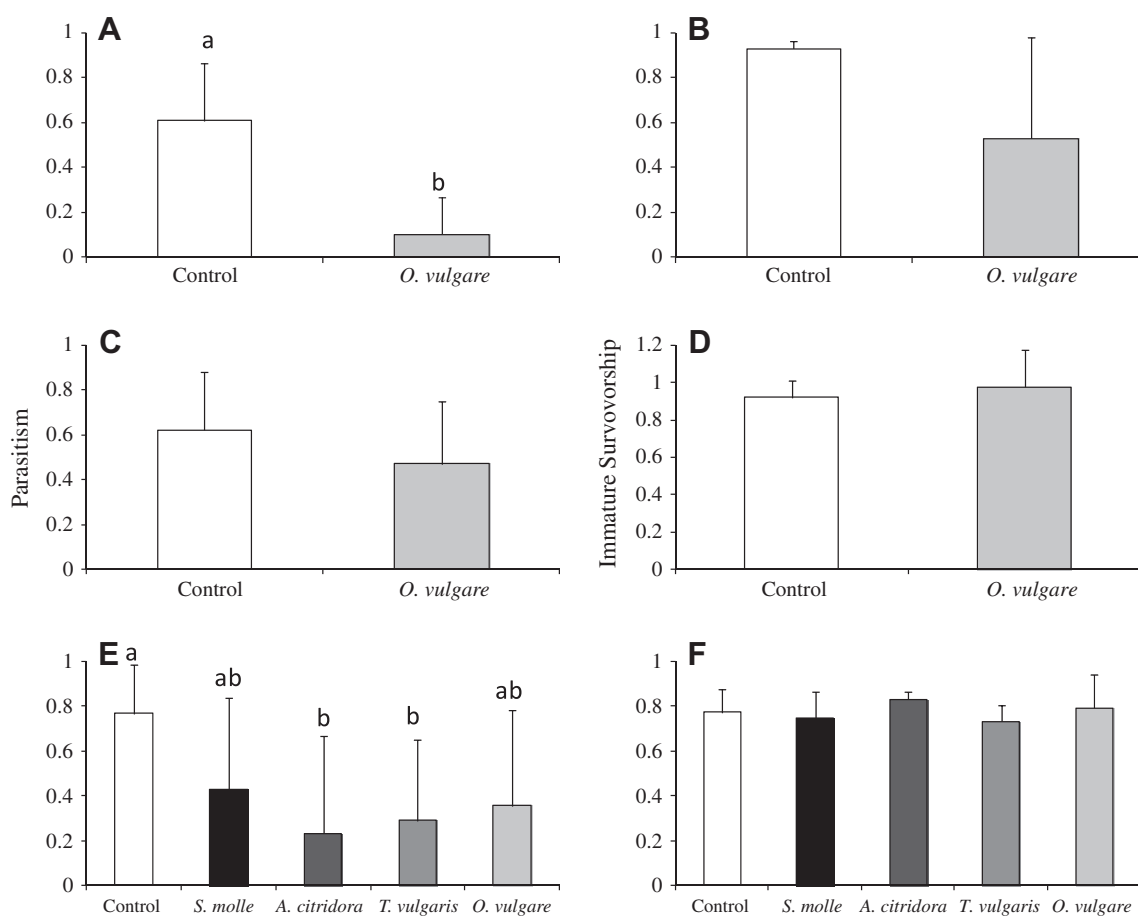


Fig. 3. Parasitism (left, proportion from offered eggs, No. eggs parasitized/total eggs, left) and survivorship of immature stages (right, proportion from parasitized eggs, No. of descendents/No. of parasitized eggs) of *T. basalis* females after contact with residues (45 μg of essential oils/ cm^2) of different ages. (A and B) parasitism and survivorship after contact with 1 d old residues of oils, (C and D) parasitism and survivorship after contact with 3 d old residues of oils, (E and F) parasitism and survivorship after contact with 7 d old residues of oils. Columns with the same minus letter indicate nonsignificant differences between means, GLM analyses for 1 and 3 d residues and Deviance and contrast analyses for 7 d residues ($P > 0.05$). Graphics with no minus letter above the bars indicate nonsignificant statistics.

also reported that the blends with large amounts of β -caryophyllene attract *T. basalis* females. In the EOs evaluated in this work, the main components of from *S. molle* var. *areira* were α -phellandrene and limonene; from *A. citridora* were citronellal and sabinene; the main components from *O. vulgare* were p-cymene and γ -terpinene and from *T. vulgaris*, thymol and p-cymene. These chemicals were not reported as induced plant volatile organic compound attacked by pentatomids, so this could be the reason that *T. basalis* do not response to this blends.

The bicyclic sesquiterpene β -caryophyllene is present in the four EO evaluated. An increase of this terpenoid was found in plants damaged mechanically, by herbivore feeding or a combination of feeding and oviposition (Conti et al., 2008) and was reported that the blends with large amounts of β -caryophyllene attract *T. basalis* females (Colazza et al., 2004). Even though, in the EOs studied, β -caryophyllene was a minor compound (less than 8.25%) so it is possible that the amount of this compound in the flow air was not enough to induce any behavioral consequence. Moreover, antagonistic effects between EOs compounds could be operating.

The effectiveness of an egg parasitoid in biological control is highly dependent upon the parasitoids ability to locate and recognize the host (Bayram et al., 2010). It is known that *T. basalis* females have a host searching behavior characterized by turning movements with an intensive antennal drumming of the substrate; when the parasitoid is exposed to specific stimulus, the orthokinesis parameters decrease and increase the klinokinesis ones

showing an arrestment response (Colazza et al., 1999; Borges et al., 2003; Laumann et al., 2009). The process of odor detection and consequent parasitoid response is dependent on neural transmissions, which is expected to be affected by neurotoxic insecticides commonly used in agro-ecosystems (Desneux et al., 2007; Bayram et al., 2010). Salerno et al. (2002) demonstrated the sublethal effect of deltamethrin against *T. basalis* on the walking behavior and on the arrestment response. Our result showed that the EOs did not influence the searching behavior of *T. basalis* because taxis and orthokinesis and klinokinesis variables were no modified. In this way, the effectiveness of *T. basalis* in controlling *N. viridula* would not be affected by the exposition to the EOs tested.

The residual toxicity bioassays showed that one day old residues from *O. vulgare*, were slightly harmful. 3 d old residues from this EO and those from *A. citridora* were harmless and slightly harmful, respectively. All 7 d old residues were harmless, excepting *S. molle* var. *areira*. The decrease in residual toxicity could be attributed to the rapid volatilization of EOs compounds.

The residues can cause lethal and sublethal effects on natural enemies' biology (Desneux et al., 2007). 1 d old residues from *O. vulgare* decreased the potential parasitism but did not alter the survivorship of immature stages of *T. basalis* while 3 d old residues from this EO and seven day residues from *O. vulgare*, *T. vulgaris* and *S. molle* var. *areira* did not modify these variables. Almeida et al. (2010) reported similar result on *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) using azadirachtin.

Table 3
Effects of EO on *T. basalis* females walking pattern parameters.

Variable	<i>S. molle</i> var. <i>areira</i>		<i>T. vulgaris</i>		<i>O. vulgare</i>		<i>A. citriodora</i>	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
Tortuosity index	0.51 ± 0.03	0.47 ± 0.03	0.62 ± 0.03	0.56 ± 0.04	0.55 ± 0.02	0.49 ± 0.02	0.50 ± 0.04	0.50 ± 0.03
Linear velocity	1.15 ± 0.08	1.03 ± 0.05	0.96 ± 0.12	1.23 ± 0.39	1.39 ± 0.16	1.37 ± 0.09	1.06 ± 0.08	1.09 ± 0.10
Angular deviation	13.84 ± 0.82	14.52 ± 1.06	14.22 ± 0.63	16.71 ± 2.26	13.60 ± 0.54	13.18 ± 0.50	13.53 ± 0.55	13.32 ± 1.02
Direction ratio	0.96 ± 0.11	1.19 ± 0.15	1.40 ± 0.12	1.25 ± 0.11	1.27 ± 0.12	1.30 ± 0.11	1.55 ± 0.12	1.23 ± 0.08

^a Significant level: ns: no significant differences were found (U Man-Whitney test).

Selectivity can be classified into ecological and physiological. The ecological selectivity is the use of insecticides selectively, namely, minimizing the exposure of natural enemies to insecticides. On the other hand, the physiological selectivity employs insecticides with low toxicity to the natural enemies or those which are more toxic to pests than to natural enemies (Bacci et al., 2006; Fernandes et al., 2010).

Insecticides from aromatic plants are an attractive alternative for pest management because they have low hazard to the environment or to human health compared to synthetic insecticides (Tavares et al., 2011). Even though studies on bioactivity of plant derivatives for pest control continue to increase, few of them explore the lethal and sublethal effects on the beneficial fauna, as natural enemies. Both strategies (natural products and biological control) are essential components of IPM.

According with our results, the EOs from *O. vulgare* and *T. vulgaris* are potential candidates to *N. viridula* control and should be use in combination with *T. basalis* as biological control agent. These EOs show a partial physiological selectivity (when they were used as fumigant) and not modify the parasitoid behavior. Moreover, 7 d old residues of these EOs were harmless and would not affect the parasitoid life-history traits. In concordance with basis of ecological selectivity, the parasitoid release would be done one week after EOs application. These EOs also probed insecticidal and repellents effects in other nonparasitoids insects (Aslan et al., 2005; Sin et al., 2006; Yi et al., 2006; Bittner et al., 2008; Pavela et al., 2009; Werdin González et al., 2011b; Kim et al., 2012).

Stink bugs are serious pests of many major crops (Panizzi, 1997). The laboratory results presented here suggest that egg EOs joint to egg parasitoids could be an alternative to stink bug management. Further work need to be conducted to test the efficacy of EOs and their compatibility with natural enemies in the field.

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