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Potential botanical pesticides from Asteraceae essential oils for tomato production: Activity against whiteflies, plants and bees

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) crops are affected by a diversity of pests. Among these pests, the whitefly *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) and the leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) cause large yield losses. The effects from these insects are often minimized by applying synthetic pesticides, although these have many drawback. To characterize novel tools for insect control, essential oils from two Asteraceae (*Artemisia absinthium* and *Eupatorium buniifolium*) were studied for their potential toxicity to beneficial insects [honey bees (*Apis mellifera* L.)] and phytotoxic effects against tomato seeds and vegetative parts. Our results show that seed germination was affected at application rates needed to control the leafminer *T. absoluta* but not at rates needed to control the whitefly *T. vaporariorum*. The same trend was found for honeybee toxicity: the use of these essential oils at the amounts needed to control *T. vaporariorum* would be not acutely toxic for bees. Finally, an experimental greenhouse trial showed that the application of the essential oil from *E. buniifolium* at 3% on whitefly-infested plants can cause whitefly adult mortality without affecting the crop yield.

1. Introduction

Worldwide tomato (*Solanum lycopersicum* L.) production has almost doubled from 1994 to 2014, being the second most important horticultural crop following potato (*Solanum tuberosum* L). Fresh fruit world production reached about 171 million tons occupying a total sown area of 5 million ha (Food and Agriculture Organization of the United Nations, 2014). Production is mainly done in open fields (Peet, 2012; Peet and Welles, 2005). Even though greenhouse production has many downsides including intensive management, enhanced insect and disease propagation and pollination issues, its practice is growing (Peet, 2012). In greenhouses, pollination does not occur naturally and there must be assisted either by physical means or the use of cultured bees (Peet and Welles, 2005). In both kinds of practices, tomato crops are affected by many pests including bacteria, fungi, insects and acari (Kennedy, 2003; Khuhro et al., 2014; Sastry and Zitter, 2014; Selvanarayanan, 2015). Among other means, the control of these pests is carried out using conventional pesticides which are well known to cause health and environmental impacts (Isman, 2006). Therefore, new means for pest and disease control as substitutes to synthetic pesticides are needed. Botanical pesticides are one of those potential alternatives (Isman et al., 2011; Regnault-Roger et al., 2012). The description of a botanical pesticide starts with the characterization of its chemical composition and bioactivity against target organisms under laboratory conditions. Afterwards, its development requires, besides the study of its effectivity under field conditions, the study of its safety to beneficial organisms and to the crop where it will be applied (Regnault-Roger et al., 2005).

The essential oils (EOs) from two asteraceous plants, *Eupatorium buniifolium* Hook. ex Arn. and *Artemisia absinthium* L. have anti-insect and anti-fungal activity against different herbivores and fungi (Aslan et al., 2005; Bachrouch et al., 2015; Bessada et al., 2015; Bouchenak et al., 2015; Gonzalez-Coloma et al., 2012; Joshi, 2013; Julio et al., 2015; Knaak et al., 2013a; Knaak et al., 2013b; Kordali et al., 2006; Kordali et al., 2005; Lancelle et al., 2009; Mihajilov-Krstev et al., 2014; Msaada et al., 2015; Riahi et al., 2015; Sosa et al., 2012; Umpiérrez

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et al., 2012). Our previous studies showed that the EOs of *E. buniifolium* and *A. absinthium* growing in Uruguay differ in chemical composition (the first being rich in sesquiterpenes, the second in β-thujone). Both products exhibited insecticidal and fungicidal activity against organisms that affect tomato crops: the whitefly *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) and the leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and the fungi *Botrytis cinerea* and *Alternaria* spp., which pinpoints them as raw material for formulations to be applied in tomato crops (Umpiérrez et al., 2012).

The objective of this work was therefore to characterize the activity of these products related to their potential toxicity against beneficial insects [honey bees (*Apis mellifera* L.)] and phytotoxicity against tomato seeds and vegetative parts as well as to test them under field conditions in experimental greenhouses.

2. Materials and methods

2.1. Plant material and essential oil production

The aerial parts of *A. absinthium* collected in Sauce, Canelones (34.65° S, 56.06° W) were vegetatively propagated (unpublished data) to obtain more plant material at 2 locations: Montevideo (34.84° S, 56.14° W) and Las Brujas, Canelones (34.38° S, 56.20° W); in an experimental crop field of the INIA (Instituto Nacional de Investigación Agropecuaria, Uruguay). At the last location, wild *E. buniifolium* plant material was collected. Both plants were collected during summer (2009–2010 and 2014–2015) and neither were flowering at the time of collection. As previously reported (Umpiérrez et al., 2012), both species were identified by Prof. Eduardo Alonso-Paz (Cátedra de Botánica), and voucher specimens were deposited at the Herbarium of Facultad de Química, Montevideo, Uruguay (*A. absinthium*: Umpierrez & Rossini s/n. MVFQ 4382 and *E. buniifolium*: Santos s/n MVFQ 4391). The EO from *A. absinthium* grown in Montevideo was obtained by hydro-distillation with *in situ* steam generation in a Clevenger apparatus. This EO was used to study toxicity on seeds and bees. In the rest of the assays EOs used were obtained by exogenously generated steam distillation using a 200-L alembic connected to a 50-L plant material container. Table 1S of supplementary material details the EOs used in this work. In all cases, after drying with anhydrous magnesium sulfate, EOs were stored in amber glass containers under nitrogen at −4 °C.

2.2. Chemical characterization

The identification of the individual compounds was carried out using a Shimadzu 2010 GC coupled to a Shimadzu QP2010 plus mass spectrometer (MS). Data were analyzed using Shimadzu Corporation GC–MS Solution v2.50 software (1999–2006). Analyses were run on a DB5MS column (30 m x 0.25 mm id, 0.25 μm film thickness) provided by Macherey–Nagel (Düren, Germany). The carrier gas was helium at 1 mL/min. The oven temperature program was as follows: 40 °C for 2 min, increase to 240 °C at 5 °C/min for 1 min, increase to 320 °C at 10 °C/min for 1 min. Injector and detector temperatures were 250 °C. Injections were performed in split mode (30:1), and the injection volume was 1 μL. MS parameters were: electron impact ionization at 70 eV ionization potential, *m/z* 40–550. The identification of the EO components was done by comparison of their retention indices with those reported by Adams (2007) and Pherobase database (El-Sayed, 2011), and of their fragmentation patterns with those contained in NIST 05 (Linstrom and Mallard, 2005) and SHIM 2205 (Adams, 2007) mass spectrometer libraries.

The composition of the EOs extracted from the different plant materials, at different extraction times and with different methods were compared by $\chi 2$ contingency analyses.

2.3. Tomato seed toxicity

Tomato (*Solanum lycopersicum* L.) seeds of two different commercial varieties were used: Mirella F1[1] (Nirit Seeds Ltd., Israel) y Cetia F1[2] (Clause, France). The assay was a modification of that reported by Sobrero and Ronco (2004). Three replicates per treatment or control were run in Petri dishes (5 cm x 1 cm) with a wet (4 mL distilled water) 0.5 cm-cotton layer on the bottom. Each replicate included 10 previously soaked seeds (distilled water, 15 h, 4 °C). A piece of filter paper (5 cm diameter) was placed on top of the cotton. Filter papers were coated (200 μL) with the EOs emulsified in 2% aqueous Tween® 20 for treatments or with the vehicle (WT from now on) for the controls. Both EOs were tested at each of 2 doses that represent the LD_{50} previously obtained for *T. absoluta* and *T. vaporariorum* respectively (Umpiérrez et al., 2012), that is, 650 and 65 μg/cm^2 for *E. buniifolium* EO, and 500 and 50 μg/cm^2 for *A. absinthium* EO (see Table 2S in Supplementary material).

The dishes were incubated at 22 ± 3 °C and 14:10 L:D to complete a time period of 10 days after initial germination. The number of seeds germinated in each dish was recorded daily and the root length of each seed was recorded at the end of the assay using ImageJ software (Schneider et al., 2012). Positive controls were performed with juglone (5-hydroxy-1,4-naphthoquinone CAS#481-39-0, Sigma-Aldrich, Germany) in WT (8 mg/dish, 125 μg/cm^2) (Gonzalez-Coloma et al., 2012). Finally, after the 10-day period, the treatments corresponding to the higher doses of both EOs and the positive control were kept under the same environmental conditions for 5 more days to check whether the inhibition effect was reversible.

Germination data were subjected to a multifactorial analysis of variance (GLM) with seed variety, time and treatment as factors.

2.4. Toxicity of the essential oils to honey bees

The toxic activity against *Apis mellifera* L. (Hymenoptera: Apidae) was tested by two bioassays. First, a bioassay of acute toxicity was performed following the EPA recommendations described in the OCSPP 850.3020 guide for the “Honey Bee Acute Toxicity Test” (US EPA, 2012) using bees from an organic apiary in Sauce (Canelones-Uruguay). The test consists of topical application to the dorsal thorax of 2-day-old adult bees of 2 μL of an acetone solution of EO (treatment, $N = 5$), acetone (solvent control, $N = 5$) or nothing (natural survival control, $N = 15$). A range of doses from 0 to 0.75 mg/bee was used. After application, bees from the same treatments were placed in groups of 5 in Petri dishes (9 cm x 1 cm) and fed with 1 cm^3 of candy, a solid mixture of powdered sugar and honey (Ruffinengo et al., 2005). Plates were maintained in darkness at 26 ± 2 °C and 58% rH. Mortality was recorded at 24 h. Second, to allow comparisons between EO activity on bees and on the tomato insect pests under the same experimental conditions, a “Complete Exposure Test” was made as described elsewhere (Ruffinengo et al., 2005). In brief, the bottom of dishes was lined with filter paper previously treated with 1 mL of the EO emulsions in WT. A dose response study was carried out using 0, 1, 0.5, 0.25, 0.125, 0.05 and 0.01 mg/cm^2 of each product. Five 3-day old adult bees were placed in each dish (N = 3-7 replicates/test), with candy *ad libitum* (Ruffinengo et al., 2005), and mortality was recorded at 24 h. Bees were obtained from an experimental apiary at Nágera (Mar del Plata, Argentina).

Lethal doses and comparisons among them were calculated by means of Probit analyses (Finney, 1971).

[1] F1 Hybrid Indeterminate tomato seeds, variety Mirella F1, Lot N° 1841079126, Germination & purity above required standards, tested 05/2012, poison treated with thiram. Produced by Nirit Seeds Ltd, Israel. Imported by Surco SA.

[2] Cetia F1 (obtaining by Clause), Pure/Germ 99%-92%, Lot N° D73073, treated thirame. Produced by Clause (Francia). Imported by Millacar SA.

2.5. Experimental greenhouse assays

The *E. buniifolium* EO was evaluated for its phytotoxic and insecticidal (against whiteflies) activities in the same assay. Tomato plants (20 cm height) were placed in experimental greenhouses (35 m^2) in 2 rows of 20 plants each. The Cetia variety was used due to its lower susceptibility to the EOs in the laboratory assay reported here. An artificial infestation was made with adult whiteflies from a laboratory colony previously established from nymphs collected on tomato crops. Once the whitefly population in the greenhouses had produced nymphs of the next generation (*ca.* 1 month) the different treatments were applied. *E. buniifolium* EO was applied as an emulsion in WT, and control plants were sprayed with WT only. The plants were then visually examined once at 48 h after each application and thereafter every week, recording the total number of adults per plant (Abbott, 1925). To assess potential phytotoxic effects leaf necrosis was recorded and at the end of the assay the number of fruits and total fruit weight were determined.

Greenhouse experiments were run twice (2014 and 2015). In 2014, a first treatment with EO at 1.5% (v/v) was done on day 6 of the trial. Since no differences were found compared with the controls (see results below) a second treatment with EO at 3% (v/v) was applied at day 37. The trial was monitored until day 58. In 2015, *E. buniifolium* EO was applied at 4.5% (v/v).

The number of adults was analyzed by ANOVA (GLM) using time and treatment as factors.

2.6. Statistical analyses

All statistical analyses were run with the MINITAB software package v17 (Minitab, 2010).

3. RESULTS and DISCUSSION

3.1. Essential oil tested

The EOs produced in different years and by different distillation methods did not differ in their content of compound types (Chi-Square, Cramer's V tests: $\chi 2 = 0.81$, $df = 3$, $P = 0.85$ for *E. buniifolium* and $\chi 2 = 4.02$, $df = 3$, $P = 0.26$ for *A. absinthium*. Table 1). The detailed chemical compositions of the tested EOs are shown in Tables 3S and 4S of supplementary material. *E. buniifolium* EO contained more hydrocarbons (both, mono- and sesquiterpenes) than *A. absinthium* EO (ANOVA, $P < 0.05$ for both compound classes, Fig. 1). On the other hand, *A. absinthium* EO is richer in oxygenated monoterpenes (ANOVA, $P < 0.05$).

3.2. Tomato seed toxicity

In the seed germination assay, juglone, included as a positive control (Gonzalez-Coloma et al., 2012), inhibited the germination of both seed varieties until day 10 (Fig. 2A and B) and this effect remained unchanged until day 15 for both seed varieties (Fig. 2C and D). In the case of the negative controls, seed germination rate was not significantly different between both seeds varieties.

At day 10, the relative germination (calculated as the% germination in each replicate/average% germination in the control x 100) of both seed varieties were similar. Both tomato seed varieties were inhibited in their germination by the EOs tested at the maximum amounts (the amounts that reproduces the LD_{50} for *T. absoluta*, $F_{5,359} = 312.1$, $P < 0.001$, Table 2). At these amounts, post-hoc contrast showed that the Cetia variety is less susceptible to the effect of the *E. buniifolium* EO than the Mirella variety. On the other hand, at the minimum amounts tested (the LD_{50} for *T. vaporariorum*) no significant inhibitory effect relative to the control was observed for either variety (Table 2). Therefore, these doses seem to be safe when applied to seeds.

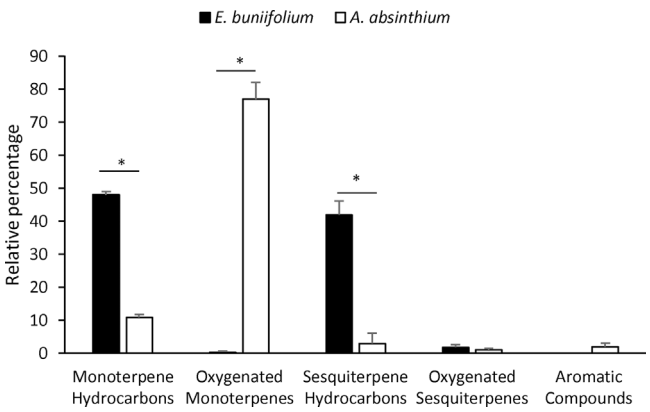

Fig. 1. Comparison between the chemical composition of the EO from *E. buniifolium* and *A. absinthium* tested in this study grouped by compound classes (* indicate significant difference by ANOVA at $P < 0.05$).

The multifactorial analysis of variance (GLM) performed on number of germinated seeds showed that all factors (time, seed variety and EO) included in the analysis had a significant effect at $P < 0.05$. There was an expected significant effect of the time on the germination ($F_{9,359} = 109.3$, $P < 0.001$). In the case of Mirella seeds, contrasts (Tukey Pairwise Comparisons) revealed that while for the control germination reached its plateau at day 5 of the assay, for the seeds treated with *A. absinthium* EO at 50 μg/cm^2 (ie. the *A. absinthium* LD_{50} for whiteflies, $AaLD_{50}$_Whitefly from now on) and with *E. buniifolium* EO at 65 μg/cm^2 (ie. the *E. buniifolium* LD_{50} for whiteflies, $EbLD_{50}$_ Whitefly from now on) the plateaus were delayed 1 and 2 days respectively (Fig. 2A). Such delay was not observed for Cetia seeds treated with any of the EOs at these lower doses (Fig. 2B). All other treatments (juglone, and both EO at the higher doses representing the LD_{50}_TUTA) did not reach plateaus, and germination onset was delayed until day 9. There was also a significant main effect of the seed variety on germination ($F_{1,359} = 15.18$, $P < 0.001$) with the Cetia variety the one less affected by the treatments.

To additionally check whether the reduction in germination exhibited by both EOs when tested at the equivalent of the LD_{50} for *T. absoluta* was produced by an herbicidal or a −static effect the germination rates at day 10 (end of the time-curse study) and at day 15 were compared by paired *t*-tests (Fig. 2C and D). Only in the case of the EO for *A. absinthium* applied to Mirella seeds germination did significantly increase in the following 5 day period ($P = 0.04$), indicating a reversible effect.

The root length reached after germination at the end of the assay

Table 1
Typical chemical composition of the EOs tested discriminated by compound family.

Essential Oil[a]	Monoterpene		Sesquiterpene		Aromatic%	NI%
	Hydrocarbons%	Oxygenated%	Hydrocarbons%	Oxygenated%		
A. absinthium	10.9 ± 0.8	77 ± 5	3 ± 3	1 ± 0.5	2 ± 1	6.2 ± 0.5
E. buniifolium	48 ± 1	0.4 ± 0.2	42 ± 4	1.8 ± 0.8	0 ± 0	0 ± 0

[a] As no significant differences were found between the EOs from the same species results are shown as means ± standard deviation of all EOs produced. NI: non-identified compounds.

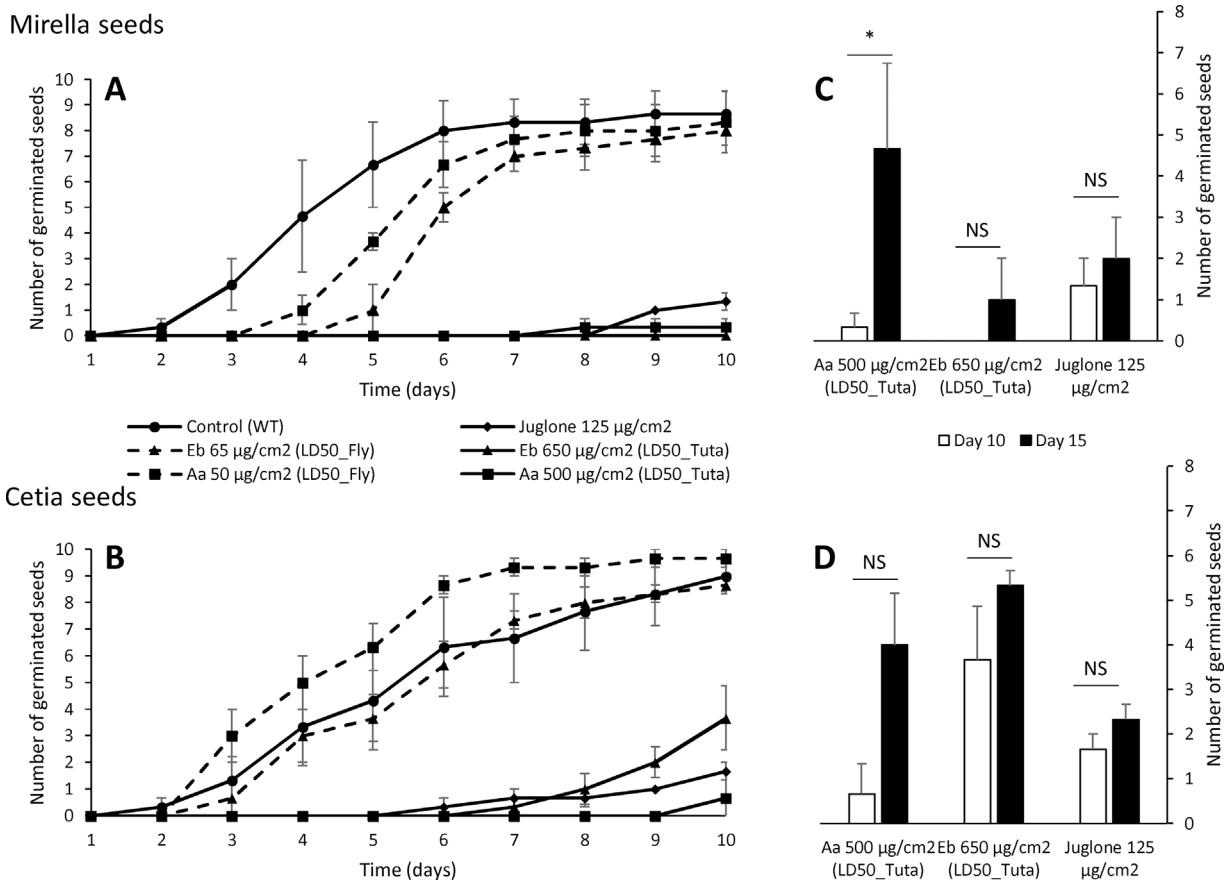

Fig. 2. Seed germination as a function of time for: Mirella (A) and Cetia (B) seeds. Insert C shows the difference in germination between day 10 and 15 for Mirella seeds, and insert D for Cetia seeds for those treatments where seed inhibition was found. All values are shown as mean ± SD. Inserts A and B show that germination was inhibited at the higher doses tested for both EO and seed variety but it was not affected at lower doses (ANOVA, GLM tests, see text for further statistical analyses). Inserts C and D show that from day 10 to day 15 only Mirella seeds increased their germination rates when treated with doses representing the AaLD$_{50}$_Tuta (NS: not significant, *: $P < 0.05$, *t*-tests). Aa: *A. absinthium*, Eb: *E. buniifolium*.

Table 2
Seed toxicity of the EOs from *E. buniifolium* and *A. absinthium* aerial parts. Treatments were applied in Water-Tween® 20 emulsions (WT). Results are shown at the end of the assay (10 days after initial germination).

Essential oil in WT[a]	Relative germination (%, mean ± SE) [b]			
	Minimum amount [c]		Maximum amount [d]	
	Mirella	Cetia[e]	Mirella[e]	Cetia
	65 µg/cm^2		650 µg/cm^2	
E. buniifolium	92 ± 7^{NS,C}	96 ± ^{NS,C}	0 ± 0 ^{*,A}	41 ± 13 $^{*;B}$
	50 µg/cm^2		500 µg/cm^2	
A. absinthium	96 ± 1 ^{NS,C}	107 ± ^{NS,C}	4 ± 4 ^{*,A}	7 ± 7 ^{*,A}

*indicates statistically significant differences from the control tests ($P < 0.05$); NS: no significant differences from the control tests.

[a] WT: Water: Tween® 20 (98:2).

[b] Relative germination is the percentage of germination compared with the respective control: [(% germination in each replicate/average% germination in the control) x 100].

[c] Concentration representing the LD$_{50}$ previously obtained for *T. vaporariorum* (Umpiérrez et al., 2012).

[d] Concentration representing the LD$_{50}$ previously obtained for *T. absoluta* (Umpiérrez et al., 2012).

[e] Different capital letters indicate statistical significant differences (ANOVA) between seed variety (ANOVA, GLM, $P < 0.05$, Tukey Pairwise Comparisons).

(Fig. 3) was also subjected to a multifactorial analysis of variance (GLM) with treatment and seed variety as factors. Seed variety did not show differences in root development ($F_{1,359} = 1.33$, $P = 0.24$). However, there was a significant effect of the treatment on root length ($F_{5,359} = 74.94$, $P < 0.001$), as well as of the interaction seed x treatment ($F_{5,359} = 3.55$, $P = 0.004$). Tukey Pairwise Comparisons revealed that root length roughly separated in two groups (Fig. 3) that correspond to the treatments at the lower amounts and those at the higher amounts.

Therefore, these results showed that both seed varieties were affected at the higher doses tested by both EOs not only in their relative germination (Table 2) and in their germination rates (Fig. 2A and B), but also in the length that roots reach when seeds germinate at those higher doses (Fig. 3). The effect of this inhibition was not reversible except in the case of the EO of *A. absinthium* on Mirella seeds. However, at lower doses (the ones that represent the LD$_{50}$ for whiteflies) both seeds performed similarly in their germination capacity as well as root length to control seeds, indicating nontoxic effects at those doses.

Even though these EOs would not be applied to seeds, from these results, a conservative prediction would indicate at least a similar (probably lower) toxicity on vegetative parts given that the seed germination assay is widely used as an indirect indicator of acute toxicity on plants (Kapustka and Reporter, 1993; Munzuroglu and Geckil, 2002).

3.3. Toxicity to honey bees

3.3.1. Honey bee acute toxicity test

The recommendation from EPA OCSPP 850.3020 guide (US EPA, 2012) indicates that products with LD$_{50}$ values greater than 25 µg/bee can be considered safe. Lethal doses calculated for this assay (Table 3) point to both EOs as non-toxic products (US EPA, 2012).

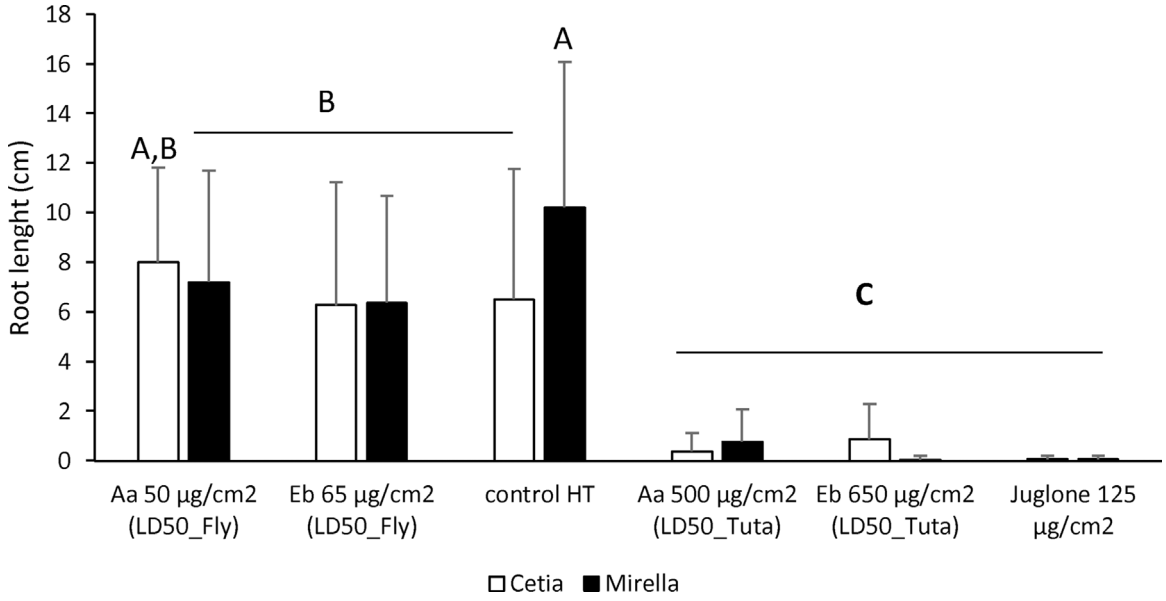

Fig. 3. Root length (mean ± SD) at the end of the 10-day assay. Capital letters above bars indicate significant differences (ANOVA, GLM, $F_{5,359} = 74.94$, $P < 0.001$). Aa: *A. absinthium*, Eb: *E. buniifolium*.

Table 3
Lethal doses at 24 h of the EOs from *A. absinthium* and *E. buniifolium* topically applied following EPA OCSPP 850.3020 guide (US EPA, 2012).

Essential oil	LD_{50} (fiducial interval) µg/bee	LD_{99} (fiducial interval) µg/bee	*P*
E. buniifolium	252 (207–318)	635 (520–828)	< 0.001
A. absinthium	197 (163–241)	457 (382–578)	< 0.001

3.3.2. Complete exposure test

Toxicity to bees was also tested in this assay to allow comparisons between EO activity on bees and on the tomato insect pests under the same experimental conditions. Table 4 shows that for both EOs the LD_{50} and LD_{99} fell between the ones reported for *T. absoluta* and *T. vaporariorum* which could indicate that the amounts needed to control the leaf miner would be toxic to bees. However, if these EOs are used at the doses needed to control the whiteflies in the conditions here tested there will be no effect on bees.

3.4. Greenhouse assays: insecticidal effect on whiteflies and toxicity on plants

Based on the results above we chose to run the greenhouse trials using Cetia plants, whiteflies and *E. buniifolium* EO. The Cetia variety was chosen due to its lower seed susceptibility to the EOs; whiteflies because its higher susceptibility to the EOs; and *E. buniifolium* because it is a local undergrowth that grows easily in our local conditions and does not contain thujone, a toxic product (Lachenmeier, 2010).

Plants treated with *E. buniifolium* EO showed a significant decrease on the number of total whiteflies (alive + dead) found on plants compared with the number on control plants (Fig. 4A, ANOVA, GLM, $F_{1,191} = 10.56$, $P = 0.001$). Populations in both greenhouses also varied with time ($F_{9,191} = 6.66$, $P < 0.001$) but no interaction between treatment and time was found ($F_{9,191} = 1.11$, $P = 0.36$). Note that the application itself caused a population decline irrespective of the product applied (Fig. 4A, checkpoints after application arrows). After the first application (day 6), populations in both greenhouses varied equally indicating no significant effect of the *E. buniifolium* EO applied at 1.5% up to day 37, when control plants showed a trend for higher adult occurrence than *E. buniifolium*-treated plants (Mann-Whitney test, $P = 0.08$). The lower number of total whiteflies in *E. buniifolium*-treated plants might be explained by a repellent effect of the EO. More studies will be carried out to investigate on this possibility. To check whether this effect could be improved at higher doses, on day 37 of the trial a second application was done with *E. buniifolium* EO (3%) in WT. This application at 3% kept the adult population lower than in control plants but the effect was not better than applying the EO at 1.5% (Fig. 4A). However, considering the whiteflies that had settled, an acute effect after this second application of *E. buniifolium* EO could be detected since the proportion of dead whiteflies was higher in these plants than in control plants (Fig. 4B, ANOVA, GLM, $F_{1,191} = 13.11$, $P < 0.001$ for treatment, $F_{9,191} = 29.53$, $P < 0.001$ for time and $F_{9,191} = 2.98$, $P < 0.005$ for the interaction treatment x time, Tukey Pairwise Comparisons).

At the end of the trial tomato yields were similar in both greenhouses (contingency analysis, $\chi^2 = 0.17$, $P = 0.92$ for yield (Kg) and

Table 4
Lethal doses of the EOs from *A. absinthium* and *E. buniifolium* for bees in the complete exposure bioassay after 24 h (Ruffinengo et al., 2005) and for tomato pests previously reported (Umpiérrez et al., 2012).

Essential oil	*A. mellifera* (this report)		*T. absoluta* (previous report: (Umpiérrez et al., 2012))		*T. vaporariorum*	
	LD_{50} mg/cm^2	LD_{99} mg/cm^2	LD_{50} mg/cm^2	LD_{99} mg/cm^2	LD_{50} mg/cm^2	LD_{99} mg/cm^2
E. buniifolium	0.15 (0.11–0.18) *	0.35 (0.29–0.45)	0.65 (0.53–0.78)	1.54 (1.22–1.92)	0.02 (0.01–0.04)	0.08 (0.04–0.13)
A. absinthium	0.26 (0.22–0.33)	0.45 (0.37–0.67)	0.50 (0.41–0.60)	1.42 (1.13–1.74)	0.08 (0.06–0.09)	0.19 (0.16–0.22)

* numbers in parenthesis indicate fiducial intervals.

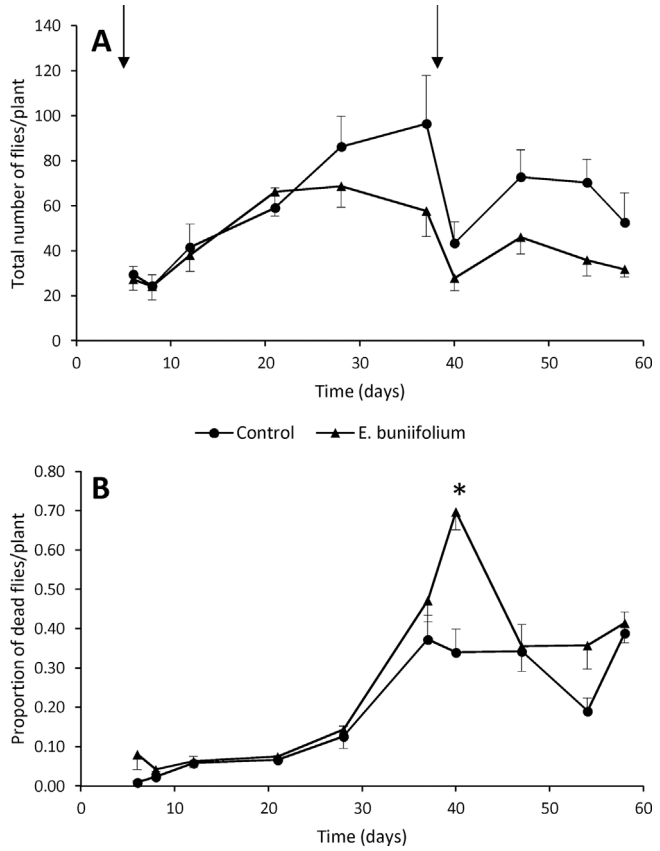

Fig. 4. Population variation as a function of time (mean ± error): total number of whiteflies settled per plant (A), and proportion of dead whiteflies per plant (B). Arrows indicate the days when applications were done (after fly counting). Significant effects (ANOVA, GLM, $P < 0.05$) of the treatment and time were found for both variables (see text for further details) and in the case of proportion (insert B) also for the interaction treatment x time.
*indicates a significant difference between treatments at day 40 (ANOVA, GLM, $P = 0.003$ by Tukey Pairwise Comparisons).

$\chi^2 = 0.08$, $P = 0.92$ for number of fruits (Lowry, 1998-2009; Lowry, 1998). Further, no leaf necrosis or other toxic effects on plants treated with the EO were observed throughout the trial. These results together suggest that EO application has no phytotoxic effects. It is noteworthy that plants treated with the EO showed a lower incidence of fungal infection than control plants (data not shown). A second greenhouse trial where EO was applied at 4.5% (WT emulsion) showed necrotic effects on leaves that did not allow for whiteflies to settled on plants and therefore the trial was not completed.

4. Conclusions

In this study, the EOs from two asteraceous plants were characterized based on their toxicity to seeds and vegetative parts of tomato plants and honey bees as model beneficial organisms. Seed germination was dose-dependent: whereas at the dose representing the LD_{50} for the whitefly *T. vaporariorum* neither seeds were affected, at the dose that represents the LD_{50} for the leafminer *T. absoluta*, germination of both seed varieties was inhibited. However, in this later situation, our results showed that Cetia seeds were less susceptible than Mirella seeds. Remarkably, these results parallel the one found for honey bees where the calculated LD_{50} fell between the LD_{50} values for *T. absoluta* and *T. vaporariorum*. Therefore, the use of these EOs to control *T. vaporariorum* would likely cause no adverse acute effects on seeds and honey bees. However, possible sublethal effects must be further investigated. As mentioned, pollination is usually assisted by bumblebees (Peet and Welles, 2005), when tomato is produced in greenhouses. Although we have not tested toxicity to bumblebees, previous reports suggest that susceptibility to pesticides is similar for bumblebees and honey bees (Thompson, 2001; Thompson and Hunt, 1999). Finally, while vegetative parts suffered necrotic effects when *E. buniifolium* EO was applied at 4.5% in greenhouses, no such effects were detected when this EO was applied at 3%. Furthermore, in this situation, the *E. buniifolium* EO showed acute toxic effects on whiteflies and the crop yield was not significantly different from the control plants. Overall, the results reported here and in the previous reports (Umpiérrez et al., 2012) indicate that *E. buniifolium* appears to be a good candidate for the development of a botanical pesticide.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.indcrop.2017.09.025.

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