Peer Community In Zoology

Side effects of essential oils on pest natural enemies

Cedric Pennetier *based on peer reviews by* ***Olivier Roux*** *and 2 anonymous reviewers*

Louise van Oudenhove, Aurélie Cazier, Marine Fillaud, Anne-Violette Lavoir, Hicham Fatnassi, Guy Pérez, Vincent Calcagno (2022) Non-target effects of ten essential oils on the egg parasitoid Trichogramma evanescens. bioRxiv, ver. 4, peer-reviewed and recommended by Peer Community in Zoology.
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Integrated pest management relies on the combined use of different practices in time and/or space. The main objectives are to better control pests, not to induce too much selective pressure on resistance mechanisms present in pest populations and to minimize non-targeted effects on the ecosystem [1]. The efficiency of such a strategy requires at least additional or synergistic effects of chosen tools against targeted pest population in a specific environment. Any antagonistic effect on targeted or non-targeted organisms might reduce control effort to nil even worst.

Van Oudenhove et al [2] raised the question of the interaction between botanical pesticides (BPs) and egg parasitoids. Each of these two strategies used for pest management present advantages and are described as eco-friendly. First, the use of parasitoids is a great example of biological control and is massively used in a broad range of crop production in different ecological settings. Second, BPs, especially essential oils (EOs) used for a wide range of activities on pests (repellent, antifeedant, antiovipositant, ovicidal, larvicidal and simply pesticidal) present low-toxicity to non-target vertebrates and do not last too long in the environment. Combining these two strategies might be considered as a great opportunity to better pest control with minimized impact on environment. However, EOs used to target a wide range of pest might directly or indirectly affect parasitoids.

Van Oudenhove et al [2] focused their study on non-target effects of 10 essentials oils with pesticide potential on larval development and egg-seeking behaviour of five strains of the biocontrol agent *Trichogramma evanescens*. Within two laboratory experiments mimicing EOs fumigation (i.e. contactless EOs exposure), the authors evaluated (1) the toxicity of EOs on parasitoid development and (2) the repellent effect of these EOs on adult wasps. They confirmed that contactless exposure of EOs can (1) induce mortality during pre-imaginal

development (more acute at the pupal stage) and (2) induce behavioural avoidance of EOs odour plume. These experiments ran onto five strains of *T. evanescens* also highlighted the variation of the effects of EOs among parasitoid strains.

The complex and dynamic interaction between pest, plant, parasitoid (a natural enemy) and their environment is disturbed by EOs. EOs plumes are also dynamic and variable upon the environmental conditions. The results of van Oudenhove et al. experimentally illustrate such a complexity by describing opposite effects (repellent and attractive) of the same EO on the behaviour of two *T. evanescens* strains. These contrasting results led us to question more broadly the non-target effects of pest management programs based on EOs fumigation on natural enemies.

Finally, the limits of this experimental study as discussed in the paper draw research avenues taking into account biotic variables such as plant chemical cues, odour plume dynamics, individual behavioural experiences and abiotic variables such as temperature, light and gravity [3] in laboratory, semi-field and field experiments. Facing such a complexity, modelling studies at fine scale in time and space have the operational objective to help farmers to choose the best IPM strategy regarding their environment (as illustrated for aphid population management in the recent review by Stell et al. [4]). But before such research effort to be undertaken, Van Oudenhove et al study [2] sounds like an alert for a cautious use of EOs in pest control programs that integrate biological control with parasitoids.

References:

[1] Fauvergue, X. Biocontrôle Elements Pour Une Protection Agroecologique des Cultures; Éditions Quae: Versailles, France, 2020.

[2] van Oudenhove L, Cazier A, Fillaud M, Lavoir AV, Fatnassi H, Pérez G, Calcagno V. Non-target effects of ten essential oils on the egg parasitoid *Trichogramma evanescens*. bioRxiv 2022.01.14.476310, ver. 4 peer-reviewed and recommended by PCI Zoology. https://doi.org/10.1101/2022.01.14.476310

[3] Victor Burte, Guy Perez, Faten Ayed, Géraldine Groussier, Ludovic Mailleret, Louise van Oudenhove and Vincent Calcagno (2022) Up and to the light: intra- and interspecific variability of photo- and geo-tactic oviposition preferences in genus *Trichogramma*, Peer Community Journal, 2: e3. https://doi.org/10.24072/pcjournal.78

[4] Stell E, Meiss H, Lasserre-Joulin F, Therond O. Towards Predictions of Interaction Dynamics between Cereal Aphids and Their Natural Enemies: A Review. Insects 2022, 13, 479. https://doi.org/10.3390/insects13050479

Reviews

Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.1101/2022.01.14.476310
Version of the preprint: v2

Authors' reply, 16 December 2022

Download author's reply

Decision by Cedric Pennetier, posted 18 November 2022, validated 18 November 2022

Dear Louise van Oudenhove and authors,
The revised preprint was reviewed by 2 reviewers who agree that the revised draft is very good. One of them suggested a minor revision.
So I invite you to address this last minor concern before recommanding your paper.
Thanks you again for your support to PCI.
Cédric Pennetier

Reviewed by Olivier Roux, 14 September 2022

All concerns and suggestions I have made were satisfactorily answered by the authors.

Nevertheless, I still have a minor concern with one point in the "statistical analysis" – "olfactometry bioassays".

To a previous comment, the authors answered: "We do not know individual location, we only have access to the number of individuals in a given zone". In that case, the use of some wording is confusing, such as: "determination of the individuals that stay in their current zone" line 265. This suggests that some identified individuals did not move while it is the number (or proportion) of individuals that has not changed.

The authors should consider slightly rewording this section as well as the corresponding results section.

Reviewed by anonymous reviewer 1, 15 September 2022

The authors have addressed all my comments and improved the manuscript with a better description of the methods and results and a more complete discussion. I have no supplementary comments.

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2022.01.14.476310

Authors' reply, 01 September 2022

Download author's reply

Decision by Cedric Pennetier, posted 26 April 2022

Dear Louise van Oudenhove and authors,
Thanks so much for submitted your preprint to PCI Zoology.
Your preprint was reviewed by 3 specialists and all of them agree that your work is highly relevant. Reviewers highlighted few major concerns that should be addressed in ordrer to improve the quality of your manuscript.
So I invite you to go through all comments and submit a revised version of your preprint.
Thanks you again for your support to PCI.
Cédric Pennetier

Reviewed by anonymous reviewer 2, 20 April 2022

Download the review

Reviewed by Olivier Roux, 14 April 2022

The manuscript by van Oudenhove et al. entitled "Non-target effects of ten essential oils on the egg-parasitoid Trichogramma evanescens" reports laboratory experiments on both parasitoid pre-imaginal development and

female movements when exposed to vapor of pure essential oils. Authors selected 5 strains of Trichogramma evanescens which were exposed to different doses of ten essential oils (EOs) either in a tube or in a 4-way olfactometer to investigate effect on development or movements, respectively. The authors used Bayesian statistics to analyze all the data.

As I am not qualified to review Bayesian analyzes, I did not make any comments on it. Nevertheless, I found it sometimes difficult to comprehend and I had to trust the authors on results interpretations.

The rationale is well steered and results support conclusions. However, I expected more discussion about the parasitoid movements and the origin of strains. Is it possible to make more link between secondary compounds found in ”mother plants”, movements of parasitoids while exposed to EOs and the inter-strain variability? I think it could underline the originality of working with several strains of a single parasitoid species.

My main concern is about the doses used to test toxicity on pre-imaginal development. Compared to a quick search in literature, doses used here seem to be very high (see comment below). The authors should explain this choice and specify why it is acceptable and/or discuss this drawback.

Hereafter are some comments intended to help the authors to improve the manuscript.

Introduction

-lines 65-66: I guess that many EOs have an insecticidal effect. Is there a reason to choose specifically these ten EOs?

-line 69: ”parasitoid” not ”paratoid”

-line 69: ”movement” is not clear enough in the context of the last section of the introduction. Please be more specific.

Materials & Methods

-lines 73-78: As for EOs, is there a reason to choose these five strains of T. evanescence? Did the authors expect different results according to the origin of parasitoids? The five strains were collected in 2015 and 2016. When were carried out the experiments? How many generations were obtained between collection and experiments?

-line 98: Please, change ”experiment” for ”exposure to EOs”

-line 99: Were all the eggs parasitized? If not known at Day 1, please reword. Is there any chance that around 50 females for around 25 eggs produced superparasitism? If yes, is it possible that it affects results on pre-imaginal development and emergence rate?

-lines 103-104: Please, provide concentrations to make them comparable to other studies. When I converted doses in concentrations, they seem to be very high. Are they relevant if used in the field? This point could be discussed.

-line 106 and throughout the manuscript: Please use ”location” instead of ”position”.

-lines 122-130: Please, use the past tense.

-line 129: What was the extraction air flow?

-lines 134-135: Did all the females climb up? What happened if too few females climbed up?

-line 136: I guess it was with the same EO.

-lines 189-191: From which data was the initial parasitism rate estimated? If I understood well, the first data collected are at day 5 to estimate the number of eggs turning black (pupal stage).

-line 239 and legend of Table 3: ”µL” instead of ”mL”

-line 240-247: In repeated measurements of individual locations, the probability that an individual is located in a given area at t(n) is dependent from its location at t(n-1). Does the model take account for the previous location of each individuals? Or, are the parasitoid movements fast and long enough (in a 2-3min laps time) to consider as equal the probability for a parasitoid to be located at any point?

Results

-lines 272-297: This section is very unclear, I had to read it several times and I am not sure I understood well. Authors provide a C50 (which is in fact a LD50, see below) which seems to correspond to the overall development (<2µl). Then, they provide probabilities for the first phase of development (α) and the second phase (β) for a dose of 5µL. Using the same estimation procedure for survival would help the reader to comprehend. Maybe the authors should consider reworking this section.

-line 272: What the authors named ”C50” is in fact a ”LD50” (if it can be considered that an absence of development into adults is related to the death of larvae, otherwise it is an ED50).

Discussion

-lines 349-350: ”emergence rate” and ”pre-imaginal development” seem to be used indifferently to refer to the same data point. Please pick one and be consistent across the manuscript or clarify.

-line 360: It could also be a consequence of stage duration as pupal stage is almost twice the larval stage duration.

-lines 386-390: Unclear

-lines 373-390: The conclusion of this section could be that exposure mode could be highly important to evaluate parasitoid susceptibility to EOs.

End of comments.

Reviewed by anonymous reviewer 1, 14 April 2022

The work presented in the manuscript by VanOudenhove and collaborators entitled ”Non-target effects of ten essential oils on the egg-parasitoid Trichogramma evanescens” investigates the fumigant effect of 10 essentials oils on the parasitoid’s development and behavior. To that purpose, authors conducted 2 experiments. First, they exposed eggs of 5 strains of T. evanescens to vapor of 3 doses of each EO and characterized the probability of further development into pupae and into adult. In a second experiment, they exposed females parasitoids

to 2 doses of each EO in a 4-ways olfactometer to investigate attractant/neutral/repellent properties of the EO. Authors found that some EO have toxic effect on the development of the parasitoids while other EO have significant effect on their behavior (repellency/attraction).

I found this work highly relevant as it could highlight adverse effect of a promising new method for pest control. Nevertheless, I have some concerns and remarks about the methodology, presentation and interpretations of the results that I have detailed in the part below.

I suggest the authors to re-organize and clarify the results presentation, particularly the effect of EO on development.

Introduction

- Are EO tested already available as fumigant bio pesticides ?

- Authors should justify the choice of the fumigant form for assessing the insecticidal and repellent potential of chosen EOs.

§ 2 : I recommend to the authors to define clearly « non-target » term as it looks confusing during reading. I first understood this term as an effect on non target species (l.36) and later as a synonym of sub lethal effect (l.37:39).

- L65:70 : Use of terms « direct » and « indirect » effect seems confusing because I first though that « direct » mean after a contact with the EO and « indirect » without contact. I suggest to authors to change these words and directly talk about toxic effect and behavioral (or other word) effect.

Method

- Insects : the descriptions of strains used and rearing conditions are well done. I suggest to the authors to justify the choice of these 5 strains.

- Essential oils : the description and origins of the EOs are well written. Full description of EO composition in supplementary Information is clearly presented.

- L89 : Typo « ; » instead of « . »

- L103 : How did the authors chose the volume of essential oil ? Are there previous experiments done that studied the volume to use? Please add a reference if any.

- L104: The volume of air in the tube seems to be very small in comparison to the volume of essential oil deposited in the cotton. Do the authors have an idea about the concentration of EOs in the tube? Is it the concentration used in the field for biocontrol method?

- As tubes were closed during all the experiment, maybe it could be a risk of saturation/increase of concentration of EO along days. I have a concern about the influence that could have on development.

- The tubes are in dark all the time, is this natural condition? If not, can't the lack of light/dark alternance and therefore, daily rhythm influence pupation or the emergence of adults?

- Olfactometry bioassays: The device is clearly and precisely described, allowing to replicate experiment if needed. I particularly appreciate the verification of air flow before experiment.

- As EOs could be toxic as vapor on adult, there was a risk of toxic effect in the olfactometer. Could authors explain their choice of the EOs volume to test?

- L131: what is meant for « lm » ?

- L133: Could authors give the age of the parasitoids used for olfactometer experiment?

- L167 : « NbB the number of parasitoid pupae NbB = EggsB) » this sentence implies the assumption of a one to one relation between the number of brown egg and pupae. As the number of females parasitoids was twice higher than the number of eggs of E. kuehniella , could it be possible that an egg could be parasitized with more than one T. evanescens egg?

Note : typo «) » at the end of the sentence.

Statistical analysis

R Scripts are not joined to the manuscript.

Pre imaginal survival :

L190 : Could authors precise how was estimated the number of parasitized eggs? In supplementary informa-

tions this parameter is in circle, meaning that it has to be estimated, but I do not understand how.
L192, 195,198 and supplementary informations : Why a binomial distribution is used for the estimation of the number of parasitized/turning brown eggs? As the binomial distribution is used for modeling the frequency of successes, I am confused. Do the authors want to say "proportion of eggs" instead of "number" ?
L193 : EO concentration is noted Cl in the text, but Ci in the equation. Is it because one model is run for each dose (5-10-20µL) ? Could the authors specify in which sense the term C should be understood?
L199 : the description of the beta parameter is a little bit confusing. Authors said it represents the difference of the sensibility between eggs/larva and pupae but that it could also be viewed as the representation of a cumulative effect. As accumulation of EO in a tube is very likely, is it possible to add a parameter to separate this cumulative effect from the real sensitivity to EO of the different stages?

Olfactometry :

L224-225: Same comment as previously about the use of a binomial distribution for number estimation.

Results

General comment:

- I am not familiar with Bayesian analysis, but I am wondering if estimated parameters could be given with an equivalent of confidence interval as in frequentist way? It would allow the reader to quickly assess the precision of the estimation.
- Raw data are not available to the reader.

L272: 276 : concentration word is used but authors talk about volume (µL), not concentration (quantity/volume). It would be more precise to talk about doses of EO.
There is no description in the method part of the C50 estimation, could the authors add a description of this point in the method part ?

Fig 4 legend :

Authors should precise the « development » term as it could be used for egg to pupae or pupae to adults etc.
Authors should precise what represents the ribbon around lines.
Authors should clarify the signification of ±0.01 » in « 0.95±0.01 ».

L286 : Authors should clarify the signification of « ±0.0005 » in « 0.997±0.0005 ».

Figure 5: I do not understand if estimation parameters are given for one given dose or for all ? Could authors clarify this point?

L298: Olfactometry bioassay.

It could be interesting to provide the total number of females that enter in the olfactometer ats the same time. Is 60 the maximum number of individuals that avoid group effect response in the olfactometer? Is group effect behaviour known for T. evanescens? Please provide a reference if any.
L302 : Authors should precise the signification of the interval.
L304 : Authors need to homogenize the terms they want to use. I suggest that dose is better than concentration in this context.

§ L329 & Fig 9: I do not really understand why authors make such hypothesis about the relation between toxicity and behavior.
First, the authors do not know the concentrations to which the development stages and adults are exposed, then the comparison doesn't seem relevant. Indeed, it is very likely that the concentration in the tube of the developmental experiment is more important than in the olfactometry bioassays because of the low volume of the tubes.
As the relation between toxicity and behavior is highly complex and dose dependent, I suggest the authors to modify this part of their results and to explicit more clearly their hypothesis in the discussion section.

Discussion

Global comment : Authors should add a paragraph to discuss the fact that they do not know the actual air concentration in both experiment.
L337 : same comment as in introduction about direct and indirect terms use.

L360-364 : Do the authors have an hypothesis about the higher sensibility to EO of pupal stage compared to larva stage ? It could be interesting to develop this point.

§ L402 : experimental design means : olfactometry design ?

I suggest to remove « but had no oviposition experience » (L403) because it suggests that females in the olfactometer are searching for egg to parasitized during the experiment and we do not know that. Authors could make this assumption if there was a signal of egg presence in the olfactometer, but it was not the case if I well understood.