IMPROVING INPUTS FOR ORGANIC FARMING

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Protocol for the optimized use of *Clitoria ternatea* extract and Orange Oil and description of prototype-shakers for vibration and playback code

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1. Executive Summary

This document addresses the optimized use of pesticides proposed for the gradual phase-out of Mineral Oil, Orange Essential Oil and *Clitoria ternatea* extracts, and reports the description of the prototype shaker usable in greenhouse for the control of the whitefly *Trialeurodes vaporariorum*.

2. State of the Art of Essential Oils and Vibrations to control Whiteflies

A sustainable approach for the control of whiteflies is based on the use of essential oils and plant extract with insecticidal properties. Several insecticides of botanical extracts have been tested at research level in recent years (Baldin, et al., 2015; Aroiee, et al., 2005). The most promising plant extracts and plant oils are under further development but according to our knowledge to date, there are **no active ingredients registered in Europe against whiteflies** based on plant extracts and the only plant oil registered is Orange Oil. Applied biotremology is a new frontier of insect control. Insect's vibrational communication can be manipulated to develop ecofriendly strategies of pest control (Nieri, et al., 2021). For two economically important whitefly species, the Greenhouse Whitefly *Trialeurodes vaporariorum* and the tobacco whitefly *Bemisia tabaci*, vibrational communication has been partially studied (Kanmiya, 1996; Kanmiya, 2005; Takanashi, et al., 2019). The fine description of the mating behavior and the associated vibrational signals has been achieved during RELACS (Fattoruso, et al., 2021). Recently, the efficacy of substrate-borne vibrations has been tested to reduce the greenhouse population density of *B. tabaci* (Yanagisawa, et al., 2021). In this experiment, the vibrational exciter was connected to tomato plants via a plastic rod and operated for 1 min every 30 min. Disruptive vibrations seems to have pushed the insects away from the plants, also interfering with the mating and inducing stress as startle responses. In this case, authors concluded that more studies are needed to improve this new technology and better understand the mechanism behind the reduction of population density obtained. At this moment, despite the relevant volume of research going on, **there are no available commercial tools or methods of pest control, based on the release of vibrational signals** into plants.

3. Part A: Essential oils and plant extracts

3.1. Characteristics of the Essential Oils and plant extracts

3.1.1. *Clitoria ternata*

Clitoria ternatea L. is a plant belonging to the family of Fabaceae (subfamily: Papilionaceae), commonly named as "butterfly pea". It is known to be an excellent forage legume that can also be used as a cover crop and green manure (Mukherjee, et al., 2008), as an ornamental plant (Gomez & Kalamani, 2003) or for medicinal purposes (Al-Snafi, 2016; Mukherjee, et al., 2008). Due to the presence of "Finotin" protein in its seeds, and other secondary metabolites, *C. ternatea* is reported to have antibacterial, antifungal, and insecticidal activities (Kelemu, et al., 2004). The insecticidal effect is associated to the presence in the plant extract of cyclotides and flavonoids, which are synthesized in response to herbivore attacks (Zanotelli, et al., 2017). Seed extracts of C. *ternatea* had shown a great efficacy against mosquitoes larvae (*Aedes aegypti, Culex quinquefasciatus* and *Anopheles stephensi*), and against the Indian earthworm *Pheretima posthuma* Kinberg (1867) (Haplotaxida: Megascolecidae); in addition the substance displayed anthelmintic activity (Nirmal, et al., 2008; Mathew, et al., 2009). *Clitoria ternatea* based oil was reported to significantly decrease the level of infestation of the cotton pest *Helicoverpa spp. Hübner* (Lepidoptera: Noctuidae), interfering with the insect feeding and oviposition behaviors and causing larvae and adults mortality by direct toxicity, with no effects on the beneficial fauna (Mensah, et al., 2015). Bioassays followed by field trials to test the efficacy of this plant extract against several pests showed promising results, particularly against the whitefly *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) and the thrips *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) (Zanotelli, et al., 2017).

As *C. ternatea* extracts contain different active compounds ("finotin" protein, cyclotides and flavonoids), several modes of actions may be at play. For example, cyclotides display insecticidal activities that seem to be mediated by selective membrane binding and disruption (Poth, et al., 2011). Meanwhile, flavonoids' antibacterial effect is the result of the inhibition of energy metabolism and nucleic acid synthesis and the damage of microorganisms' cytoplasmic membrane (Ahmad, et al., 2015).

No phytotoxicity caused by *C. ternatea* extracts has been observed or reported.

3.1.2. Orange Oil

Citrus essential oils have been largely used for medicinal purposes, as well as for the protection of stored crops and as a pesticide (Mahmoudvand, et al., 2011; Palazzolo, et al., 2013). Citrus essential oils, and more precisely Orange Essential Oil (OEO), are gaining importance as prominent biopesticides, widely available, economically affordable, and effective for pest management (Campolo, et al., 2016; Malacrinò, et al., 2016).

The insecticidal activity of Orange essential oil has been associated to its content of monoterpenes, specifically Limonene (Zarubova, et al., 2015; Malacrinò, et al., 2016). D-Limonene was reported to be effective also against nematodes, bacteria, fungi as well as mites, by displaying acute and sub-lethal effects, especially on adult females, with a decrease in oviposition and interference on mites' movement (Ibrahim, et al., 2001). In addition, limonene was reported to show a great efficacy against some whiteflies, mealybugs, and scale insects (Hollingsworth, 2005).

3.2. Technical Feasibility and Protocol

BPA044I, the product based on *C. ternatea* extract, is an emulsifiable concentrate highly soluble in water. The product can be easily diluted and sprayed on the crops with all the standard devices used for conventional pesticides (e.g., back sprayers, atomizers). Moreover, the proteins contained in the extract are stable over heat.

Orange oil product is formulated as a micro-emulsion, is diluted in water, and applied to the crop with standard spray devices used for conventional pesticides. For the control of whiteflies in open-field and greenhouse, the label dose rate suggested is 400 mL/hL.

BPA044I did not show a good efficacy against the adult stage neither on zucchini nor on tomato plants. It showed a good efficacy against nymphs on tomato but not on zucchini plants. The Orange Oil preparation showed a significant efficacy on *T. vaporariorum* adults both on zucchini and tomato plants, while against nymphs the efficacy was low on tomato, but high on zucchini plants. Concerning the egg stage, none of the tested products showed a relevant efficacy.

To conclude, the most vulnerable stages are adults and nymphs, while the eggs appear to be not significantly affected by treatments with the tested essential oils.

The two products **are physically and chemically compatible.** When mixed together in tap water we did not observe residues, deposits, formation of phases or any other negative trait. BPA044I and Orange Oil were used at **20 and 4 mL/L**, respectively, as final concentration in the spray solution. Tap water was used to dilute the products and application was performed with a pressurized hand sprayer.

The mixture should be applied as soon as the first signs of pest infestation (presence of adults) appear on the crop and applications can be repeated with intervals of approximatively 7-10 days and according to the pest pressure. The products can be also sprayed in the field with a 15 Bar atomizer, at a rate of 2.7L/min until runoff.

In experimental trials, the mixture produced a significant reduction of populations of *T. vaporariorum* (in the greenhouse), *Aleurocanthus spiniferus* and *Planococcus citri* (in the field).

4. Part B: Vibrational signals

4.1. Spectral and temporal characteristics of the Disruptive Signals

The playback signal (PbS) is emitted by an electromagnetic transducer (mini shaker) and has been synthesized on the base of *T. vaporariorum*'s mating signals' characteristics (Fattoruso, et al., 2021). The PbS has been designed with the precise aim to disrupt whiteflies communication (currently the target is *T. vaporariorum*, but the method is potentially extendable to other species such as *Aleurocanthus spiniferus* and *Planococcus* spp.). The PbS is characterized with 6 main peaks of amplitude corresponding with the 3rd to 8th harmonics of the signals used by the insects to communicate, around 50 Hz and 200 Hz for the fundamental and the dominant frequency, respectively. More specifically, the peaks are at 152, 200, 248, 296, 352, 400 Hz (Fig1a). The signal duration is a sequence of 1s-long, which is played in loop. The desired intensity of the PbS, measured as velocity of the substrate at the peak of frequency, must be at least 0.5 mm/s (measured from the top of the vibrating plate).

4.2. The Device and its characteristics

"Vibro-Plate" is the name of the device developed to control the greenhouse whitefly. It consists of a square plate made of wood (side length: 20 cm, thickness: 1 cm). The plate is covered by a plastic layer that makes it waterproof and prevents damage from plant watering. The plate is provided with 4 iron legs (h: 6.5 cm). Under the plate, in the center, is placed a mini-shaker (model Tremos, CBC-Biogard) which is electrically powered (12V) (Fig. 1b, c, d). The emission of the PbS is allowed by a microchip installed inside the mini-shaker and which is associated to the mechanical part.

4.3. Technical feasibility: applications in the greenhouse and in the field

The designed prototype can be easily applied in the greenhouse thanks to its versatility. The plate dimension can be modified and adapted to the bench size, or it can be used as support for the plants grown on the ground. The plants can be put singularly directly on the plate or in groups on a larger board (made of wood, plastic or polystyrene). To reinforce the effects of the PbS, groups of two or more mini shakers could be used to transmit the PbS to a single large board. As an alternative, the PbS could be spread through metallic wires for those crops that use wires as a part of the trellis system. The latter can be more practical for the use in the field, when the method would be applied to bush and trees for large surfaces. In the greenhouse, the energy will be provided by electric cables plugged in the facility, whereas in the field it is supplied by a solar panel. The calibration of the system is made by measuring the PbS with an accelerometer and by analyzing the spectral properties of the vibrations once the prototype is installed, to ensure a correct transmission of the signals.

5. Conclusion

Our trials indicated that the essential oils provide a substantial protection from whitefly, adults and nymphs, but also that, especially in the case of nymphs, performances are different according to the target plant (either tomato or zucchini). In this way, the use of an optimized mixture of the two products conferred higher efficiency that was positively tested against not only whiteflies, but also *Planococcus*. On the other hand, the device to produce a disruptive vibrational signal, characterized by spectral and temporal parameters suitable to interfere with the intraspecific communication of the whitefly, is now available to be employed, together with essential oils. The combination essential oils-vibrations aim at increasing the stress caused to the target pests and thus significantly reduce their populations and the associated damage to the crop.

6. Dissemination activities related with the Deliverable

Part of this research has been published in a peer reviewed article:

Mokrane, S., Cavallo, G., Tortorici, F., Romero, E., Fereres, A., Djelouah, K., Verrastro, V., Cornara, D. (2020). Behavioral effects induced by organic insecticides can be exploited for a sustainable control of the Orange Spiny Whitefly *Aleurocanthus spiniferus*. Scientific reports, 10(1), 1-12.

This research has also been presented and discussed on several occasions:

- Talk at a Scientific Conference: "Sustainable alternatives to gradually phase out mineral oil for pests control" Fattoruso V, Cornara D, Keciri S, Berardo A, Mazzoni V, Verrastro V. - Second International Congress of Biological Control (ICBC2), Davos, 26-30 April 2021.
- Talk at a dissemination event organized by Ecovalia 'alternativas a los aceites minerales en control de plagas de cítricos'. Las vibraciones como estrategia nueva, alternativa y sólida para el control de plagas. Fattoruso V., Mazzoni V. (https://www.ecovalia.org/index.php/comunicacion/noticias/16-ambito-internacional/428-ecovalia-organiza-un-taller-on-line-para-presentar-alternativas-a-los-aceites-minerales-en-control-de-plagas-de-citricos)
- Discussion and workshop with stakeholders on Replacement of Copper in Viticulture at the annual event 'Metodi innovativi per la sostituzione degli oli minerali in agricoltura' (Italian), Mach 'Efficacy of BPA044I and 030-S-1-D against the Greenhouse whitefly Trialeurodes vaporariorium (Westwood) (Homoptera: Aleyrodidae)' Fattoruso V., Mazzoni V. San Michele all'Adige, Trento, Italy (https://www.fmach.it/CRI/info-generali/comunicazione/eventi-CRI/Controllo-di-insetti-e-microrganismi-dannosi-e-fertilizzazione-studi).

The vibrational device is described in a RELACS practice abstract to be soon available on: https://relacs-project.eu/resources/practical-guidelines/. A dossier on the use of disruptive vibrations was presented during a RELACS national workshop on Mineral Oil.

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Figures

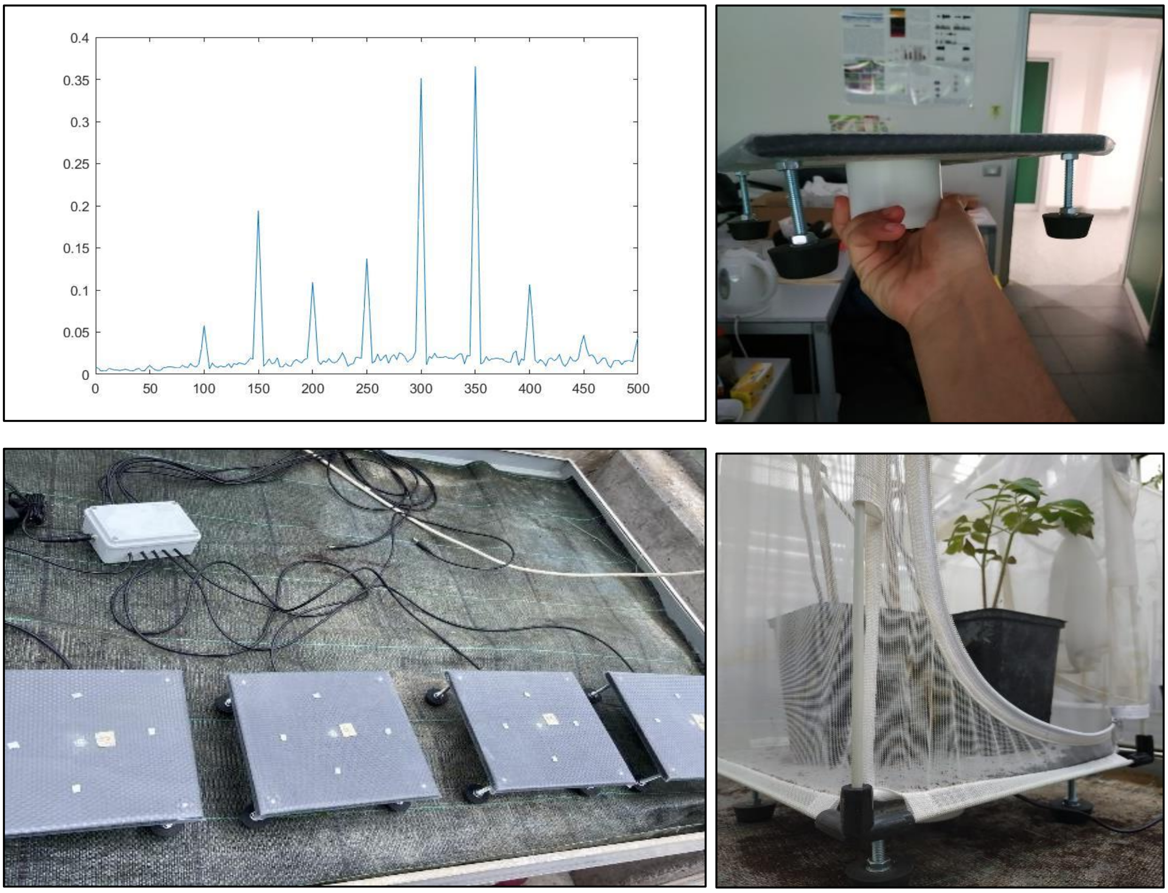

Figure 1: a) Power spectrum of the PbS with the main peaks of frequency; b) detail of the mini-shaker placed under the square wooden plate; c) vibrational plates; d) experimental setup.