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Chemical properties and efficacy of Sweet orange essential oil nanoemulsion applied as cold aerosol against two stored product beetles

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Abstract

Common control strategies to manage stored product pests are mainly based on the use of synthetic insecticides and fumigants. Consumer's demand for pesticide-free food, and the increasing resistance of pests to traditional insecticides, dictate the need to evaluate alternative control methods. For this purpose, many sustainable techniques have been tested for the control of stored product pests. Among them, Citrus essential oils can represent a valid alternative to synthetic insecticides. The effects of Sweet Orange essential oil (EO) nanoemulsion applied as cold aerosol were evaluated against adults of *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) and *Cryptolestes ferrugineus* Stephens (Coleoptera: Cucujidae). Both chemical and physical characterization of the EO-based formulation was carried out. The developed formulation had an average size belonging to the nanometer scale and a low polydispersity index. The relatively high zeta potential value confirms the stability over time of the developed formulation. The efficacy of the tested formulation showed a dose-dependent response and the cumulated mortality of the exposed insects increased until 24h of exposure for *C. ferrugineus* and until 120h for *T. confusum*. The tested formulation was more effective against *T. confusum* adults (LD₅₀= 86.30 ppm) than *C. ferrugineus* ones (LD₅₀= 36.79 ppm). The results of this study coupled with the large availability at reasonable costs of Sweet orange EO, are promising for the potential development of new tools against stored product pests.

Keywords: Citrus, essential oil, *Tribolium confusum*, *Cryptolestes ferrugineus*, control, fumigation

Introduction

Although their high toxicity and non-biodegradable nature have already been acknowledged worldwide, the use of synthetic pesticides is still increasing (Koul et al. 2008). However, the environmental consequences, the negative impact on non-target species and the development of resistance have stimulated the interest in alternative control strategies, such as the use of naturally derived chemical compounds (biopesticides), which have selective toxicity and are easily biodegradable (Kordali et al. 2006; Regnault-Roger et al. 2012).

Among botanicals, essential oils (EOs) are effective biopesticides, due to their promising laboratory results in term of toxicity against insect pests, bacteria and other pathogens (Romeo et al. 2008; Ali et al. 2012; Russo et al. 2013; Campolo et al. 2014).; Essential oils are volatile natural compounds, synthesized by many species of plants as secondary metabolites (Bakkali et al. 2008), which may act against insects as larvicidal, antifeedant, growth inhibitor, adulticidal, fertility reducer, oviposition deterrent and repellent (Cardiet et al. 2012; Ibrahim et al. 2001; Werdin-González et al. 2011; Licciardello et al. 2013). Furthermore, EO toxic activity against mammals is quite reduced (rat oral LD₅₀ = 2–5 g × kg⁻¹) (Regnault-Roger et al. 2012), guaranteeing product specificity and safety. Although most essential oils are exempt from registration, standardization and quality control are key issues for registration (Isman 2000; Koul et al. 2008). Moreover, EO chemical composition can negatively affect their application in operative conditions, since EO-based insecticides generally show high volatility and poor solubility in water (Moretti et al. 2002).

In this context, the development of a stable formulations containing EOs is a pivotal requisite for the application of these technique in field conditions. Nanoemulsions are defined as emulsions (i.e. mixtures of two or more liquids that are normally immiscible) in which the micelles of the dispersed phase show nanometric dimensions. In this study, we developed and characterized a nanoemulsion oil in water (i.e. the dispersed phase was EO and the dispersion medium was water) of sweet orange (SOR) [*Citrus sinensis* (L.) essential oil. Thus, the aim of this study was to assess the insecticidal activity of SOR-EO against two stored product pests, *Tribolium confusum* du Val and *Cryptolestes ferrugineus* Stephens, testing its toxicity as cold aerosol (i.e. cold fumigation) against adult insects.

2. Materials and Methods

2.1. Insects

The confused flour beetle *T. confusum* and the rusty grain beetle *C. ferrugineus* were reared for several generations in the Stored Products Laboratory of the Department of Agriculture on wheat flour mixed with yeast (10:1, w: w). The rearing conditions were: 25 ± 1°C, 65±5% r.h., with a

photoperiod of 16h:8h (L: D). To obtain adults of the same age, about 100 unsexed adults were placed inside 5 l glass containers each provided with 500 g of non-infested rearing medium. After 2 days the specimens were removed and the newly emerged adults (2–8 days old) were used in the trials. Insects were collected from cultures using a 450- μ m sieve (Technotest; Modena, Italy) and a mouth aspirator.

2.2. EO extraction

SOR-EO was extracted from the fruit peels, pesticide-free certified (Capua SRL, Campo Calabro Italy). The essential oil was extracted with the cold pressing technique (Citroflor, Condofuri Marina, Italy) (Lahlou 2004), from fruits cultivated in Calabria (Italy) and harvested from November to March following the harvest calendar for the species.

2.3. Nanoemulsion formulation and characterization

TWEEN 80 (Polyoxyethylene (20) sorbitan monooleate) was purchased from Sigma-Aldrich (Italy). The EO-NPs were prepared following Werdin-González et al. (2011), with some modifications. In brief, EO was mixed with Tween 80 and stirred for 30 min. Then, nanoemulsion was realized using the mechanism of spontaneous emulsification that is created between an organic phase and an aqueous phase when they are mixed. Double-distilled water was added to the homogeneous solution of citrus essential oil and a hydrophilic surfactant and then stirred for 60 min. The oil in water nanoemulsion was composed by 5% Tween 80[®], 15% essential oil and 80% water. Lastly, to reduce micelles, the formulation was sonicated using an ultrasonic immersion homogenizer, stored at 25 \pm 0.5 °C in an airtight container and used for the bioassay within the following 48h. In order to measure the characteristics of the realized nanoemulsion, qualitative analyses such as particle size, polydispersion and emulsion stability were measured using the Zetasizer Nano (Malvern[®]) instrument. The dimensional and polydispersion analyses were carried out in cuvettes, model DTS0012 in polystyrene latex at 25°C. The nanoemulsion was diluted in double-distilled water in a ratio of 1/200 and 1 ml of the diluted solution was inserted into the cuvette. The measurement of each sample involved 3 replicas of 14 cycles. Three samples were tested as replicates. The stability analysis (potential ζ) was carried out by inserting 730 μ l of the diluted solution in DTS1070 polystyrene latex cuvettes and tested at 25°C. The measurement of each sample involved 3 replicas of 14 cycles. Three samples were tested as replicates.

2.4. Cold aerosol trials

Toxicity trials were carried out in laboratory conditions at 25 \pm 1°C, 65 \pm 5% r.h. with a photoperiod of 16h:8h (L:D). Test specimens were placed inside a Perspex cage (25 x 25 x 25 cm), presenting a hole on one side (highness from the bottom 20 cm; diameter 14 mm) where was allocated an aerosol glass ampule. A known quantity of SOR-EO nanoemulsion (2 mL for *C. ferrugineus* and 4 mL for *T. confusum*) was put inside the aerosol ampule, which was connected to an air delivery system blowing purified air at 2 L min⁻¹ constant flow. The air flow was turned off when the ampule was empty. Tested insects were maintained inside the cage for an exposure time of 24 h. After exposure time, specimens were removed from the cage and gently placed in a clean glass Petri dish, in which was added a plastic container containing 1g of wheat flour mixed with yeast (10:1, w: w). The dosages tested against *C. ferrugineus* were: 300, 150, 75, 37.5, 18.75 and 9.38 ppm of SOR-EO. For every dose 3 replicates (i.e. 15 insects each) were performed. The mortality was recorded after 24 from the beginning of the cold aerosol treatment. The dosages employed against *T. confusum* were: 600, 300, 150, 75 and 37.5 ppm of SOR-EO. For every dose 3 replicates (i.e. 10 insects each) were performed. For *T. confusum*, the mortality was counted after 24, 48, 72, 96 and 120h.

To exclude the impact of surfactant on insect mortality, control trials were carried out using formulations of Tween in water at the same concentrations tested as EO-nanoemulsions. In addition, additional control trials using only distilled water were performed for both *T. confusum* and *C. ferrugineus*.

2.5. Data analysis

Statistics were carried out using SPSS® V. 20 (IBM). The efficacy of the tested formulations was corrected for control mortality using Abbott's formula (Abbott 1987). Probit analysis was performed in order to estimate the median lethal concentrations for both tested insect species (LD50 and LD99).

3. Results

The average size of the developed formulation belonged to the nanometer scale (average size \pm standard error= 230.3 \pm 14.65 nm) (Figure 1), with low polydispersity index (Pdi 0.274). The stability over time of the tested formulation was confirmed by the relatively high zeta potential value obtained (ζ = 26.93) (Figure 2).

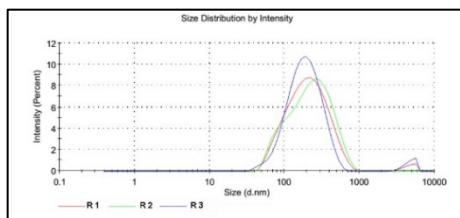


Fig. 1 Size and Pdl values for the SOR-EO nanoemulsion

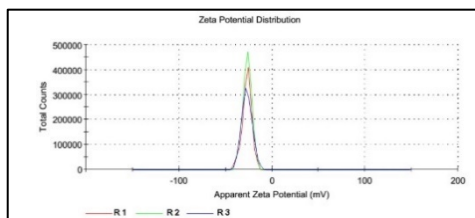


Fig. 2 ζ potential of the SOR-EO nanoemulsion

The efficacy of the tested formulation showed a dose-dependent response and the cumulated mortality of the exposed insects increased until 24h of exposure for *C. ferrugineus* (Figure 3) and until 120h for *T. confusum* (Figure 4). No mortality was recorded for control with distilled water, while little toxic activity was recorded for Tween solutions. From statistical analyses *T. confusum* adults proved less susceptible to the tested formulation than *C. ferrugineus* specimens. Lethal dose values for *C. ferrugineus* were LD50= 36.79 ppm and LD99= 209.7 ppm after 24h from the exposure. In contrast, the LD50 and LD99 values recorded for *T. confusum* at 24h from the exposure were 86.30 ppm and 631.14 ppm, respectively.

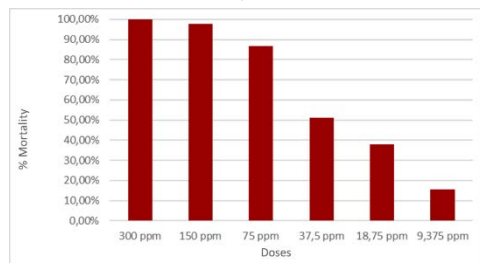


Fig. 3 Insecticidal activity of SOR-EO nan emulsion as cold aerosol against *C. ferrugineus* adults after 24h from the exposure.

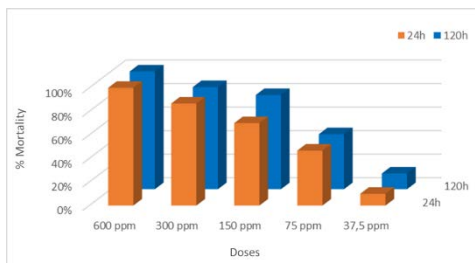


Fig. 4 Insecticidal activity of SOR-EO nanoemulsion as cold aerosol against *T. confusum* adults after 24h and 120h from the exposure.

4. Discussion

Several studies had demonstrated the fumigant activity of essential oils to several stored product pests (Polatoğlu and Karakoç 2016), highlighting higher susceptibility of adults than of pre-imaginal stages (Koul et al. 2008). Terpenes are key compounds for the bioactivity against insects, bacteria and fungi, acting as contact, fumigant and ingestion insecticides (Malacrino et al. 2016; Prates et al. 1998). The formulation of EOs as nanoemulsions improves both the stability and effectiveness of botanical insecticides. Indeed, nano-formulations can solve problems related to EO volatility, poor water solubility, and the tendency to oxidize (Campolo et al. 2017; Werdin-González et al. 2014). Furthermore, these formulations are able to release the active compounds at the site of action

gradually (de Oliveira et al. 2014), and concurrently minimize the toxic effects on non-target organisms (Gogos et al. 2012).

Both the size and the polydispersion index obtained in our study are adequate for nanoemulsion and the zeta potential obtained can be considered an indicator of the extent of EO loading in the emulsion. Furthermore, our results highlighted the good insecticidal activity of the citrus peel essential oils against the stored product pests *T. confusum* and *C. ferrugineus*. In this study, we also tested a novel administration method of EOs, using cold fumigation as aerosol. Indeed, this promising technique may allow the development of efficient and effective control strategies based on the application of plant-derived compounds to protect stored products in the food industries. The results obtained in these trials, together with the availability of SOR-EO at reasonable cost, are promising for the potential development of new tools against stored product pests.

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Fogging loads of California fresh citrus for control of Asian citrus psyllid, *Diaphorina citri*

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Abstract

Contact insecticides are commonly applied as fogs to disinfest and disinfect spaces. Recently, these fogs have been adapted to treat commodity within the spaces, and much has been learned regarding the efficacy of this process. When considering fresh citrus in California, fogs are applied to control both insects and microbes. One insect pest, the Asian citrus psyllid (ACP), *Diaphorina citri*, is a quarantine pest in California and limiting its geographic distribution is a major goal of the California citrus industry. While a variety of phytosanitary measures can be used to control adult ACP once fruit is at a packing house, ultimately, a treatment must be developed to disinfest field-run fruit prior to its exiting the grove. High-pressure fogging with 1,100-L of an aqueous mixture containing 0.2% Evergreen® (6% pyrethrins & 60% piperonyl butoxide) and 0.5% (v/v) BreakThru® (polysiloxane surfactant) was explored in laboratory-, pilot-, and commercial-scale trials as an approach to disinfest a 48-bin trailer load of fresh citrus. Laboratory-scale studies were conducted to quantify, and subsequently model, insecticidal coverage as a function of temperature, surface area, droplet size, and fog volume. Results are discussed in the context of experimental variability across confirmatory trials and continued efforts to optimize the technical and economic feasibility of fogging as a postharvest control strategy.

Keywords: food security, food safety, pyrethrins, postharvest fogging

1. Introduction

Asian citrus psyllid (ACP), *Diaphorina citri*, which transmits citrus greening disease (Huanglongbing or HLB)-associated liberibacter (*Candidatus Liberibacter asiaticus*; Las), has the potential to devastate the production of fresh citrus in California (Grafton-Cardwell et al. 2016). Moreover, the presence of ACP in the marketing channel can create a phytosanitary barrier for exports, which are key to the industries profitability. While ACP adults are removed from fresh citrus that has been subjected to cleaning and packing procedures standard to commercial production and distribution, State and Federal quarantines often restrict movement of fruit from ACP infested orchards to packhouses (CDFA, 2018).

Accordingly, a treatment must be developed to disinfest field-run fruit prior to its exiting the grove to control any incidental transportation of ACP and potential spreading of the insect and its associated disease. This work describes the development of a high-pressure fogging system using Evergreen® to control ACP in trailer loads of field-run fruit. The proposed treatment will reduce the number of psyllids in bulk citrus and reduce the insecticides applied to the grove, which will in turn improve worker safety, reduce environmental impacts, and improve IPM of ACP and other pests.

2. Materials and Methods