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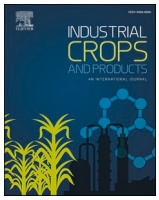

Effects of essential oil-based formulation on biopesticide activity

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ABSTRACT

Essential oils (EOs) represent a promising source of biopesticides, given their compositional complexity which bestows them high insect specificity and low risk of inducing resistance. However, their use in agriculture remains limited by their rapid degradation, limited duration of effect and non-target toxicity. These issues largely result from the under-optimized methods currently used to formulate EOs, in which their volatile organic compounds (VOCs) are poorly protected. In this study we compared pure Artemisia and Rosemary EOs to EOs formulated in three, low-cost, relatively simple, and easily applicable manners: as nanoemulsions, atomized powders, and Natural Deep Eutectic Solvents (NaDES). 24 hours after formulation, the entomotoxicity and phytotoxicity of the EOs were tested on *Bemisia tabaci* infested tomato plants. The identity and relative abundance of VOCs present in all formulations were also assessed 24 hours post-preparation using GC-MS and GC-FID. Nanoemulsions proved the most entomotoxic of formulations, followed by NaDES and pure EOs, while atomized powders were not significantly more entomotoxic than the control. Entomotoxicity was coupled with phytotoxicity for all formulations, except NaDES which induced particularly high rates of *B. tabaci* mortality when prepared with Rosemary EO, in addition to reducing damages on treated plants. Total VOC abundance depended on VOC release kinetics, determined by formulation, which although higher for pure EOs, was more gradual for NaDES. These results show the importance of EO formulation and the potential for NaDES to provide effective, sustained pest control.

1. Introduction

Today, agriculture finds itself faced with the significant challenge of feeding an ever-growing world population (UN World Population Prospects report, 2017). This must be achieved without increasing the use of chemical pesticides, which are gradually being placed under stricter control in light of the risks to human and environmental health (Desneux et al., 2007; Aktar et al., 2009; Damalas and Eleftherohorinos, 2011; Köhler and Triebskorn, 2013). However, without crop protection, losses due to pest damage would rise from 27% to 42% to 48–83% (Oerke and Dehne, 2004); Alternative means to control pest populations are thus required. Botanical pesticides are a promising alternative means of pest control based on the use of natural plant materials (Miresmailli et al., 2014; Siegwart and Lavoir, 2022). Although widely used before the mid-twentieth century, they were largely replaced by the advent of synthetic pesticides (carbamates, organochlorides, organophosphorous). Renewed interest in their usage began a mere thirty years ago, stimulated by a wakening awareness of the threats of non-target toxicity and resistance development posed by chemical pesticides (Lavoir et al., 2022; Regnault-Roger, 1997). In Europe for instance, a small number of botanical substances have been authorized for usage e.g. the pyrethrins, neem-based products (*Azadirachta indica*), or sweet orange essential oil, sold as Limocide (©Pre-Vam).

Among the potential botanical pesticides, essential oils (EOs) represent one of the most promising options (Isman, 2000; Pavela and Benelli, 2016). They are hydrophobic liquids essentially composed of volatile compounds belonging mainly to the phenylpropanoid (e.g., trans-anethol) and terpenoid (e.g., monoterpene and sesquiterpene) families (Burger et al., 2019; Pavela and Benelli, 2016). They are used in contact application or fumigation thanks to their volatile nature (Ikbal

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and Pavela, 2019). Although it is true that contact application is more practical, its use includes risks for farmers, consumers (Bajwa and Sandhu, 2014; Chavarri et al., 2004; Ecobichon, 2001) and plants (Hajjar et al., 2014; Touzout et al., 2021) as shown for chemical pesticides. Fumigation methods are often used in the control of stored pest species, allowing for homogeneous diffusions of volatile compounds in confined areas (Dubey et al., 2008; Kavallieratos et al., 2021; Rajendran and Sriranjini, 2008). However, its use can be extended to enclosed cultivation, such as greenhouses. Even though application of this method can prove challenging, uniform diffusion of VOCs and reduced phytotoxicity, in addition to a reduced remanence on crops makes it a worthwhile option compared to contact application or pesticide spraying. This is a definite advantage to synthetic pesticide, as it is safer for farmer and consumer health especially as classic pesticides used in greenhouse system are known to cause a multitude of health problems to farmers acute and chronic exposition (Cimino et al., 2017).

For biopesticides, whose market penetration remains limited mainly due to their variable and insufficient efficiency, rapid degradation, and difficult handling and application, formulation represents an important, yet largely unexplored, means of amelioration (Gasic and Tanovic, 2013; Glare et al., 2012; Isman, 2020). Recent studies have demonstrated the benefits of formulation for EO-based biopesticides, including increased dispersion, improved stability and a more persistent release, most notably for the release of active terpenes (Campolo et al., 2020a; Donsì and Ferrari, 2016; Werdin González et al., 2015). Another element of great importance in biopesticide formulation is the identification of non-toxic, environmentally friendly inactive ingredients required for formulation (Martin et al., 2011). Many classically employed formulation methods involve toxic solvents and other dangerous additives used to improve solubility and stability, such as tensioactives (e.g. alcohol ethoxylates) rendering formulations hazardous to human, animal and environmental health (Athanassiou et al., 2018; Campolo et al., 2020b). There now exists a number of innovative, "green" methods for the encapsulation of biopesticides, notably: emulsification, coacervation, spray drying and ionic gelation (Gasic and Tanovic, 2013; Maes et al., 2019). In addition, Natural Deep Eutectic Solvents (NaDES), a recently discovered family of solvents of intermediate polarity found in living organisms (Choi et al., 2011), show great potential for use in the formulation of biopesticides.

Previous studies have demonstrated the efficiency of EO-based biopesticides against numerous important crop pests (Aslan et al., 2004; Campolo et al., 2020a; De Clerck et al., 2021; Dunan et al., 2021; Ikbal and Pavela, 2019; Isman, 2020; Isman et al., 2011; Kim et al., 2011; Regnault-Roger, 1997), including tobacco whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae), a major tomato pest. In these studies, EOs were applied, either pure or diluted, with ethanol (Aslan et al., 2004; Baldin et al., 2013; Kim et al., 2011). In order to further investigate the ability to use formulated EOs as biopesticides, the aims of the present study are (i) to assess the feasibility of innovative formulations of two common Mediterranean plant-based EOs, Artemisia (*Artemisia vulgaris*) and Rosemary (*Rosmarinus officinalis*) formulated as nanoemulsions, atomized powders and NaDES, (ii) to investigate and compare their potential for use as fumigant biopesticides against *B. tabaci*, and (iii) to address their phytotoxicity to tomato plants. To do so, we compared the baseline entomotoxicity of pure and formulated EOs when indirectly applied by fumigation to whole tomato plants infested with *B. tabaci*, allowing only volatile compounds to reach the plants and insects. In addition to bioassays, a chemical determination of formulated EOs was performed by gas chromatography coupled with mass spectrometry (GC-MS) and flame ionized detection (GC-FID). The results of this study will provide a comparative assessment of the stability of EO-based biopesticides formulated with NaDES, nanoemulsions, and spray drying with the aim of finding a "green" formulation method that is not only sufficiently entomotoxic and persistent but also suffciently low-cost and easy to prepare so as to envision large-scale agricultural use.

2. Material and methods

2.1. Chemical material

2.1.1. Essential oils

Artemisia and Rosemary essential oils used for all formulations were obtained from Esperis s.p.s (Milan, Italy). These EOs were selected according to the results of a preliminary screening which showed Artemisia to be the most entomotoxic of six Mediterranean EOs tested against *B. tabaci* and Rosemary to be the only one not inducing any phytotoxicity from a concentration of 5 $\mu L/L_{air}$ (Supp. Mat. 1). LC_{50} (lethal concentration for 50% of pest population) for Artemisia and Rosemary EOs were preliminarily determined to be 2.28 $\mu L/L_{air}$ and 5.80 $\mu L/L_{air}$, respectively. In the interest of simplification, in this study, the quantity of EO used, whether pure or formulated, was rounded to 2.5 $\mu L/L_{air}$ for Artemisia EO and 6 $\mu L/L_{air}$ for Rosemary EO, when applied in a climatic chamber (543 L_{air}); Concentrations close enough to respective EOs LC_{50} (LC_{56} for Artemisia EO & LC_{53} for Rosemary EO; Supp. Mat. 1, See the dose-response curves).

2.1.2. Chemical material for formulation

All chemical materials used for formulation were purchased from Sigma Aldrich (Saint-Quentin-Fallavier, France). This included Tween 80 and analytical grade ethanol for nanoemulsions, DL-malic acid and L-serine for NaDES, as well as maltodextrine (dextrose equivalent 13.0–17.0) and gum arabic for atomized powders. Water was distilled using a distilling apparatus from Schott Instruments.

2.2. Essential oil formulations

2.2.1. Nanoemulsions

Nanoemulsions were prepared according to the formulation determined by previous work from our lab and collaborators (Campolo et al., 2020a; See also Suppl. Mat. 1). Briefly, EO (1% of the final solution), Tween 80 (3%) and ethanol (3%) were successively added to a glass flask (Table 1), after which the mixture was agitated with a magnetic stir bar for 5 min at 250 rpm. Then, distilled water (93%) was added using a micropipette and the solution was stirred for 10 min at 250 rpm followed by 10 more minutes at 500 rpm. Characterization has been carried out as detailed in a previous study (Campolo et al., 2020a). Values of 217 ± 1 nm, 0.36 ± 0.01 and − 18.63 ± 0.14 mV were obtained for the droplet size, polydispersion index and zeta potential of nanoemulsion prepared with Rosemary EO. Values of 199 ± 2 nm, 0.42 ± 0.02 and − 12.27 ± 0.26 mV were obtained for the droplet size, polydispersion index and zeta potential of nanoemulsion prepared with Artemisia EO. A lid was placed on the flask to prevent evaporation and the emulsion was stored in the laboratory, away from light, until use in chemical analysis or in entomotoxic bioassays (24 h after preparation). Nanoemulsions were found to be stable over three days, covering the duration of experiments.

2.2.2. Spray drying

Atomized powders were prepared according to the procedure described by Bringas-Lantigua et al. (2011). Formulation began with the preparation of a liquid emulsion by combining gum Arabic (4%), maltodextrin (12%) and distilled water (80%) (Table 1) using a magnetic stir bar for 15 min at 500 rpm. Then, EOs (4%, LC_{50} value) were added and the mixture was stirred for 10 min at 500 rpm until an emulsion was formed. For further homogenization a T18 Digital ULTRA-TURRAX® was used to stir the emulsion for one minute at each of the following speeds: increasing from 11000 rpm to 16000 rpm, then19000 rpm, following by a decrease at 16000 rpm again, and finally 11000 rpm again. Once prepared, the liquid emulsion was introduced into the atomizer Büchi B-290 where it was drawn up by a peristaltic pump and then dispersed as fine droplets by a flow of nitrogen gas heated to 170 °C. The water in the droplets then evaporated, leaving a dry powder containing EO fixed to maltodextrine in the atomizer's

Table 1
Quantity of chemical materials used to prepare enough of each of the tested formulations to treat one climatic chamber (4 plants, 543 L_{air}). Each formulation contains a quantity of EO equal to roughly LC_{50} calculated in Suppl. Mat. 1.

	Pure EOs		Nanoemulsions[a]		NaDES[b]		Atomized Powder[c]	
	Product	**Quantity**	**Product**	**Quantity**	**Product**	**Quantity**	**Product**	**Quantity**
Artemisia EO formulations	Artemisia EO LC_{56}: 2.5 µL/L_{air}	1.37 mL	Artemisia EO	1.37 mL	Artemisia EO	1.30 g	Artemisia EO	1.30 g
			Tween 80	4.12 mL	Malic Acid	24.28 g	Gum Arabic	1.30 g
			Ethanol	4.12 mL	Serine	19.03 g	Maltodextrin	3.90 g
			Water	127.76 mL	Water	9.78 g	Water	26.02 g
Rosemary EO formulations	Rosemary EO LC_{53}: 6 µL/L_{air}	3.26 mL	Rosemary EO	3.26 mL	Rosemary EO	2.95 g	Rosemary EO	2.95 g
			Tween 80	9.79 mL	Malic Acid	26.95 g	Gum Arabic	2.95 g
			Ethanol	9.79 mL	Serine	21.13 g	Maltodextrin	8.84 g
			Water	303.40 mL	Water	10.85 g	Water	58.94 g

a - 1% EO, 3% Tween 80, 3% ethanol and 93% distilled water; b – Malic acid, serine, and water were added to a glass vial, according to the molar ratio of 1:1:3; c- 4% EO, 4% gum arabic, 12% maltodextrin and 80% distilled water

collector. The outflow temperature was set to 100 °C.

2.2.3. NaDES

NaDES were prepared using the heat and stir method, according to the formulas presented by Mouden et al. (2017). Resulting NaDES were evaluated according to their viscosity and their solubility limit for Artemisia and Rosemary EOs (Suppl. Mat. 2). Viscosity was determined at 20, 25, 30, 35, 40, and 45 °C using a Rheometer Physica MCR 51 (Anton Paar). Solubility assays were carried out by adding increments of mg of EO into 1 g of DES. Solubility limit of EO was qualitatively determined when two phases were observed visually.

NaDES with overly high viscosity or low EO solubility were discarded. Two NaDES were thus selected, namely [malic acid: sorbitol: water] and [malic acid: serine: water], both with a molar ratio of [1:1:3]. The latter was preferred because of a slightly greater EO solubility limit.

To prepare this NaDES, malic acid, serine, and water were added to a glass vial, according to the molar ratio of [1:1:3]. The mixture was then heated at 70 °C for 30 min with magnetic stirring at 500 rpm. Next, the vial was placed in an ultrasound bath at 55 °C for another 30 min. If the solution obtained was not completely homogenous, it was heated for another 5 min with stirring and then for 5 more minutes in the ultrasound bath. Once a homogenous solution was achieved, the NaDES was allowed to cool for 1 h. Then, a quantity of the EO corresponding roughly the LC_{50} (Table 1) was added to the NaDES with a micropipette and the mixture was vortexed for 20 s. Next, the EO-NaDES mixture was transferred to a Falcon tube and centrifuged at 3000 rpm for 5 min. After, the mixture was placed in a sealed glass vial, away from light and at room temperature, until use for kinetic analysis (1, 4, 11, 18, 25 days after preparation) or for entomotoxic bioassays (24 h after preparation).

2.3. Headspace solid-phase microextraction (HS-SPME)

A headspace solid-phase microextraction (HS-SPME) method combined with gas chromatography - mass spectrometry (GC-MS) and gas chromatography - Flame Ionized Detection (GC-FID) was developed and optimized for the extraction and the analysis of volatile organic compounds (VOCs) released after 24 h by pure and formulated EOs. Kinetic analyses performed at 1, 4, 11, 18 and 25 days after preparation were also performed. To do this, first a quantity of the formulation, containing the LC_{50} corresponding to 0.5 L_{air}, was placed in a SPME vial (20 mL). The vial was then hermetically sealed and the volatile components contained in the formulation were allowed to fill the headspace during an incubation period of 30 min. Next, a SPME fiber (Stableflex gray, 24 Ga, 50/30 µm, Supelco), composed of divinylbenzene / carboxen / polydimethylsiloxane (DVB / CAR / PDMS), was introduced into the headspace so as to extract only the volatile EO components which had saturated the headspace during incubation. Fibers were conditioned before being used for the first time at 270 °C for 30 min and again after each extraction at 250 °C for 15 min.

2.4. Gas chromatography–mass spectrometry (GC-MS) and flame ionized detection (GC-FID) analyses

After 30 min of extraction, the fiber was introduced into a gas chromatographic injector lined with a SPME Injection Sleeve (0.75 mm ID, Supelco). Injection was accompanied by a split of 100:1 due to chromatogram peak oversaturation in pretests of varying extraction times. Subsequent desorption of the EO volatiles absorbed to the SPME fiber during extraction was realized during a 4 min desorption period at 250 °C with a helium gas flow of 94.9 mL/min. Analysis of desorbed volatiles was performed on an Agilent 6890N gas chromatograph model coupled to a mass-selective detector (5973N) equipped with an HP-1 (0.2 mm × 50 m x 0.33 µm) column. The method used for analysis involves an initial oven temperature of 40 °C increased first to 200 °C (2 °C/min), then from 200 °C to 270 °C (20 °C/min), and finally held at 270 °C for 5 min, giving a total analysis time of 88.5 min. Helium gas flow during analysis was set to 15 mL/min.

The relative abundance of identified volatiles contained in the formulations was determined by means of GC-FID. The extraction procedure, as well as the GC analysis parameters, were the same as those used for GC-MS. The GC-FID analyses were performed in triplicate for each EO formulation at five points in time during the month following formulation preparation (days 1, 4, 11, 18, 25).

Compounds were identified by comparison of the mass spectra with those recorded by internal or commercial mass-spectral libraries (NIST02 and Wiley6n) and by comparison of their linear retention indices (LRI) with those of literature (NIST, ESO). Retention indices (RI) were calculated using a formula according to Van den Dool and Kratz and according to the retention times of a standard *n*-alkanes C_6-C_{24} mixture (SUPELCO). Two drops of the alkanes mixture diluted to 0.1% in dichloromethane were analyzed by HS-SPME GC-MS/FID according to the method described previously.

2.5. Biological materials

2.5.1. Tomato plants

Solanum lycopersicum var. Nano, used for entomotoxic and phytotoxic experiments, were grown under laboratory conditions (24 ± 2 °C, 40 ± 10% RH, 16:8 L.D.) for 4–5 weeks until reaching a height of 20 ± 5 cm.

2.5.2. Silverleaf whiteflies, pest

Bemisia tabaci Gennadius, 1889 (Hemiptera: Aleyrodidae) is a phloem sap-feeding herbivore which has become a major invasive pest, particularly on tomato crops. It is a highly diversified species complex, presumably originated from Middle East–Asia Minor region, currently globally-distributed as a result of on several invasion events (De Barro et al., 2011). The *B. tabaci* colony (MED) was raised on tobacco plants (*Nicotiana tabacum*) at the Sophia Agrobiotech Institute (ISA, INRAe, Sophia-Antipolis) since 2009 (24 ± 2 °C, 40 ± 10% RH, 14:10 L.D.). The adult individuals used for bioassays were collected by means of a vacuum pump which allowed transfer into glass vials for introduction into the experimental system without harming the insects.

2.6. Bioassay experimental set-up: entomotoxicity and phytotoxicity

In order to compare the potential entomotoxic and phytotoxic effects of formulated EOs, bioassays were performed at ISA (INRAe, Sophia-Antipolis) in climatic chambers (543 L_{air}, 24 ± 1 °C, 80 ± 10% RH, 14:10 L.D.) 24 h after preparation of the applied formulation.

At the outset of the experiment, tomato plants were generously watered and then placed into nylon cages (28 cm × 28 cm × 42 cm). A circular disk of red paper (12.5 cm) was placed inside the cage, around the base of the plants, covering the soil, to facilitate the counting of insects by preventing them from falling into the soil. Next, groups of 10 *B. tabaci* individuals were collected in glass vials. Four vials were then placed open in each of the nylon cages, at the base of the tomato plant (allowing individuals to freely settle on the plant) before closing the cages. Four cages were placed into each of the three climatic chambers (543 L_{air}) with two cages on the upper shelf and two on the lower shelf (Fig. 1).

The calculated quantity of the formulation (Table 1) containing roughly the LC_{50} of a given EO for the volume of air contained in the climatic chamber was divided among eight Petri dishes. Formulations were placed outside the cages, allowing only volatile EO components to enter the cages by fumigation (i.e. no contact between the formulation and the plants or insects). One petri dish was placed in front of and behind each cage so as to equally distribute the volatiles contained in the formulations inside the climatic chamber. The control consisted of the same formulation as that used to prepare the EOs, but without the addition of any EO. For bioassays with pure EOs, untreated filter paper was used as a control treatment. After formulations were placed in the climatic chambers, the chambers were closed.

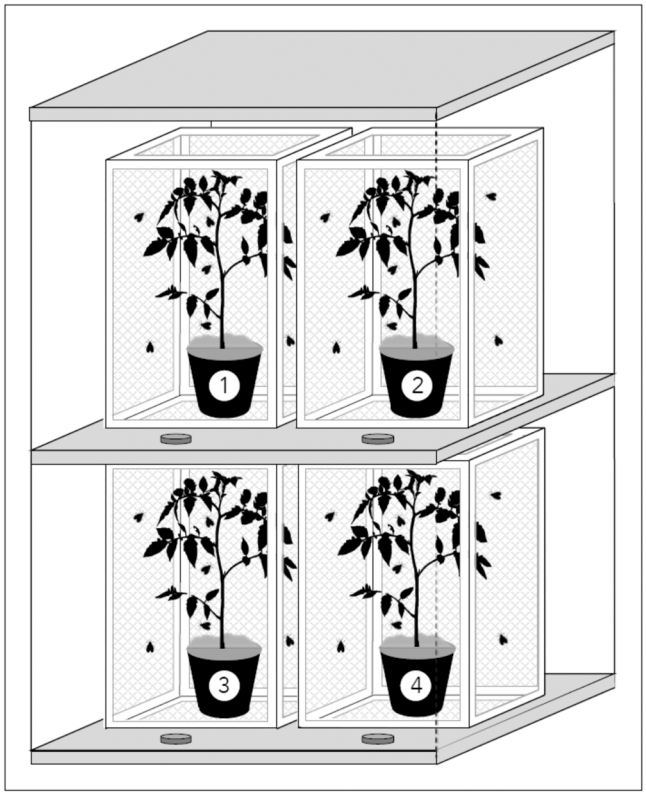


Fig. 1. Experimental setup used for entomotoxic and phytotoxic bioassays in climatic chambers. Each chamber holds four nylon cages (two per shelf), each containing one tomato plant and 40 *B. tabaci*. The formulation containing roughly the LC_{50} of either Artemisia or Rosemary EO is divided between eight petri dishes, one behind and one in front of each cage.

Three climatic chambers were used to test the formulation of both Artemisia and Rosemary EOs vs the control treatment. This experimental set-up was repeated three times in order to avoid a climatic chamber effect. Treatments were rotated from one climatic chamber to another in each of the different repetitions. Twelve replicates were carried out per treatment.

After 24h, cages were removed from the chambers, living and dead whitefly individuals were counted, and the mortality rate was calculated for each treatment group:

$$Mortality\ \ rate = \frac{Number\ \ of\ \ dead\ \ individuals\ \ after\ 24\ hours}{Total\ \ number\ \ of\ \ individuals\ \ dead\ \ and\ \ alive\ \ found\ \ at\ \ the\ \ end\ \ of\ \ the\ \ experiment}$$

In addition, the level of phytotoxicity of each treatment was assessed in terms of the percentage of necrotized leaf surface (0–100%), determined visually. This experimental procedure was performed in triplicate for each of the three formulations: nanoemulsions, atomized powders, and NaDES.

2.7. Statistical analyses

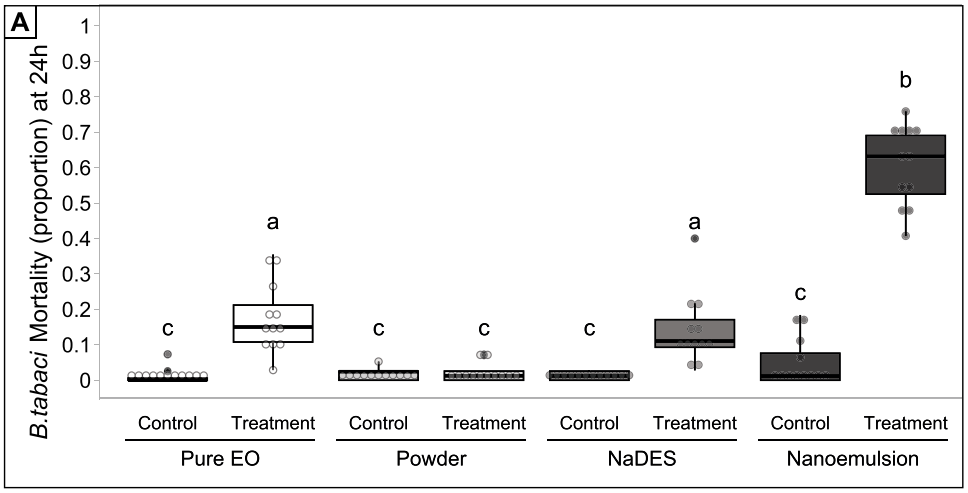
All statistical analyses were performed using R-64 programming system (version 4.1.2) R core Team (2012). A Generalized Linear Model (GLM) (with a binomial error structure) was used to assess differences in entomotoxicity and phytotoxicity between EOs and formulations. For the assessment of EOs, VOC relative abundance by class or all together, data normality was first checked using the Shapiro test in order to determine a suitable method of analysis. Normally distributed data were analyzed using a one-way analysis of variance (ANOVA) and data with non-normal distribution were analyzed using a GLM (with a poisson error structure). Finally, evolution of the relative abundance of major classes of EO VOCs over time was analyzed by fitting a Generalized Estimating Equation (GEE) with a poisson error structure, to take into account the interdependence of data (package "geepack", Halekoh et al., 2006). For all analyses, significant treatment effects were considered for p-value < 0.05 and followed-up by Tukey's HSD post hoc test (function "lsmeans", package "lsmeans", Lenth, 2016) to investigate statistical differences between groups.

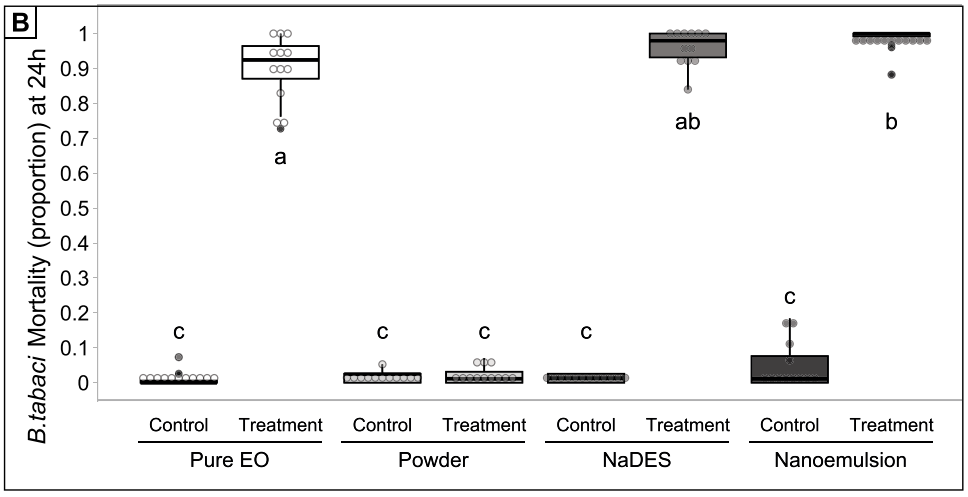
3. Results

3.1. Across formulation comparison of entomotoxicity & phytotoxicity

Both Rosemary and Artemisia EOs consistently induced higher rates of *B. tabaci* mortality across all formulations (nanoemulsions, NaDES and pure EOs) with the exception of atomized powders compared to the controls (Fig. 2).

Artemisia treatments resulted in an average entomotoxicity of 25%. Nanoemulsions proved to be the most efficient of all formulations tested (X_3^2 =12.706, p-value < 0.05), with *B. tabaci* mortality averaging 60% (Fig. 2A). The NaDES formulation and pure EOs proved to be significantly less efficient than the nanoemulsions, causing respectively 14% and 17% mortality on average. Atomized powder had no effect, inducing mortality rates equal to those of the control. Phytotoxicity across all





C

	Pure EO		Powder		NaDES		Nanoemulsion	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
Artemisia	0	5	0	0	0	0	0	9
Rosemary	0	23	0	0	0	11	0	37

Fig. 2. *B. tabaci* mortality 24 h after application of atomized powders, NaDES, nanoemulsions, pure EOs and control treatments, prepared with either Artemisia (A) or Rosemary (B) EOs. Different letters represent significant differences in entomotoxicity between formulations (X^2_3 =12.706, p-value < 0.05, n = 12). (C) represent phytotoxicity (%) on tomato plants according to formulation & EO treatment.

Artemisia formulations was minimal and did not differ from the control – no EOs (all p-values > 0.05; Fig. 2 & 3).

Rosemary-induced mortality across all tested formulations averaged 70%. The EO formulations (Fig. 2B) induced significantly higher mortality than the controls, apart from powder formulations ($X^2_3 = 59.143$, p-value < 0.05). The highest average mortality of 98% resulted from the Rosemary nanoemulsion treatment, closely followed by the NaDES treatment with 96% mortality. Pure Rosemary EO induced a slightly lower average mortality of 90% (Fig. 2B). Similarly to Artemisia, Rosemary atomized powder was no more entomotoxic than the control, inducing an average of 2% mortality. In terms of phytotoxicity, the highest phytotoxicity resulted from the Rosemary nanoemulsion treatment, equal to that of the pure EO (Fig. 3). NaDES-formulated Rosemary EOs show lower phytotoxicity and Rosemary atomized powder resulted in no visible phytotoxicity (Fig. 3). Globally, phytotoxicity indexes were higher for Rosemary EOs than for Artemisia EO.

3.1.1. Identification of EO components

A HS-SPME method combined with GC-MS and GC-FID was developed for the extraction and the analysis of volatile organic compounds released by both EOs. A list of the compounds identified can be found in Table 2 along with their literature and calculated linear retention indexes, as well as the percentage of peak area of a given compound in the total peak area. A total of 36 and 31 compounds were detected for Artemisia and Rosemary EOs, respectively. The majority of identified compounds belong to the monoterpenoid and monoterpene families. VOCs emitted by Artemisia EO were mainly composed of monoterpenoids (81.20%) such as α-thujone (34.33 ± 0.19%), β-thujone (17.09 ± 0.19%) and camphor (17.73 ± 0.07%), while Rosemary EO was characterized by monoterpenes (33.30%) and monoterpenoids (61.63%) such as α-pinene (11.87 ± 0.66%) and eucalyptol (51.61 ± 1.11%). Other molecule classes, including sesquiterpenes and sesquiterpenoids, were present in very minimal quantities in both EOs (less than 6%). Artemisia and Rosemary EOs share 4 major compounds: camphene, m-cymene, eucalyptol, and camphor. Compounds found exclusively in Artemisia EO were the monoterpenoids α-thujone, β-thujone, and chrysanthenone. Compounds found uniquely in Rosemary EO were the monoterpenes α-pinene, β-pinene, and β-myrcene.

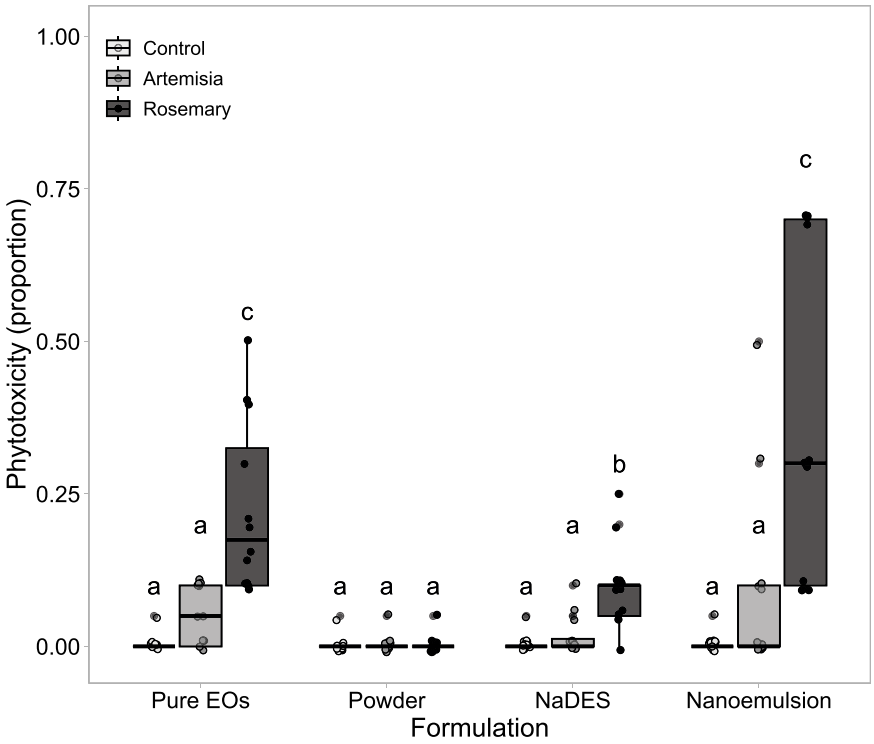


Fig. 3. Phytotoxicity proportion induced by the different treatments (i.e. control condition, Artemisia EO or Rosemary EO) for all formulations. Colors represent different treatments and letters indicates different significance between treatments and formulations (respectively, $X^2 = 12.547$, df = 2, n = 12, p-value < 0.001, $X^2 = 7.865$, df = 3, n = 12, p-value < 0.001).

Volatile Organic Compounds (VOCs) detected by SPME-GC-MS in Artemisia EO (Art.) and Rosemary EO. (Ros). Major compounds are highlighted in bold. LRI HP-1 (lit.) and LRI HP-1 (calc.): literature and calculated linear retention index on a DB-5 column. Area means percentage of peak area of a given compound in the total peak area. SD means Standard Deviation

Ros.	Compound	Class	LRI HP-1 (lit.)	LRI HP-1 (calc.)	Area (% ± SD)
1	**α-pinene**	**monoterpene**	**932**	**933**	**11.87 ± 0.66**
2	camphene	monoterpene	946	947	3.86 ± 0.16
3	sabinene	monoterpene	968	970	0.86 ± 0.04
4	β-pinene	monoterpene	972	973	6.44 ± 0.21
5	β-myrcene	monoterpene	981	990	2.31 ± 0.01
6	α-phellandrene	monoterpene	996	1002	0.17 ± 0.01
7	δ-3-carene	monoterpene	1003	1011	1.34 ± 0.02
8	α-terpinene	monoterpene	1010	1014	0.08
9	*p*-cymene	monoterpene	1017	1018	5.84 ± 0.03
10	**eucalyptol**	**monoterpenoid**	**1025**	**1027**	**51.61 ± 1.11**
11	trans-β-ocimene	monoterpene	1033	1046	0.06
12	γ-terpinene	monoterpene	1051	1055	0.30 ± 0.01
13	terpinolene	monoterpene	1079	1087	0.17
14	linalool	monoterpenoid	1085	1099	0.26 ± 0.01
15	**camphor**	**monoterpenoid**	**1123**	**1129**	**8.45 ± 0.09**
16	borneol	monoterpenoid	1154	1162	0.25 ± 0.01
17	terpinen-4-ol	monoterpenoid	1162	1173	0.28

(*continued on next column*)

(*continued*)

Ros.	Compound	Class	LRI HP-1 (lit.)	LRI HP-1 (calc.)	Area (% ± SD)
18	α-terpineol	monoterpenoid	1175	1187	0.29 ± 0.01
19	bornyl acetate	monoterpenoid	1273	1282	0.49 ± 0.02
20	α-ylangene	sesquiterpene	1370	1380	0.02
21	α-copaene	sesquiterpene	1374	1384	0.08
22	longifolene	sesquiterpene	1416	1414	0.01
23	β-caryophyllene	sesquiterpene	1421	1427	0.87 ± 0.04
24	α-humulene	sesquiterpene	1451	1461	0.08
25	γ-muurolene	sesquiterpene	1488	1482	0.03 ± 0.01
26	δ-cadinene	sesquiterpene	1521	1528	0.02
27	unknown	other	/	1358	0.02 ± 0.01
28	unknown	sesquiterpene	/	1436	0.02
29	unknown	sesquiterpene	/	1448	0.03
30	unknown	sesquiterpene	/	1525	0.02
31	unknown	other	/	1358	0.01
					area (%)
		Monoterpenes			33.30
		Monoterpenoids			61.63
		Sesquiterpenes, Sesquiterpenoids			1.19
		Other compounds			0.03

LRI (calc.), linear retention index calculated according to the retention times of a standard n-alkane C_6-C_{24} mixture.
LRI (lit.), linear retention index reported in the literature (NIST, Adams 2017).

3.1.2. Within and across formulation quantification of the relative abundance of major classes of EO VOCs

HS-SPME combined with GC-FID allowed the determination of the relative abundance of each of the major classes of VOCs present in Artemisia and Rosemary EOs in their pure form and when formulated as NaDES, nanoemulsions, and atomized powders, 24 h after formulation.

Disregarding the formulation, the global profile of the EOs is

Table 2
Volatile Organic Compounds (VOCs) detected by SPME-GC-MS in Artemisia EO (Art.) and Rosemary EO. (Ros). Major compounds are highlighted in bold. LRI HP-1 (lit.) and LRI HP-1 (calc.): literature and calculated linear retention index on a DB-5 column. Area means percentage of peak area of a given compound in the total peak area.

Art.	Compounds	Class	LRI HP-1 (lit.)	LRI HP-1 (calc.)	Area (% ± SD)
1	santolina triene	monoterpene	910	911	0.22 ± 0.01
2	tricyclene	monoterpene	921	923	0.20 ± 0.02
3	α-pinene	monoterpene	932	934	0.46 ± 0.02
4	camphene	monoterpene	946	946	3.37 ± 0.27
5	thuja-2,4(10)-diene	monoterpenoid	947	953	0.04
6	sabinene	monoterpene	968	971	0.52 ± 0.02
7	β-pinene	monoterpene	972	974	0.10
8	1,2,4-trimethylbenzene	other	986	986	0.47 ± 0.01
9	β-myrcene	monoterpene	981	992	0.10
10	unknown	monoterpenoid	/	1001	0.12 ± 0.07
11	dehydro paracymene	monoterpene	1011	1009	0.08 ± 0.02
12	1,2,3-trimethylbenzene	other	1014	1013	0.30 ± 0.01
13	*m*-cymene	monoterpene	1010	1017	1.88 ± 0.04
14	eucalyptol	monoterpenoid	1025	1027	4.31 ± 0.08
15	γ-terpinene	monoterpene	1051	1056	0.10
16	butanoic acid	other	1044	1070	0.13 ± 0.05
17	safranal	monoterpenoid	1175	1078	0.15 ± 0.01
18	Artemisia alcohol	monoterpenoid	1069	1085	0.13 ± 0.02
19	**α-thujone**	**monoterpenoid**	**1086**	**1093**	**34.33 ± 0.19**
20	**B-thujone**	**monoterpenoid**	**1097**	**1104**	**17.09 ± 0.19**
21	chrysanthenone	monoterpenoid	1099	1109	5.84 ± 0.29
22	**camphor**	**monoterpenoid**	**1123**	**1129**	**17.73 ± 0.07**
23	trans-pinocarveol	monoterpenoid	1135 *	1136	0.32 ± 0.01
24	pinocamphone	monoterpenoid	1144	1161	0.17
25	borneol	monoterpenoid	1154	1164	0.45 ± 0.01
26	erpinene-4-ol	monoterpenoid	1162	1173	0.28
27	myrtenal	monoterpenoid	1168	1182	0.20
28	bornyl formate	monoterpenoid	1222	1224	0.06 ± 0.01
29	carvone	other	1226	1235	0.05
30	unknown	other	/	1257	3.89 ± 0.16
31	unknown	other	/	1282	0.32 ± 0.01
32	unknown	monoterpene	/	1288	0.12 ± 0.01
33	unknown	other	/	1354	0.04
34	unknown	sesquiterpenoid	/	1375	0.24 ± 0.12
35	α-copaene	sesquiterpene	1374	1384	0.11
36	unknown	other	/	1398	0.09 ± 0.04

	area (%)
Monoterpenes	7.16%
Monoterpenoids	81.20%
Sesquiterpenes, Sesquiterpenoids	0.35%
Other compounds	5.29%

LRI (calc.), linear retention index calculated according to the retention times of a standard n-alkane C_6-C_{24} mixture.
LRI (lit.), linear retention index reported in the literature (NIST, Adams 2017).

maintained: In Artemisia formulations, the relative abundance of monoterpenes was approximately one-eighth that of the monoterpenoids and in Rosemary formulations the relative abundance of monoterpenes was half that of the monoterpenoids (Fig. 4). Sesquiterpenes, sesquiterpenoids, and other molecule classes were present in very minimal quantities in all formulations of both EOs.

Despite their similarities in compositional breakdown, the different formulations tested varied greatly in terms of the relative abundance (peak area) accounted for by emitted VOCs (for Artemisia and Rosemary EOs respectively, $X^2_3 = 1.22e{+}9$, p-value < 0.05 and $X^2_3 = 1.50e{+}9$, p-value < 0.05). Pure EOs contained the greatest total VOC abundance, followed by nanoemulsions, NaDES, and, finally atomized powders (Fig. 4). The dominant VOC classes, monoterpenoids and monoterpenes, accounted for most of the total variation observed. The relative abundance of monoterpenes differed significantly between all formulations of both EOs (F-value=689, 3, p-value < 0.05), except between Artemisia atomized powder and Artemisia NaDES (p-value $= 0.97$) (Fig. 4). Monoterpenoid relative abundance differed significantly between all formulations of both EOs (F-value=967.4, 3, p-value < 0.05). The relative abundance of sesquiterpenes/sesquiterpenoids differed significantly between all Artemisia and Rosemary formulations (F-value=120, 3, p-value < 0.05), except between atomized powders and NaDES (p-value $= 0.91$ (Artemisia), $p > 0.05$ (Rosemary)). The relative abundance of all molecules belonging to other classes differed significantly between all formulations of Artemisia ($X^2_3 = 7.36e{+}7$, p-value < 0.05) but did not differ between any of the different formulations of Rosemary.

3.1.3. Evolution of the relative abundance of major classes of VOCs in NaDES formulation

Results of the kinetic analysis of changes in the relative abundance of the major classes of EO VOCs over time after formulation preparation can be found in Fig. 5. Here, we included only the kinetic analysis of the NaDES, as this was the most relevant formulation entomotoxic efficiency and low induced phytotoxicity on tomato plants, as showed in Fig. 2 and 3.

For both EOs, a decrease in the relative abundance of the majority of VOC classes over time was observed (for Artemisia and Rosemary EOs respectively, $X^2_1 = 16$, p-value $= 7.1e{-}05$ and $X^2_1 = 11$, p-value $= 0.00094$). Rosemary NaDES displayed the most important decrease in monoterpene relative abundance between day 1 post-preparation and the end of the month-long kinetic analysis (from $1.0\ 10^{-7}$ to $7.6\ 10^{-6}$ peak area, slope estimate $= -0.03403$). The two EOs presented a significant decrease in monoterpene abundance over the course of the analysis (Respectively for Artemisia and Rosemary EOs, p-value $= 1.9e{-}10$ and p-value $= 2.7e{-}11$; Fig. 5 – top left). Monoterpenoid relative abundance tended to decrease over time for both EOs, however this decrease was not significant for Rosemary EO (p-value $= 0.18$; Fig. 5 – top right). Sesquiterpene relative abundance proved more variable as an increase in abundance was observed for Rosemary EO (p-value $= 0.0057$, slope estimate $= 0.0333$), while a significant decrease in abundance was observed for Artemisia EO (p-value $= 0.0024$, slope estimate $= -0.0305$; Fig. 5 – bottom right). The relative abundance of molecules belonging to other classes only displayed a significant decrease over time for Rosemary EO (p-value $= 0.014$; Fig. 5 – bottom left).

Overall, the trend observed is a rapid decrease in monoterpene abundance, followed by a less rapid decrease in monoterpenoids and an increase of the heavier sesquiterpenes for NaDES-formulated Rosemary EO. Regarding Artemisia EOs, monoterpenes and sesquiterpenes decreased over time, while monoterpenoids and other compounds seemed to remain constant.

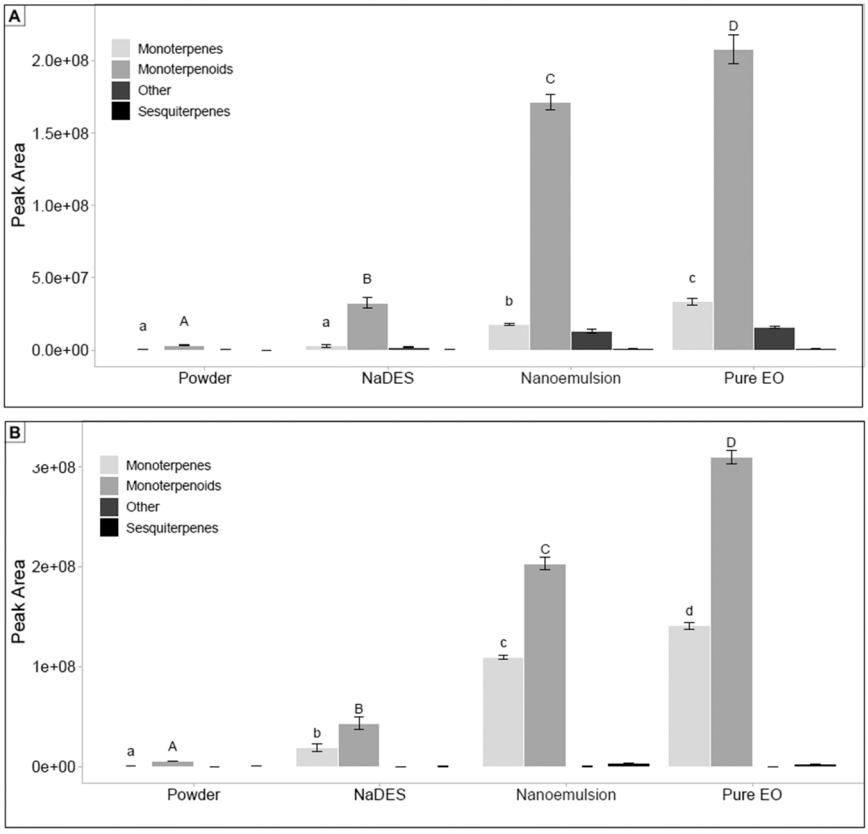


Fig. 4. Relative abundance of the major classes of VOCs in Artemisia (A) and Rosemary (B) atomized powders, NaDES, nanoemulsions and pure EOs one day after formulation. Different letters indicate significant differences in relative abundance between formulations. Capital letters are used for monoterpenoids and lowercase for monoterpenes. Differences for sesquiterpenes and molecules of other classes are not shown, given their low abundance (F-value=689, 3, p-value < 0.05, n = 3 (monoterpenes); F-value= 967.4, 3, p-value < 0.05, n = 3 (monoterpenoids) except for Rosmarinus powder-EO where n = 1 due to technical issues).

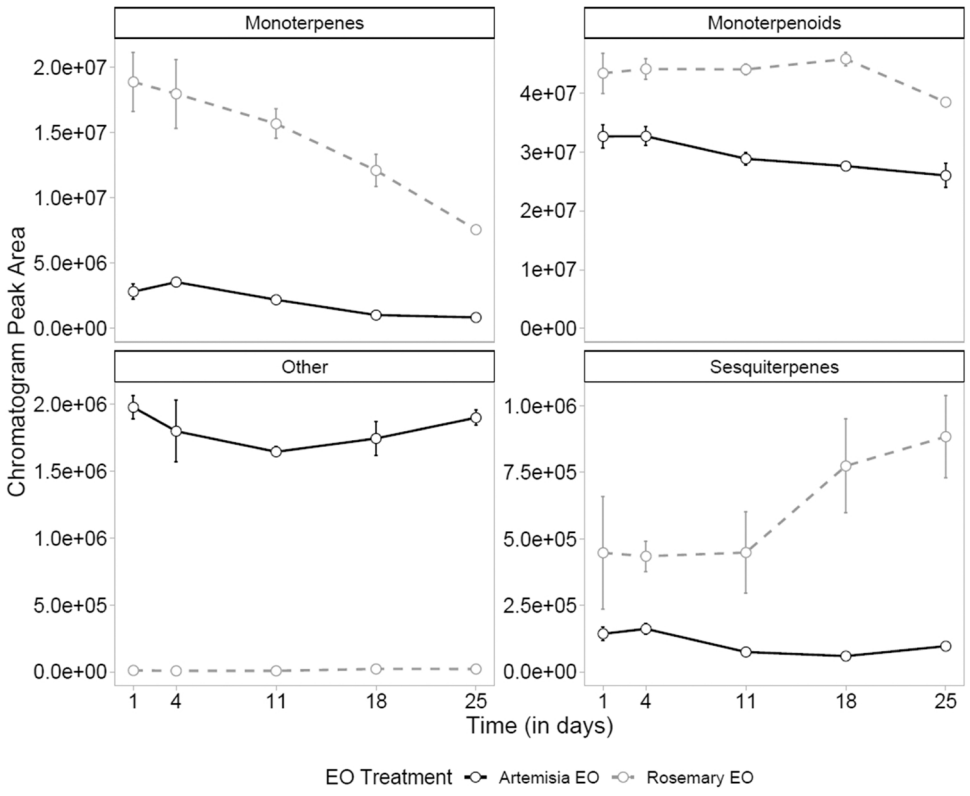


Fig. 5. Evolution of the abundance of monoterpenes, monoterpenoids, sesquiterpenes, and other classes of VOCs in Artemisia and Rosemary NaDES over the course of a month-long period. Error bars indicate SE.

4. Discussion

4.1. Expected efficacy and phytotoxicity of pure EOs

In this study, we undeniably demonstrated the efficacy of Artemisia and Rosemary EOs against *B. tabaci.* As proven here and in many other studies, EO VOCs possess an insecticidal activity. VOCs belonging to the terpene families, such as monoterpene or monoterpenoids, proved to be the most efficient type of VOCs and the most represented in EO composition (Mossa, 2016; Pavela and Benelli, 2016). The selected EOs for this study were mainly characterized by monoterpenoids for Artemisia EO and by a ratio two-third/one-third - monoterpenoids/ monoterpenes for Rosemary EO (Table 2; Fig. 4). Fumigated VOCs act on insects through inhalation (Regnault-Roger, 1997) and the identified compounds of our tested EOs have been shown to be neurotoxic (Devrnja et al., 2022; Jankowska et al., 2018; Mossa, 2016) or cytotoxic (Bakkali et al., 2008) for insects. All of Artemisia EO's major VOCs (i.e. α-thujone, β-thujone and camphor) are known to have a neurotoxic mode of action affecting the Gamma-amminobutyric acid (GABA) receptors, by attaching to the receptor and inhibiting neurotransmission in the nervous system and muscles (Jankowska et al., 2018; Mossa, 2016). However, Rosemary EO's major compounds (i.e. eucalyptol (also called 1,8-cineole), α-pinene and camphor) as well as β-thujone have proven to inhibit the activity of Acetylcholinesterase (AChE), an enzyme involved in the breakdown and deactivation of acetylcholine, one of the primary neurotransmitters in neuro-neuronal and neuromuscular junctions (Devrnja et al., 2022; Jankowska et al., 2018). According to a review from Jankowska et al. (2018), α-pinene was found to be one of the most effective VOCs in inhibiting AChE potentially explaining the greater entomotoxic activity of Rosemary EO in which α-pinene is the second most abundant compound. Several other compounds such as camphor, eucalyptol and α-pinene (all present in Rosemary EO) have also been reported to have a cytotoxic mode of action which induces cell membrane damage (Bakkali et al., 2008).

Previous studies have tested the entomotoxic properties of EOs against *Bemisia tabaci* (Aslan et al., 2004; Kim et al., 2011). Aslan et al. (2004) evaluated the efficiency of *Satureja hortensis*, *Ocimum basilicum* and *Thymus vulgaris,* finding promising control by fumigation, requiring less than 3 $\mu L/L_{air}$ of EO to kill at least 50% of individuals in 24 h. All three EOs were from the Lamiaceae family mainly characterized by a composition dominated by monoterpenes and phenylpropene. Although none of the major compounds in these EOs were also present in Artemisia and Rosemary EOs, they were found to have similar mode of action. They too affected the GABA receptors, inhibited AChE and had cytotoxic properties, just like the major VOCs contained in Artemisia and Rosemary EOs (Bakkali et al., 2008; Jankowska et al., 2018). The only difference was for *Satureja hortensis* containing carvacrol, which provides an additional neurotoxic mode of action by acting on octopamine receptors, affecting neurotransmission in the central nervous system of insects (Jankowska et al., 2018). Kim et al. (2011), screened an extensively large panel of EOs in order to investigate their insecticidal activity against *Bemisia tabaci* when fumigated. The results of their study allowed them to identify *Allium sativum* and *Satureja hortensis* as the most insecticidal EOs after 24 h of fumigation. Their findings were in line with those of Aslan et al. (2004), who had previously demonstrated *Satureja hortensis* as greatly entomotoxic against *Bemisia tabaci*. However, the LC_{50} found by Kim et al. (2011) was much higher (i.e. 170 mL/L_{air}). This could be explained by the method they used to expose the insects to the tested EO which was diluted in ethanol and then applied to filter paper. The filter paper was then left to dry in a fume hood for 1.5 min before being placed in contact with the insects for 24 h. VOCs could have been released in the fume hood or could here been prevented from being released by the ethanol.

Overall *Bemisia tabaci* seems to be susceptible to a variety of VOCs from the monoterpene and monoterpenoid families whose modes of action may explain the different efficiency of a range of EOs. EO efficacy has been reported to be linked to their chemical composition but also to the proportion of these compounds (Mossa, 2016; Regnault-Roger, 1997). The presence of synergetic interactions between compounds could also explain differences in EO insecticidal activity. For example, eucalyptol (or 1,8-cineole) has been reported to have synergetic effects when combined with carvone or ɣ-terpinene, both found in Rosemary EO, (Table 2) allowing for a synergistic interaction of major and minor components, potentially explaining its greater control of *Bemisia tabaci* (Attia et al., 2016; Mossa, 2016). Finally, the insecticidal activity of Artemisia and Rosemary EOs is not simply limited to *Bemisia tabaci*. Rosemary EO was shown to have great entomotoxicity against a range of aphid species (Ikbal and Pavela, 2019), thrips and other stored pest species such as *Callosobruchus maculatus* or *Tribolium castaneum* (both beetles) (Jahanian et al., 2022). Likewise, Artemisia EO has been used against stored product beetles (Sharifian et al., 2013) as well as plant-feeding mites (Aslan et al., 2004).

Yet the efficacy of Artemisia and Rosemary EOs observed in the present study on *Bemisia tabaci* differed from preliminary work (Suppl. Mat. 1): Initial screening revealed high efficacy of Artemisia EO against *B. tabaci* in leaflet assays when applied pure while Rosemary EO showed less entomotoxicity as well as high phytotoxicity. Here, when tested pure in whole-plant assays, EOs yielded different mortality than the 50% anticipated. Artemisia EO had a lower entomotoxic effect whereas Rosemary EO reached a mortality rate of almost 1 (Fig. 2). Phytotoxicity effects are also reversed compared to preliminary results (Suppl. Mat. 1): Here, Rosemary EO is more phytotoxic than Artemisia (Fig. 3). In addition to being performed on a full-functioning organism (whole plant), interacting with soil, changing the experimental scale modifies the abiotic conditions. Thus, an increase in efficiency could be explained by a biostimulatory effect of the EOs triggering a response from the plant, consequently adding to the EO effect on the insects. The emission of VOCs, specifically monoterpenes, has been shown to trigger plant defenses, either by transcription of defense genes (Vergnes et al., 2014) or directly by inducing the production and emission of monoterpenes from the plant itself (Erb et al., 2015). Moreover, introducing whole plants in a closed system also creates a microclimate with new abiotic conditions, causing an increase in humidity from plant transpiration in the climatic chamber. It is known that high humidity can either increase or decrease VOC emission: Vallat et al. (2005) found that with an increase in relative humidity, emission of α-pinene (a major compound in Rosemary EO) increased whereas other compounds had their emissions reduced with higher relative humidity.

Overall, even before discussing the efficacy of formulated EOs in comparison to pure EOs, these findings reinforce the importance and the advantage of formulating EOs due to their instability.

4.2. Degradation of pure EOs and variability among encapsulated EOs

Pure unformulated EOs are subject to degradation by numerous environmental factors, notably atmospheric oxygen, moisture, light and temperature, decreasing the abundance of available VOCs (Erb et al., 2015; Vallat et al., 2005). Wholly unprotected pure EO volatiles may be released extremely rapidly or may be lost, in part, during application at the outset of the experiment. These hypotheses are supported by the results of GC-FID relative quantification of total VOC abundance one day after formulation. These data show that pure EOs release the largest quantity of VOCs of all the formulations tested (Fig. 4). This may indicate that unformulated EOs quickly release VOCs, resulting in a short-lived treatment effect over time when used against *B. tabaci*.

One of the key challenges in increasing the number of EO-based biopesticides is the development of efficient stabilization processes (e.g., encapsulation, coacervation) in order to avoid the degradation of EO components by isolating them from the surrounding environment. Furthermore, entrapping active compound allows a better dispersion in the final formula, and a better controlled release, especially for volatile terpenes (Campolo et al., 2020a; Donsì and Ferrari, 2016; Werdin

González et al., 2015). In this study, three methods chosen for their "green" raw materials were evaluated for the formulation of EO-based biopesticides, used as fumigants. Depending upon their protection in the formulation, volatile compounds are released in a more or less controlled manner. Of all the approaches tested here, nanoemulsions of both EOs proved the most entomotoxic against *B. tabaci,* topping the effect of pure EOs, followed by NaDES and then by atomized powders, which demonstrated almost no entomotoxicity. Phytotoxicity followed the same trend as entomotoxicity, with nanoemulsions inducing the most phytotoxicity (higher than pure EOs). NaDES produced less phytotoxicity than pure EOs and finally atomized powder did not yield any. These results could be explained by the different forms of EO encapsulation provided by the different methods.

4.3. Nanoemulsions

Oil-in-water nanoemulsions consist of small oil droplets (0.1–10 μm) containing the active ingredient, dispersed homogenously throughout a hydrophilic solvent (Gasic and Tanovic, 2013). When prepared as nanoemulsions, EOs are encapsulated in direct micelles formed by Tween 80 surfactant (Kfoury et al., 2019). The advantages of nanoemulsions include their simple preparation and low risk of active component loss due to evaporation. Furthermore, encapsulation within micelles allows for increased EO stability and a more sustained release over time (Turek and Stintzing, 2013). Potential disadvantages include a risk of phytotoxicity when applied by direct contact and a limited shelf life (Gasic and Tanovic, 2013).

In our study, nanoemulsions released a large, yet significantly less important quantity of VOCs than pure EOs, one day after preparation and showed high entomotoxicity. EOs nano-emulsions (garlic) were also shown to successfully control another tomato herbivore *Tuta absoluta* (Tortorici et al., 2022). The present results which show a higher entomotoxicty and phytotoxicity of Rosemary EOs compared to the corresponding pure EO are difficult to explain. It is probably due to the fact that nanoemulsified volatile compounds may be released in a slower, more controlled manner allowing for persistence of effect thus causing higher *B. tabaci* mortality than pure EOs after 24 h. The sustained release of important amounts of volatiles proposed by nanoemulsions would result in a longer exposure time of plants to phytotoxic VOCs, indeed creating higher phytotoxicity than pure EOs. In addition, depending on the structure of an EO compound and on its affinity within other molecules within the droplet, its release might differ slightly from its release using unformulated EO. For instance, interactions between TWEEN 80 and a given EO compound, such as monoterpenoids or monoterpenes, might influence their ability to be released. This could explain the difference in relative abundance between monoterpenes and monoterpenoids observed between pure Rosemary EO and the corresponding nanoemulsions of this EO, as shown in Fig. 4 and the different results observed here between pure EO and nanoemulsions.

4.4. Atomization

Spray drying, or atomization, involves passing a hot gas through a liquid preparation of a desired active ingredient in order to obtain a powder in which the active ingredient is nanoencapsulated (Gharsallaoui et al., 2007). Often, the initial preparation is a nanoemulsion which, when atomized, forms a fine powder (particle size: 10–50 μm) in which the active ingredient finds itself fixed to a solid matrix. This technique is widely used in the food industry because of high equipment availability and low cost (Gharsallaoui et al., 2007). In crop protection, nanoencapsulated active ingredients (nanoparticles) present numerous advantages over existing biopesticide formulations, including improved solubility, increased stability (decreased volatility, degradation), controlled release, and decreased non-target toxicity (Athanassiou et al., 2018; Campolo et al., 2020b). As a result, nanoencapsulated biopesticides present greater efficiency and thus reduced active ingredient requirements, lowering costs compared to their nonencapsulated equivalents (Luiz De Oliveira et al., 2018; Pascual-Villalobos et al., 2017).

When formulated as atomized powders, EOs are found both fixed to the surface, as well as inside, of maltodextrine capsules, permitting greater EO stability and controlled release (Mohammed et al., 2020). In regard to atomized powders, although they should theoretically provide protection and controlled release of VOCs as do nanoemulsions, the process of atomization, which passes heated gas through an EO emulsion, likely results in the loss of most volatile compounds. This might explain the ineffective control of *B. tabaci* and the absence of phytotoxicity. Atomized powders were indeed shown to release an extremely low quantity of VOCs one day after preparation (Fig. 3), demonstrating that atomization is not an adequate process for the formulation of an effective EO-based biopesticide. The limited release of VOCs by atomized powders may be aggravated by their hygroscopic tendency to absorb water in which VOCs may be trapped and unable to volatilize.

4.5. NaDES

NaDES discovered a little more than a decade ago by Choi et al. (2011), possess great potential to replace harsh organic solvents currently used in biopesticides. NaDES are composed of two or more inexpensive, non-toxic and biodegradable solids that form a liquid phase when combined together in a specific molar ratio (Cao et al., 2017; Chagnoleau et al., 2021; Espino et al., 2016; Mouden et al., 2017). Depression of the mixtures melting point always occurs when mixing two solid compounds, but this decrease in melting point is significantly larger when hydrogen bonding between so-called hydrogen bond acceptor and donor compounds occurs (Mouden et al., 2017; Ruesgas-Ramón et al., 2017). A large number of natural solid compounds, with different polarity ranges, can be employed, allowing NaDES to be tailored to the properties of the active molecule to be formulated with (Cao et al., 2017). The vast network of hydrogen bonds is expected to stabilize the active ingredient, protecting it from degradation by heat and light (Mouden et al., 2017). The protection NaDES provide to active molecules, in addition to their versatility, biodegradability, low cost, and easy preparation, makes them a promising bioformulation solution (Ruesgas-Ramón et al., 2017). However, very little research on such an application is available to date (Dai et al., 2013). Ruesgas-Ramón et al. (2017) attempted to apply NaDES to bioformulation, testing their ability to dissolve six insecticidal metabolites, individually. Even so, formulation with NaDES of more complex insecticidal mixtures remains, to date, untested. Only one recent study by Mariappan et al. (2015), has attempted using NaDES in the formulation of a complex mixture of secondary metabolites contained in EO, however this study focused on the antioxidant and antimicrobial properties of ginger essential oil (*Zingiber offficinale*), rather than on its pesticide properties.

Formulation with NaDES results in the solubilization of a larger amount of EO than in other formulations, most probably due to the extensive network of hydrogen bonds present in NaDES (Dai et al., 2013). One day after preparation, NaDES released a small quantity of VOCs (Fig. 4) which may indeed be explained by interactions occurring between volatiles and NaDES compounds, hindering volatilization. This could explain the lower relative abundance of VOCs released with NaDES formulation (Fig. 4) as well as the resulting lower phytotoxicity and *B. tabaci* control when compared with results obtained for nanoemulsions of Artemisia EO over a 24 h period. On the contrary, despite the small quantity of Rosemary EO volatiles released from the prepared NaDES, a strong entomotoxic effect was provided by this formulation. This may be due to the fact that Rosemary EO contains a greater amount of VOCs than Artemisia EO or that Rosemary EO dissolved more efficiently than Artemisia EO in the NaDES (Fig. 4), resulting in a greater number of volatiles released into the experimental headspace. It would be interesting to test this hypothesis with other EOs which, similarly to

Rosemary, release a high abundance of VOCs, in order to evaluate their entomotoxicity when formulated as NaDES.

Our results suggest that despite their slow release of VOCs, NaDES may in fact provide an effective control over the long-term, achieving roughly the same entomotoxicity as EOs when used pure but avoiding the combined phytotoxicity. Releasing much smaller amounts of volatiles would result in a more limited exposure of plants to VOCs and thus less phytotoxicity. Kinetic analysis revealed the progressive release of volatiles over the course of time when an EO was formulated in a NaDES (Fig. 5). According to their molecular mass, lightweight monoterpenes are released first, followed by monoterpenoids and then by the heavier sesquiterpenes.

5. Conclusion and perspectives

In conclusion, the formulation of EOs for use as biopesticides resulted in variable success. Furthermore, phytotoxicity is one of the major constraints of EOs. This result demonstrates the need for considerable precautions to be taken in product formulation (Werrie et al., 2020). Atomized powders, although easy to prepare as well as to apply, provided no mortality of insects and required the availability of an atomizer. Conversely, nanoemulsions and NaDES proved more entomotoxic than the pure unformulated EOs. Of all of the formulations, nanoemulsions proved to be, by far, the most entomotoxic, when formulated with both Artemisia and Rosemary EOs, as well as the easiest to prepare. However, nanoemulsions, especially the most entomotoxic, resulted in important levels of phytotoxicity. In addition, nanoemulsions are formulated using Tween (polysorbate), a synthetic molecule, which although used in prepared foods and cosmetics, remains neither nature-derived nor nature-inspired. NaDES, on the other hand, are prepared with amino acids and sugars found in a number of commonly consumed foods. They additionally represent the only formulation that allows an important control of *B. tabaci* without inducing a correspondingly high level of phytotoxic damages. Continued work is needed to conceptualize a method of application adapted to the high viscosity of NaDES, which complicated NaDES application in the current study. Such future studies would allow for the greater conceptual characterization and formulation fine-tuning currently lacking, which prevent EO NaDES from representing a viable biopesticide option. Once improved, EO NaDES have the potential to revolutionize the biopesticide market, providing efficient pest control without posing risks to treated plants, nor potentially, to non-target species or the environment, thanks to their slowly released VOCs.

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CRediT authorship contribution statement

Lana Dunan: Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Tara Malanga:** Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Nicolas Papaiconomou:** Formal analysis, Writing – review & editing. **Nicolas Desneux:** Conceptualization, Funding acquisition, Methodology. **Anne-Violette Lavoir:** Conceptualization, Funding acquisition, Methodology, Formal analysis, Writing – review & editing. **Thomas Michel:** Formal analysis, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Lana Dunan reports financial support, administrative support, and equipment, drugs, or supplies were provided by French National Institute for Agricultural Research INRAE.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2023.117006.

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