Toxicity and repellent activity of a carlina oxide nanoemulsion toward the South American tomato pinworm, Tuta absoluta

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Research Article

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Abstract

Plant essential oil (EO)-based insecticides represent a promising tool for IPM, though their formulation is limited by poor physio-chemical properties. EO encapsulation into stable formulations, like nano emulsions (NEs), could boost EO efficacy and stability. *Carlina acaulis* roots contain an EO recently studied for its excellent insecticidal activities, and chiefly composed by carlina oxide (>97%). Herein, we developed two carlina oxide NEs (0.25% and 0.5% w/w) through ultrasounds exposure and characterized them by dynamic light scattering. The NE insecticidal and repellent activities were investigated against *T. absoluta* eggs and larvae, and adults, respectively. 0.25% and 0.5% NEs showed a monomodal size distribution with a Z-average size of 113.87±1.32 nm and 127.27±0.47 nm, respectively. The polydispersity indexes measured at 0.15±0.01 and 0.16±0.01 indicate a low grade of polydispersity. The 0.25% NE showed significant contact toxicity on *T. absoluta* eggs, with a maximum egg hatching inhibition of 85.7% 11 days post-treatment. The highest larvicidal effect was observed in translaminar toxicity tests, with complete mortality after 24 h. The NE did not achieve significant oviposition deterrence. Overall, the tested NE showed promising effectiveness as ovicide and larvicide on *T. absoluta*, highlighting the need of further research shedding light on its modes of action, as well as to evaluate lethal and sublethal effects on tomato biological control agents and pollinators.

Key Message

- Nanoemulsion (NE) can boost the stability and the efficacy of insecticidal essential oils.
- Here we developed carlina oxide NEs for insecticidal applications against *Tuta absoluta*.
- 0.25% (w/w) carlina oxide NE was stable for 3 months and slightly phytotoxic to tomato.
- The NE was toxic towards *T. absoluta* eggs and larvae.
- No oviposition deterrence effects were detected on adults.

Introduction

Nanotechnology is a rapidly growing field that holds great promise and is being widely utilized in various applied sciences (Yousef et al. 2023). Its employment in agriculture is significantly increased over the last years (Pavoni et al. 2019; Kavallieratos et al. 2022; Yousef et al. 2023). Thanks to the nanometric size of the dispersed phase, nano-systems facilitate the delivery of active compounds (e.g., insecticides) more efficiently and with minor health and environmental impact (An et al. 2022; Bamisaye et al. 2023, Geng et al. 2017; Schulz 2004). In addition, they provide a viable way to use plant-borne biocides with limited physicochemical qualities, high volatility, and rapid environmental degradation for managing pests of economic importance (Pavela et al. 2021a; Pavoni et al. 2020). Colloidal dispersions, characterized by the diffusion of a core phase within a continuous medium, have garnered significant attention as a highly adaptable nano-system (Pavoni et al. 2019). Nanoemulsions (NEs) are a type of self-emulsifying colloidal systems. They are composed of two immiscible liquid phases and possess the unique ability to effectively transport lipophilic or poorly water-soluble chemicals, such as essential oils (EOs) (Pavela et al. 2021a). Because of their simplicity of formulation and handling, NEs are considered one of the most promising nano-systems for pest control purposes. The insecticidal activity of EO-based NEs has been investigated on several insect species of agricultural and medical interest, such as aphids (Heydari et al. 2020; Pascual-Villalobos et al. 2017; Sciortino et al. 2021), mosquitoes (Jesser et al. 2020a; Mahran 2022; Pavela et al. 2021a), stored-product beetles (Giunti et al. 2019; Kavallieratos et al. 2022), and some Lepidoptera (Benelli et al. 2020; Jesser et al. 2020a; Louni et al. 2018). According to recent findings, a highly promising NE is that based on *Carlina acaulis* L. (Asteraceae) EO. *Carlina acaulis* EO is isolated from the roots of the plant and mainly includes carlina oxide, whose concentration usually overcomes 97% of the overall chemical composition and is the primary cause of the EO's efficacy (Spinozzi et al. 2023a). In general, compounds belonging to the polyacetylenes' family are well known as a phytoalexins, or plant defence molecules produced in reaction to living microorganisms, microbial products, and environmental stressors (Spinozzi et al. 2023b). Both *C. acaulis* EO and carlina oxide demonstrated a variety of biological activities, including a highly promising insecticidal potential (Benelli et al. 2019, 2022; Kavallieratos et al. 2020; Pavela et al. 2020; Rizzo et al. 2021), as well as antimicrobial and antiprotozoal activities (Herrmann et al. 2011; Stojanović-Radić et al. 2012; Spinozzi et al. 2023a). In addition, they have a limited impact on beneficial aquatic and terrestrial organisms (Benelli et al. 2022; Spinozzi et al. 2023a). Recent studies investigated *C. acaulis* EO and carlina oxide NE insecticidal activity and they both elicited high toxicity on 3rd -4th instar larvae of mosquitoes (Pavela et al 2021a), on 1st instar larvae of *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae) (Benelli et al. 2020), on the invasive xylomicetophage ambrosia beetle *Xylosandrus compactus* (Eichhoff) (Coleoptera: Curculionidae, Scolytinae) (Gugliuzzo et al. 2023), and on the root knot nematode *Meloidogyne incognita* (Kofoid & White) (Tylenchida: Heteroderidae) (Ntalli et al. 2023). Although both NEs have been proven to be effective as green insecticides, their bioactivity has only been studied on a limited number of insect species. Hence, the objective of the present study was to assess the bioactivity of carlina oxide NE on the South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), a key pest of tomato crops both in greenhouses and in open field (Campolo et al. 2017). The endophytic habitat of *T. absoluta* larval stages shields them from the majority of chemical compounds (Biondi et al. 2018). This has led to an increased use of synthetic insecticides (Silva et al. 2011; Roditakis et al. 2015), that has also led to the development of resistance in *T. absoluta* (Roditakis et al. 2018).

For these reasons, recent research focused on eco-friendly tools to manage this moth pest, including botanical based NEs (Campolo et al. 2017; Ricupero et al. 2022; Salazar et al. 2022). In this framework, formulating effective and highly stable NEs can be a valuable strategy to enable the practical use of carlina oxide as an active component in the development of commercial green pesticides to manage the South American tomato pinworm. Here, through ultrasonic exposure, we develop two carlina oxide NEs (0.25% and 0.5% w/w carlina oxide) and characterised them using dynamic light scattering (DLS). Since carlina oxide is phytotoxic at high concentration for many plant species (Álvarez-Rodríguez et al. 2023), we assessed the potential phytotoxicity of carlina oxide NEs (0.25 and 0.50%) on tomato plants before starting our experiments. According to phytotoxicity trials, we investigated the insecticidal and repellent properties of the 0.25% (w/w) carlina oxide NE against *T. absoluta* eggs and larvae, as well as adults, respectively.

Materials and methods

Plant laboratory rearing

Tomato plants used in all the experiments were cultivated at the CREA - Research Centre for Plant Protection and Certification (CREA-DC) of Palermo (Italy). *San Marzano nano* cultivar seeds (ITALSEMENTI S.N.C.®) were sown and grown in 1 L pots (10x10x15 cm) with peat, topsoil (Gramoflor®, GmbH & Co. KG) and expanded vermiculite (VIC, Italiana®) and were placed in fine mesh net cages (45x45x90 cm) to prevent other undesirable insect species. Water fertilization was done once every two weeks with *Sangue di bue* (COMPO Group) and no pesticides were used to manage pest infestations. The plants were placed in a chamber with controlled environmental conditions at 25 ± 2°C, 50 ± 10% relative humidity (RH) and a photoperiod (L:D) of 16:8. Plants of about 50 cm height and with at least five true leaves were used for testing and *T. absoluta* rearing.

Insect laboratory rearing

A laboratory stock culture, maintained at the CREA-DC of Palermo, supplied the insects utilised in this study. The original culture was set up in 2008, in the laboratory of the Department of Agriculture, Food and Environment (Di3A) of the University of Catania, with infested tomato plants from commercial farms in eastern Sicily. To reduce the genetic drift, once every 6 months, the colony is bred with new individuals collected in Sicilian tomato crops. The rearing is placed in a chamber with controlled conditions of temperature (25 ± 2°C) and relative humidity (50 ± 10%) with 16:8 L:D of photoperiod. Twice a week, 200 newly emerged adults of *T. absoluta* were launched in a screened cage with fine mesh (45x45x45 cm) with three plants. After three days, the adults were removed to avoid eggs of different age. Approximately after two weeks, when the 4th instar larvae became pupae, all the vegetal material was cut and put in another screened cage for the emergence of the new adults. 2nd instar larvae and newly emerged coetaneous adults were used for the experimental activities.

Extraction of carlina oxide

Carlina oxide was extracted from *C. acaulis* roots following the procedure reported by Rizzo et al. (2023) with a yield of 0.5% (w/w). The plant was purchased from Minardi & Figli S.r.l. (Bagnacavallo, Ravenna, Italy; https://www.minardierbe.it; batch no C-26102101, collected in 2021). The compound's structure and purity (97.4%, Fig. 1) were confirmed by GC-MS and NMR analyses accordingly to protocols previously published and the data were linear to those reported in literature (Benelli et al. 2019). Once obtained, the compound was stored at -20°C protected from light before biological assays.

Preparation and characterization of tested carlina oxide nanoemulsions

Carlina oxide NEs were prepared through a high energy method by employing ultrasounds. Specifically, the corresponding amounts of carlina oxide to have a final concentration in the formulation of 0.25% and 0.5% (w/w) were mixed with ethyl oleate in a 3:1 weight ratio. Then, the oil phases were included dropwise in polysorbate 80 aqueous solutions with a final concentration of 0.16 and 0.33% (w/w) for the 0.25% and 0.5% NE, respectively, under high-speed stirring (Ultraturrax T25 basic, IKA® Werke GmbH and Co.KG, Staufen, Germany) for 5 min at 13500 rpm.

Finally, the two obtained emulsions were sonicated into a 2L-Ultrasound Extractor U2020 (170W, 230V, 50Hz) (Albrigi Luigi Srl Verona, Italy) for 40 min using the H + M (high power + homogenization) program to reduce the droplet size. The NEs' particle size was determined through DLS using a Zetasizer nanoS (Malvern Instrument, Malvern, UK) following the procedure by Benelli et al. (2020). The stability of NEs, stored at 4°C in tight closed vials, was assessed by checking their mean droplet size (Z-average) and polydispersity index (PDI) at different time points: 0 day (T_0), 15 days (T_1), 30 days (T_2), and 90 days (T_3).

Phytotoxicity on tomato

To evaluate the potential phytotoxicity of the NEs, we applied two different doses of carlina oxide NE (0.25% and 0.5%) to tomato plants using a hand sprayer (V = 100 mL) from 20 cm until the solution ran off. We also included the respective control NE solutions (i.e., the NE without carlina oxide at the 0.25% and 0.5%) and a negative control (distilled water); each tested dose was repeated on 5 tomato plants. Treated plants were, then, placed in a greenhouse and checked 1, 3, 7, 14 days after the treatment. Following the methodology of Campolo et al. (2017), we noted the percentage of damaged leaves and the severity of the damage. The latter has been classified as: (i) 0, no damage; (ii) 1, leaf surface partially damaged with chlorosis but without necrosis; (iii) 2, leaves with evident necrosis; (iv) 3, dead leaves. The phytotoxicity index (P_i) was calculated as follows:

$$P_i = \sum_{j=0}^{n} \left(\frac{DL}{TL} x \frac{DC}{n-1} \right)$$

In which DL is the number of damaged leaves for each damage severity class j; TL is the total number of sprayed leaves; DC is the damage severity class; n is the number of damage severity classes.

P_i ranges from 0 (no damage) to 1 (dead leaves).

Contact toxicity on eggs

Based on the results obtained in phytotoxicity trials, we assessed the ovicidal effect of carlina oxide NE (0.25%) by directly spraying the NE on the eggs and measuring the percentage of egg mortality. In a cage (45x30x45 cm) with a fine mesh net, healthy tomato sprouts were exposed to 100 unsexed newly emerged mated adults of *T. absoluta* for 24 h. After 48 h, the sprouts with coetaneous laid eggs were sprayed as above described and were let dry for 60 min in laboratory conditions. After drying, with a fine brush a total number of 70 treated eggs per treatment were moved in new healthy tomato sprouts placed in a two-cup experimental arena as described by Biondi et al. (2012) to wait for the hatching. Every two days after the treatment the sprouts were checked to verify egg hatching, the overall egg hatching after 11 days was noted. At the same time, we tested a negative control (i.e., distilled water) and a control NE (i.e., the NE without carlina oxide).

Topical toxicity on larvae

The larvicidal effect of carlina oxide NE (0.25%) was evaluated in topical toxicity trials. The larval survival (%) and the adult emergence (%) were recorded. In addition, the negative control and the control NE were tested. For each treatment, 50 *T. absoluta* 2nd instar larvae from the insect rearing were collected and isolated in absorbent paper sheet (to prevent larval drowning caused by excess of solution after the treatment). Subsequently, 15 mL of NE were sprayed on larvae as above described (see “Phytotoxicity on tomato” paragraph). After 15 min, the treated larvae were moved with a fine brush and placed in healthy tomato sprouts arranged in the two-cup experimental arena as described above. The experimental arenas were checked at 24, 48, and 72 h after the release of the larvae for the evaluation of larval survival and after 12 days for adult emergency.

Ingestion toxicity on larvae

Ingestion toxicity tests were performed to assess the larvicidal effect of NE carlina oxide. The tested NE and the parameters noted were the same as above mentioned. Healthy tomato sprouts were treated as described above (see “Phytotoxicity on tomato” paragraph) and dried in laboratory conditions for 60 min. The treated sprouts were fixed in the two-cup experimental arena and 50 *T. absoluta* larvae were released on each sprout. As for the previous experiment of the larval toxicity by topical contact, the experimental arenas were checked at 24, 48, and 72 h after the release of the larvae for the evaluation of larval survival and were checked after 12 days for adult emergency.

Translaminar toxicity on larvae

The potential translaminar effect of the NE on *T. absoluta* larvae within the tomato leaf was assessed. The same experimental setting was used as in the previous paragraph for this test. However, the larvae were released on healthy sprouts 24 h before the treatment to give them time to get inside the leaves. The experimental arenas were checked at 24, 48, and 72 h after the treatment of the tomato sprouts for the evaluation of larval survival.

Ovideterrence trials

The repellent activity of NE carlina oxide on *T. absoluta* adults was evaluated in the two-choice assays. Three different theses were compared: (i) distilled water *vs* carlina oxide NE, (ii) distilled water *vs* control NE and (iii) carlina oxide NE *vs* control NE. For this trial, 24 h before the experiment, *T. absoluta* newly emerged adults were collected and placed inside a screened cage for the mating. Tomato sprouts, with two true leaves, were sprayed (see “Phytotoxicity on tomato” paragraph) and let dry for 30 min in laboratory conditions. After drying, the sprouts were placed in a plastic box (45x30x45 cm) aerated with a fine mesh net, and a total number of 10 adults were released inside to lay eggs. After 72 h, adults were removed and the total number of laid eggs on each tomato sprout was noted. Five replicates (cages) for each two-choice assay were performed for a total of 50 tested *T. absoluta* adults.

Data analysis

Contingency analysis was used to evaluate differences in hatchability rates among treatments ($p = 0.05$). Data from topical, ingestion, translaminar toxicity, and adult emergence experiments were not normally distributed (Shapiro-Wilk test, $p > 0.01$) and homoscedastic (Levene test, $p > 0.01$). As a result, the Kruskal-Wallis test was used to assess treatment differences ($p = 0.05$). Because ovi-deterrence data were normally distributed (Shapiro-Wilk test, $p > 0.01$) and homoscedastic (Levene's test, $p > 0.01$), two-choice assay findings were subjected to paired *t*-tests ($p = 0.05$) (Tomè et al. 2013). The phytotoxicity index (P_i) was analysed with univariate analysis of variance, with concentrations and time after the treatment as fixed factors and P_i as dependent variable. The Steel-Dwass test and Tukey test were utilized as a post-hoc analysis when suitable ($p < 0.05$). For statistical analysis, JMP Pro 17 and SPSS® V. 20 (IBM) were employed, whereas GraphPad PRISM (10.0.3) was used for graphing.

Results

Characterization of tested carlina oxide nanoemulsions

The nanometric size range of the carlina oxide NE oil droplets was assessed by DLS measurements. Both 0.25% and 0.5% (w/w) formulations showed a monomodal size distribution with Z-average size of 113.87 ± 1.32 nm and 127.27 ± 0.47 nm, respectively (Fig. 2). The quality of the two systems with respect to the size distribution was checked on the bases of the PDI. The latter showed values of 0.15 ± 0.01 and 0.16 ± 0.01 for 0.25 and 0.5% (w/w) NEs, respectively, which confirmed that the systems had a low grade of polydispersity.

The physical stability of carlina oxide NEs was evaluated for a storage period of 3 months, after which the sizes of the dispersed phases showed a slight variance. For instance, the average particle size of 0.25% NE obtained after 3 months (133.77 ± 6.05 nm) of storage is increased by only 20 nm compared to the T_0. Similarly, 0.5% NE particle size slightly increased from 127.27 ± 0.47 (T_0) to 145.63 ± 5.92 (T_3). Moreover, the PDI values obtained for the NEs at T_3 were really similar to those at T_0, confirming that this type of formulations remains stable for at least a period of 3 months. The Z-average size and PDI values of oil droplets in the two NEs obtained from the measurements at T_0, T_1, T_2, and T_3 are shown in Fig. 3.

Phytotoxicity of carlina oxide nanoemulsions

The toxic effect of the different concentrations of carlina oxide NE on plants was expressed as the phytotoxicity index (P_i) and it is shown in Fig. 4. No phytotoxicity was recorded in the distilled water treatment (P_i=0) and a slight effect was observed for control NE 0.25% (P_i=0.006) and control NE 0.5% (P_i=0.022). Carlina oxide NE at the concentration of 0.25% had slight toxic effects on tomato plants (P_i=0.170), compared to the higher concentration of 0.5%, where heavier toxic effects were recorded

(P_i=0.443). Only the concentration at 0.5% of carlina oxide NE showed significant damage in the leaves with evident necrosis and dead leaves. The data analysis highlights that the phytotoxicity index significantly depends on the treatment ($F_{4,25}$=386.75; $p < 0.001$) and on the days after the treatment ($F_{3,20}$=5.961; $p < 0.001$). In all the treatments, the new vegetation grown in the 14 days after the beginning of the trial, did not show any phytotoxic effect.

Contact toxicity on eggs

Eggs sprayed with carlina oxide NE 0.25% (w/w) showed lower hatching rates after 11 days (χ^2 = 133.042, $p < 0.0001$). Most eggs failed to hatch when exposed to carlina oxide NE (unhatched: 85.7±2.0%), whilst a high percentage of eggs hatched when treated with control NE (hatched eggs: 99.0±0.5%) and distilled water (hatched eggs: 81.0±5.5%) (Fig. 5).

Topical toxicity on larvae

Topical toxicity results indicated that carlina oxide NE 0.25% (w/w) was scarcely effective till 72 h post-treatment if compared to positive and negative controls (24 h: χ^2 = 2.333, *d.f.*=2, p = 0.311; 72 h: χ^2 = 2.533, *d.f.*=2, p = 0.282) (Fig. 6a, b). No significant reduction in adult emergence was found after 12 days (χ^2 = 0.267, *d.f.*=2, p = 0.875) (Fig. 6c).

Ingestion toxicity on larvae

Carlina oxide NE 0.25% (w/w) showed a toxic effect after 24 h (χ^2 = 27.671, *d.f.*=2, $p < 0.0001$) in ingestion toxicity trials (Fig. 7a). Larval mortality was observed only in treated specimens. Adult emergence differed significantly from positive and negative control after12 days (χ^2 = 24.728, *d.f.*=2, $p < 0.0001$) (Fig. 7b).

Translaminar toxicity on larvae

In translaminar toxicity trials, after 24 h, larval mortality was recorded on 0.25% (w/w) NE-treated sprouts (χ^2 = 26.238, *d.f.*=2, $p < 0.0001$) (Fig. 8). Control NE mortality rates increased after 72 h, but it still significantly differed from the NE-treated sprouts (χ^2 = 25.530, *d.f.*=2, $p < 0.0001$) (Fig. 7b).

Ovideterrence effect

In two-choice experiments, carlina oxide NE 0.25% (w/w) do not cause considerable egg-laying deterrence in mature females of *T. absoluta* (Fig. 9). There was a significant difference in the number of eggs laid between the NE-treated sprout and the negative control (distilled water) ($F_{1,9}$=19.997, p = 0.002). By contrast, there were no differences in the number of eggs laid on sprouts treated with carlina oxide NE over the NE control ($F_{1,9}$=0.613, p = 0.456).

Discussion

Nanoformulations can improve the stability and efficiency of botanical insecticides by addressing difficulties such as EO volatility and poor water stability, as well as gradually and effectively spreading the insecticide over the target plant, potentially reducing the impact on non-target species (Pavoni et al. 2020). NEs can be prepared with different techniques; the high energy methods are the most widespread. Earlier, carlina oxide NEs were developed using the high-pressure homogenization methodology (Benelli et al. 2020; Pavela et al. 2021a; Kavallieratos et al. 2022; Ntalli et al. 2023). In this study, we relied to ultrasounds to assist in the preparation of two formulations. Ultrasounds are a high energy method commonly used for creating nanoformulations (Bamisaye et al. 2023). Although different methodologies have been used for the preparation of carlina oxide NEs, the nanometric size of dispersed phase was achieved in both cases. For instance, the Z-average size of the oil droplets in this study was remarkably similar to size measured for the NE prepared using a high-pressure homogenization method (Pavela et al. 2021a). Both had a size of around 127 nm and 142 nm, respectively, when the concentration of oil and surfactant was the same. Herein we also focused whether the NEs prepared with ultrasounds had long-lasting stability. The slight variance of Z-average and PDI values obtained from DLS analysis at different time points confirmed that the ultrasounds are suitable for the preparation of relatively stable carlina oxide NEs.

EOs and their major compounds have a significant impact on plant biological activities by directly interacting with the lipid layers of biological membranes (Campolo et al. 2017, Synowiec et al. 2017). As herbicides, EOs applied to the leaves can cause noticeable damage in just a matter of hours (Álvarez-Rodriguez et al. 2023). Our study found that the phytotoxic effect of carlina oxide NEs increased with the dosage and became more pronounced over time. Additionally, the P_i index showed a significant increase 24 h after treatment. At the highest concentration (0.5% w/w), NE-treated plants showed damaged and dead leaves, and evident necrosis on the leaf surface. Instead, at the lowest concentration (0.25%), the leaf damage was considerably less. For this reason, carlina oxide NE (0.25%) was chose for further investigations.

From an entomological point of view, we observed an acute effect of carlina oxide NE on larvae at 24 and 72 h, as well as a long-lasting effect on egg hatching and adult emergence. Our results suggests that carlina oxide NE (0.25% w/w) is effective towards both eggs and larvae of *T. absoluta*. After 11 days, less than 15% of eggs hatched after topical treatments with carlina oxide NE. If compared with other nano-systems, carlina oxide NE seems to be more effective. Indeed, less than 50% of eggs reached the adult stage when sprayed with sweet orange-EO nanoparticles (NPs) at the highest dose (Campolo et al. 2017). *Tuta absoluta* larvae survival rates in topical, ingestion and translaminar toxicity trials were also assessed. Topical experiments revealed no significant impact of carlina oxide NE on *T. absoluta* larval mortality, probably due to a low ability of NE carlina oxide to permeate the insect body (Tortorici et al. 2022). However, the potential ability of *T. absoluta* larvae to detoxify the NE should be also considered (Ghanim & Abdel Ghani 2014). By contrast, the results of ingestion and translaminar experiments were encouraging. In both experiments, *T. absoluta* larval mortality was significantly higher carlina oxide NE-treated sprouts than the ones sprayed with control NE and distilled water. Translaminar toxicity results indicate that using NEs as a nano-system is highly recommended. Indeed, the toxic effect on *T. absoluta* larvae in translaminar tests utilizing citrus peel EO-NP was considerably less effective than the respective EOs (Campolo et al. 2017). The toxic effect could be due to carline oxide's ability to damage insect cells by forming carbocations that react with -SH groups and amino groups of proteins and other molecules (Kavallieratos et al. 2022). In addition, the phototoxicity of carlina oxide induced by UV exposure may enhance this potential (Kavallieratos et al. 2022). Furthermore, Spinozzi et al. (2023a) suggested different hypothesis for carlina oxide insecticidal mechanism of action, such as inhibition of acetylcholinesterase, modulation of gamma-aminobutyric acid receptors A (GABA-A), and inhibition of octopamine and P450 cytochromes receptors. Unfortunately, the mechanism of action of this carlina oxide remains still unknown. Lastly, carlina oxide NE has a weak ovideterrence effect; indeed, when compared its control (control NE) no significant differences were found. A possible explanation is the presence of ethyl oleate as a stabilizer component of both NE and its control which acts as an attractant or a repellent depending on the dose (Hafez et al. 2014; Chen & Dai 2015). The strong repellent effect of the NE is probably due to the potential bioactive properties of this polyacetylene. Indeed, active ingredients, such as EOs, can elicit a repellent effect towards several arthropod species (Benelli et al. 2021). Further studies are still required to assess the repellent and/or attractive activity of carlina oxide NE using electroantennography (EAG) and olfactory bioassays, which might be a promising element to better understand the compound's potential. An oviposition repellence caused by NEs has also been observed when tomato sprouts were treated with LC_{50} dose of *Allium sativum* EO-based NE (GEO-NE) (Ricupero et al. 2022).

Overall, our results support the possible use of carlina oxide NE as an effective ovicide and larvicide against *T. absoluta*, in the framework of the severe insecticide resistance quickly developed by this important tomato pest worldwide (Guedes et al. 2019; Desneux et al. 2022). One may argue that the phytotoxicity detected on tomato plants may represent a problem for practical implementation. To face this challenge, we are focusing on other nano-objects to allow highly stable carlina oxide formulation, such as zein nanocapsules and polymeric micelles (Athanassiou et al. 2018; Sanchez-Gomez et al. 2022). Further research is still required to understand its method of action and bioactivity on non-target species (Jesser et al. 2020b; Giunti et al. 2022; Yerguerdan et al. 2022), with a focus on tomato biological control agents and pollinators.

Declarations

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This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

Author Contributions

ST, FM, GB, and RR conceived and designed the study. ST, VZ, DRP, MF, ES, FM, GB, and RR conducted the experiments and/or analysed data. ST, VZ, DRP, MF, ES, and RR wrote the original draft. All authors revised the manuscript and approved its final version.

Declaration of competing interest

The authors report no declarations of interest.

References

1. Álvarez-Rodríguez S, Spinozzi E, Sánchez-Moreiras AM, López-González D, Ferrati M, Lucchini G, Maggi F, Petrelli R, Araniti F (2023) Investigating the phytotoxic potential of *Carlina acaulis* essential oil against the weed *Bidens pilosa* through a physiological and metabolomic approach. Ind Crops Prod 203:117149. https://doi.org/10.1016/j.indcrop.2023.117149
2. An C, Sun C, Li N, Huang B, Jiang J, Shen Y, Wang C, Zhao X, Cui B, Wang C, Li X, Zhan S, Gao F, Zeng Z, Cui H, Wang Y (2022) Nanomaterials and nanotechnology for the delivery of agrochemicals: strategies towards sustainable agriculture. J Nanobiotechnology 20:11. https://doi.org/10.1186/s12951-021-01214-7
3. Athanassiou CG, Kavallieratos NG, Benelli G, Losic D, Usha Rani P, Desneux N (2018) Nanoparticles for pest control: current status and future perspectives. J Pest Sci 91:1–15. https://doi.org/10.1007/s10340-017-0898-0
4. Bamisaye A, Adegoke KA, Alli YA, Bamidele MO, Idowu MA, Ogunjinmi OE (2023) Recent advances in nanoemulsion for sustainable development of farm-to-fork systems. J Clean Prod 139226. https://doi.org/10.1016/j.jclepro.2023.139226
5. Benelli G, Ceccarelli C, Zeni V, Rizzo R, Lo Verde G, Sinacori M, Boukouvala MC, Kavallieratos NG, Ubaldi M, Tomassoni D, Benvenuti F, Roy P, Petrelli R, Cappellacci L, Spinozzi E, Maggi F, Canale A (2022) Lethal and behavioural effects of a green insecticide against an invasive polyphagous fruit fly pest and its safety to mammals. Chemosphere 287:132089. https://doi.org/10.1016/j.chemosphere.2021.132089.
6. Benelli G, Pavela R, Petrelli R, Nzekoue FK, Cappellacci L, Lupidi G, Quassinti L, Bramucci M, Sut S, Dell'Acqua S, Canale A, Maggi F (2019) Carlina oxide from *Carlina acaulis* root essential oil acts as a potent mosquito larvicide. Ind Crops Prod 137:356–366. https://doi.org/10.1016/j.indcrop.2019.05.037
7. Benelli G, Pavoni L, Zeni V, Ricciardi R, Cosci F, Cacopardo G, Gendusa S, Spinozzi E, Petrelli R, Cappellacci L, Maggi F, Pavela R, Bonacucina G, Lucchi A (2020) Developing a Highly Stable *Carlina*

acaulis Essential Oil Nanoemulsion for Managing *Lobesia botrana*. Nanomaterials 10(9):1867. https://doi.org/10.3390/nano10091867

8. Benelli G, Rizzo R, Zeni V, Govigli A, Samková A, Sinacori M, Sinacori M, Lo Verde G, Pavela R, Cappellacci L, Petrelli R, Spinozzi E, Morshedloo MR, Maggi F, Canali A (2021) *Carlina acaulis* and *Trachyspermum ammi* essential oils formulated in protein baits are highly toxic and reduce aggressiveness in the medfly, *Ceratitis capitata*. Ind Crops Prod 161:113191. https://doi.org/10.1016/j.indcrop.2020.113191
9. Biondi A, Desneux N, Siscaro G, Zappalà L (2012) Using organic certified rather than synthetic pesticides may not be safer for biological control agents: selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. Chemosphere 87:803–812. https://doi.org/10.1016/j.chemosphere.2011.12.082
10. Biondi A, Guedes R, Wan F, Desneux N (2018) Ecology, worldwide spread, and management of invasive south tomato pinword, *Tuta absoluta*: past, present, future. Annu Rev Entomol 63:239–258. https://doi.org/10.1146/annurev-ento-031616-034933
11. Campolo O, Cherif A, Ricupero M, Siscaro G, Grissa-Lebdi K, Russo A, Cucci LM, Di Pietro P, Satriano C, Desneux N, Biondi A, Zappalà L, Palmieri V (2017) Citrus peel essential oil nanoformulations to control the tomato borer, *Tuta absoluta*: chemical properties and biological activity. Sci Rep 7:13036. https://doi.org/10.1038/s41598-017-13413-0
12. Chen Y, Dai G (2015) Acaricidal, repellent, and oviposition-deterrent activities of 2, 4-di-tert-butylphenol and ethyl oleate against the carmine spider mite *Tetranychus cinnabarinus*. J Pest Sci 88:645–655. https://doi.org/10.1007/s10340-015-0646-2
13. Desneux N, Han P, Mansour R, Arnó J, Brévault T, Campos MR, Chailleux A, Guedes RNC, Karimi J, Konan KAJ, Lavoir A, Luna MG, Perez-Hedo M, Urbaneja A, Verheggen FJ,Zappalà L, Abbes K, Ali A, Bayram Y, Cantor F, Cuthbertson AGS, De Vis R, Erler F, Firake DM, Haddi K, Hajjar MJ, Ismooilov K, Jaworrski CC, Kenis M, Liu H, Madadi H, Martin T, Mazih A, Messelink GJ, Mohamed SA, Nofemela RS, Oke A, Ramos C, Ricupero M, Roditakis E, Shashank PR, Wan F-H, Wang M, Wang S, Zhang Y-B, Biondi A (2022) Integrated pest management of *Tuta absoluta*: practical implementations across different world regions. J Pest Sci 95:17–39. https://doi.org/10.1007/s10340-021-01442-8
14. Geng Y, Ma J, Zhou R, Jia R, Li C, Ma X (2017) Assessment of insecticide risk to human health in groundwater in Northern China by using the China-PEARL model. Pest Manag Sci 73:2063–2070. https://doi.org/10.1002/ps.4572
15. Ghanim NM, Abdel Ghani SB (2014) Controlling *Tuta absoluta* (Lepidoptera: Gelechiidae) and *Aphis gossypii* (Hemiptera: Aphididae) by aqueous plant extracts. Life Sci J 11:299–307
16. Giunti G, Palermo D, Laudani F, Algeri GM, Campolo O, Palmeri V (2019) Repellence and acute toxicity of a nano-emulsion of sweet orange essential oil toward two major stored grain insect pests. Ind Crops Prod 142:111869. https://doi.org/10.1016/j.indcrop.2019.111869
17. Giunti G, Benelli G, Palmeri V, Laudani F, Ricupero M, Ricciardi R, Maggi F, Lucchi A, Guedes RNC, Desneux N, Campolo O (2022) Non-target effects of essential oil-based biopesticides for crop

protection: Impact on natural enemies, pollinators, and soil invertebrates. Biol Control 176:105071. https://doi.org/10.1016/j.biocontrol.2022.105071

18. Guedes RNC, Roditakis E, Campos MR, Haddi K, Bielza P, Siqueira HAA, Tsagkarakou A, Vontas J, Nauen R (2019) Insecticide resistance in the tomato pinworm *Tuta absoluta*: patterns, spread, mechanisms, management and outlook. J Pest Sci 92:1329–1342. https://doi.org/10.1007/s10340-019-01086-9
19. Gugliuzzo A, Francardi V, Simoni S, Roversi PF, Ferrati M, Spinozzi E, Perinelli DR, Bonacucina G, Maggi F, Tortorici S, Tropea Garzia G, Biondi A, Rizzo R (2023) Role of plant essential oil nanoemulsions on host colonization by the invasive ambrosia beetle *Xylosandrus compactus*. Ind Crops Prod 195:116437. https://doi.org/10.1016/j.indcrop.2023.116437
20. Hafez M, Dimetry NZ, Abbass MH (2014) Insecticidal and biological efficacy of two vegetable oils against *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) under laboratory conditions. Arch Phytopathol Plant Prot 47:194. https://doi.org/10.1080/03235408.2013.862943
21. Herrmann F, Hamoud R, Sporer F, Tahrani A, Wink M (2011) Carlina oxide: a natural polyacetylene from *Carlina acaulis* (Asteraceae) with potent antitrypanosomal and antimicrobial properties. Planta Med 77:1905–1911. https://doi.org/10.1055/s-0031-1279984
22. Heydari M, Amirjani A, Bagheri M, Sharifian I, Sabahi Q (2020) Eco-friendly pesticide based on peppermint oil nanoemulsion: Preparation, physicochemical properties, and its aphicidal activity against cotton aphid. Environ Sci Pollut Res 27:6667–6679. https://doi.org/10.1007/s11356-019-07332-y
23. Jesser E, Lorenzetti AS, Yeguerman C, Murray AP, Domini C, Werdin-González JO (2020a) Ultrasound assisted formation of essential oil nanoemulsions: Emerging alternative for *Culex pipiens pipiens* Say (Diptera: Culicidae) and *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) management. Ultrason Sonochem 61:104832. https://doi.org/10.1016/j.ultsonch.2019.104832.
24. Jesser E, Yeguerman C, Gili V, Santillan G, Murray AP, Domini C, Werdin-González JO (2020b) Optimization and characterization of essential oil nanoemulsions using ultrasound for new ecofriendly insecticides. ACS Sustain Chem Eng 8:7981–7992. https://doi.org/10.1021/acssuschemeng.0c02224
25. Kavallieratos NG, Boukouvala MC, Ntalli N, Skourti A, Karagianni ES, Nika EP, Kontodimas DC, Cappellacci L, Petrelli R, Cianfaglione K, Morshedloo MR, Tapondjou LA, Rakotosaona R, Maggi F, Benelli G (2020) Effectiveness of eight essential oils against two key stored-product beetles, *Prostephanus truncatus* (Horn) and *Trogoderma granarium*. Everts Food Chem Toxicol 139:111255. https://doi.org/10.1016/j.fct.2020.111255
26. Kavallieratos NG, Nika EP, Skourti A, Boukouvala MC, Ntalaka CT, Maggi F, Spinozzi E, Petrelli R, Perinelli DR, Benelli G, Canale A, Bonacucina G (2022) *Carlina acaulis* essential oil nanoemulsion as a new grain protectant against different developmental stages of three stored-product beetles. Pest Manag Sci 78:2434–2442. https://doi.org/10.1002/ps.6877

27. Louni M, Shakarami J, Negahban M (2018) Insecticidal efficacy of nanoemulsion containing *Mentha longifolia* essential oil against *Ephestia kuehniella* (Lepidoptera: Pyralidae). J Crop Prot 7:171–182.
28. Mahran HA (2022) Using nanoemulsions of the essential oils of a selection of medicinal plants from Jazan, Saudi Arabia, as a green larvicidal against *Culex pipiens*. PLOS ONE 17:e0267150. https://doi.org/10.1371/journal.pone.0267150
29. Ntalli N, Zochios G, Nikolaou P, Winkiel M, Petrelli R, Bonacucina G, Perinelli DR, Spinozzi E, Maggi F, Benelli G (2023) *Carlina acaulis* essential oil nanoemulsion for managing *Meloidogyne incognita*. Ind Crops Prod 193:116180. https://doi.org/10.1016/j.indcrop.2022.116180
30. Pascual-Villalobos MJ, Cantó-Tejero M, Vallejo R, Guirao P, Rodríguez-Rojo S, Cocero MJ (2017) Use of nanoemulsions of plant essential oils as aphid repellents. Ind Crops Prod 110:45–57. https://doi.org/10.1016/j.indcrop.2017.05.019
31. Pavela R, Maggi F, Petrelli R, Cappellacci L, Buccioni M, Palmieri A, Canale A, Benelli G (2020) Outstanding insecticidal activity and sublethal effects of *Carlina acaulis* root essential oil on the housefly, *Musca domestica*, with insights on its toxicity on human cells. Food Chem Toxicol 136:111037. https://doi.org/10.1016/j.fct.2019.111037
32. Pavela R, Pavoni L, Bonacucina G, Cespi M, Cappellacci L, Petrelli R, Spinozzi E, Aguzzi C, Zeppa L, Ubaldi M, Desneux N, Canale A, Maggi F, Benelli G (2021a) Encapsulation of *Carlina acaulis* essential oil and carlina oxide to develop long-lasting mosquito larvicides: microemulsions versus nanoemulsions. J Pest Sci 94:899–915. https://doi.org/10.1007/s10340-020-01327-2
33. Pavela R, Maggi F, Mazzara E, Torresi J, Cianfaglione K, Benelli G, Canale A (2021b) Prolonged sublethal effects of essential oils from non-wood parts of nine conifers on key insect pests and vectors. Ind Crops Prod 168:113590. https://doi.org/10.1016/j.indcrop.2021.113590
34. Pavoni L, Pavela R, Cespi M, Bonacucina G, Maggi F, Zeni V, Canale A, Lucchi A, Bruschi F, Benelli G (2019) Green Micro- and Nanoemulsions for Managing Parasites, Vectors and Pests. Nanomaterials 9:1285. https://doi.org/10.3390/nano9091285
35. Pavoni L, Perinelli DR, Bonacucina G, Cespi M, Palmieri GF (2020) An Overview of Micro-and Nanoemulsions as Vehicles for Essential Oils: Formulation. Nanomaterials 10:135. https://doi.org/10.3390/nano10010135
36. Ricupero M, Biondi A, Cincotta F, Condurso C, Palmeri V, Verzera A, Zappalà L, Campolo O (2022) Bioactivity and physico-chemistry of garlic essential oil nanoemulsion in tomato. Entomol Gen 42:921–930. https://doi.org/10.1127/entomologia/2022/1553
37. Rizzo R, Pistillo M, Germinara GS, Lo Verde G, Sinacori M, Maggi F, Petrelli R, Spinozzi E, Cappellacci L, Zeni V, Canale A, Benelli G (2021) Bioactivity of Carlina acaulis essential oil and its main component towards the olive fruit fly, *Bactrocera oleae*: Ingestion toxicity, electrophysiological and behavioral insights. Insects 12:880. https://doi.org/10.3390/insects12100880
38. Rizzo R, Ragusa E, Benelli G, Lo Verde G, Zeni V, Maggi F, Petrelli R, Spinozzi E, Ferrati M, Sinacori M, Tsolakis H (2023) Lethal and sublethal effects of the polyacetylene carlina oxide on *Tetranychus*

urticae (Acari: Tetranychidae) and *Neoseiulus californicus* (Acari: Phytoseiidae). Pest Manag Sci. https://doi.org/10.1002/ps.7827

39. Roditakis E, Vasakis E, Grispou M, Stavrakaki M, Nauen R, Gravouil M, Bassi A (2015) First report of *Tuta absoluta* resistance to diamide insecticides. J Pest Sci 88:9–16. https://doi.org/10.1007/s10340-015-0643-5
40. Roditakis E, Vasakis E, García L, Martínez M, Rison J, Haxaire M, Nauen R, Tsagkarakou A, Bielza P (2018) A four-year survey on insecticide resistance and likelihood of chemical control failure for tomato leaf miner *Tuta absoluta* in the European/Asian region. J Pest Sci 91:421–435. https://doi.org/1007/s10340-017-0900-x
41. Salazar AM, Arismendi N, López MD, Vargas M, Schoebitz M, Palacio DA, Becerra J, Cedeño B, Zapata N (2022) Stability of the oil-based nanoemulsion of *Laureliopsis philippiana* (Looser) and its insecticidal activity against tomato borer (*Tuta absoluta* Meyrick). Ind Crops Prod 188:115635. https://doi.org/10.1016/j.indcrop.2022.115635
42. Sánchez-Gómez S, Pagán R, Pavela R, Mazzara E, Spinozzi E, Marinelli O, Zeppa L, Morshedloo MR, Maggi F, Canale A, Benelli G (2022) Lethal and sublethal effects of essential oil-loaded zein nanocapsules on a zoonotic disease vector mosquito, and their non-target impact. Ind Crops Prod 176:114413. https://doi.org/10.1016/j.indcrop.2021.114413
43. Schulz R (2004) Field studies on exposure, effects, and risk mitigation of aquatic non point-source insecticide pollution: A review. J Environ Qual 33:419–448. https://doi.org/10.2134/jeq2004.4190
44. Sciortino M, Scurria A, Lino C, Pagliaro M, D'Agostino F, Tortorici S, Ricupero M, Biondi A, Zappalà L, Ciriminna R (2021) Silica-microencapsulated orange oil for sustainable pest control. Adv Sustainable Syst 5:2000280. https://doi.org/10.1002/adsu.202000280
45. Silva G, Picanço M, Bacci L, Crespo A, Rosado J, Guedes R (2011) Control failure likelihood and spatial dependence of insecticide resistance in the tomato pinworm, Tuta absoluta. Pest Manag Sci 67:913–920. https://doi.org/10.1002/ps.2131
46. Spinozzi E, Ferrati M, Cappellacci L, Caselli A, Perinelli DR, Bonacucina G, Maggi F, Strzemski M, Petrelli R, Pavela R, Desneux N, Benelli G (2023a) *Carlina acaulis* L. (Asteraceae): biology, phytochemistry, and application as a promising source of effective green insecticides and acaricides. Ind Crops Prod 192:116076. https://doi.org/10.1016/j.indcrop.2022.116076
47. Spinozzi E, Ferrati M, Baldassarri C, Maggi F, Pavela R, Benelli G, Aguzzi C, Zeppa L, Cappellacci L, Palmieri A, Petrelli R (2023b) Synthesis of carlina oxide analogues and evaluation of their insecticidal efficacy and cytotoxicity. J Nat Prod 86:1307–1316. https://doi.org/10.1021/acs.jnatprod.3c00137
48. Stojanović-Radić Z, Čomić L, Radulović N, Blagojević P, Mihajilov-Krstev T, Rajković J (2012) Commercial Carlinae radix herbal drug: botanical identity, chemical composition and antimicrobial properties. Pharm Biol 50:933–940. https://doi.org/10.3109/13880209.2011.649214
49. Synowiec A, Kalemba D, Drozdek E, Bocianowski J (2017) Phytotoxic potential of essential oils from temperate climate plants against the germination of selected weeds and crops. J Pest Sci 90:407–

419. https://doi.org/10.1007/s10340-016-0759-2

50. Tomé HVV, Martins JC, Corrêa AS, Galdino TVS, Picanço MC, Guedes RNC (2013) Azadirachtin avoidance by larvae and adult females of the tomato leafminer *Tuta absoluta*. Crop Prot 46:63–69. https://doi.org/10.1016/j.cropro.2012.12.021
51. Tortorici S, Cimino C, Ricupero M, Musumeci T, Biondi A, Siscaro G, Carbone C, Zappalà L (2022). Nanostructured lipid carriers of essential oils as potential tools for the sustainable control of insect pests. Ind Crops Prod 181:114766. https://doi.org/10.1016/j.indcrop.2022.114766
52. Yeguerman CA, Urrutia RI, Jesser EN, Massiris M, Delrieux CA, Murray AP, & González JOW (2022) Essential oils loaded on polymeric nanoparticles: bioefficacy against economic and medical insect pests and risk evaluation on terrestrial and aquatic non-target organisms. Environ Sci Pollut Res 29:71412–71426. https://doi.org/10.1007/s11356-022-20848-0
53. Yousef HA, Fahmy HM, Arafa FN, Abd Allah MY, Tawfik YM, El Halwany KK, El-Ashmanty BA, Al-anany FS, Mohamed MA, Bassily ME (2023) Nanotechnology in pest management: advantages, applications, and challenges. Int J Trop Insect Sci 43:1387–1399. https://doi.org/10.1007/s42690-023-01053-z

Figures

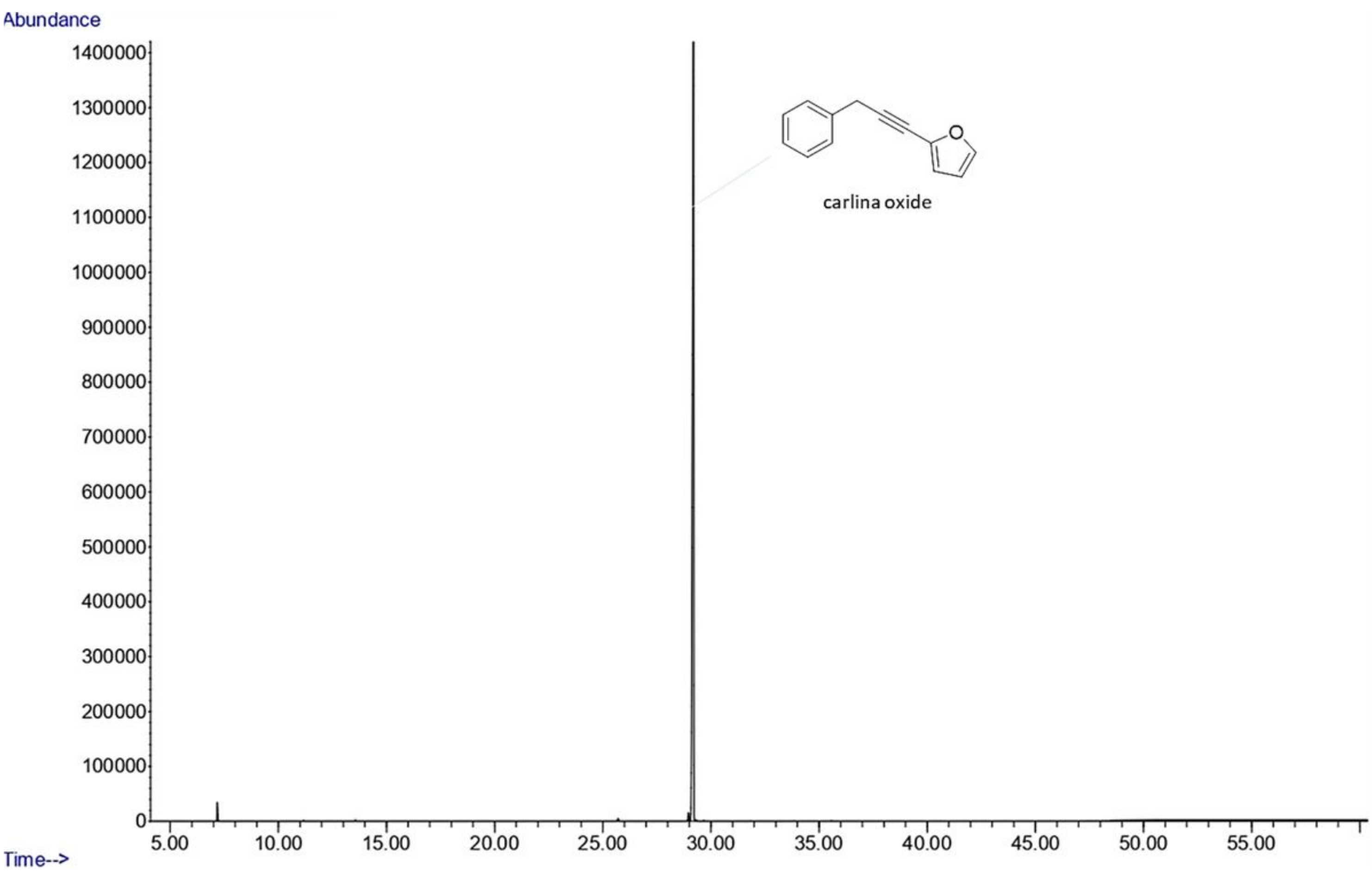


Figure 1

GC-MS chromatogram of the isolated carlina oxide

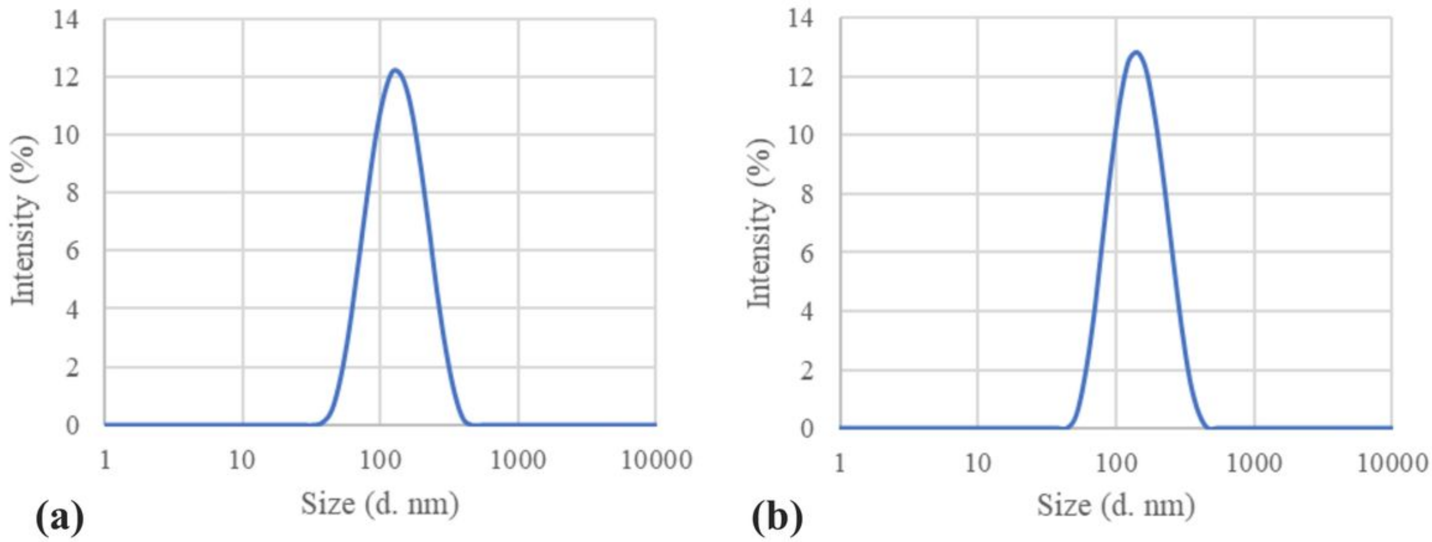


Figure 2

Dynamic light scattering (DLS) traces of 0.25% (w/w) (a) and 0.5% (w/w) carlina oxide NE (b)

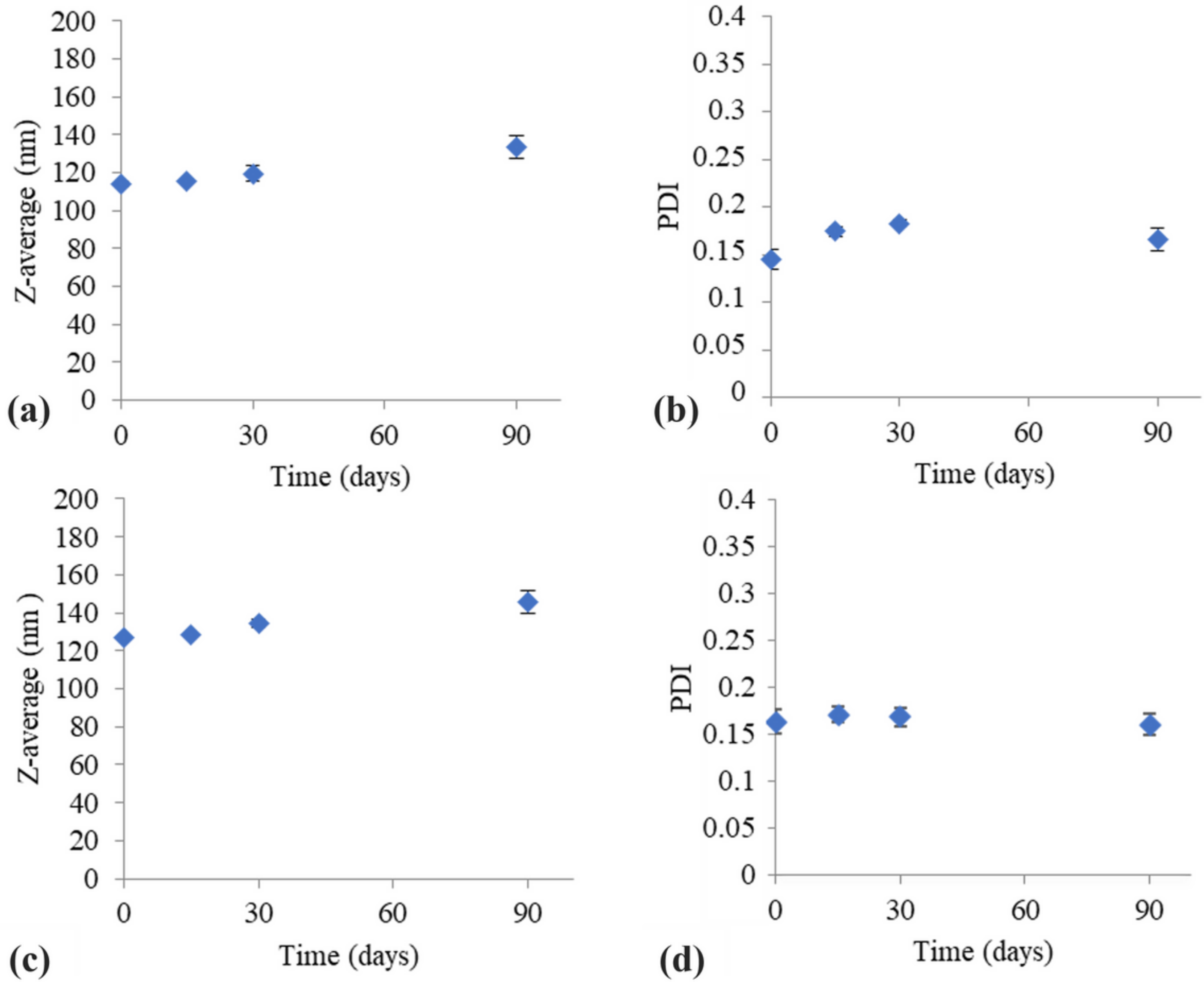


Figure 3

Z-average and polydispersity index (PDI) values obtained from dynamic light scattering (DLS) analysis of carlina oxide nanoemulsions (NEs) at timepoints T_0, T_1, T_2, and T_3. (a) and (c) represent the Z-average values for 0.25 and 0.5% (w/w) NEs, respectively. (b) and (d) showed variation of PDI for 0.25 and 0.5% (w/w) NEs, respectively. Data are presented as mean ± standard deviation (SD) of three measurements

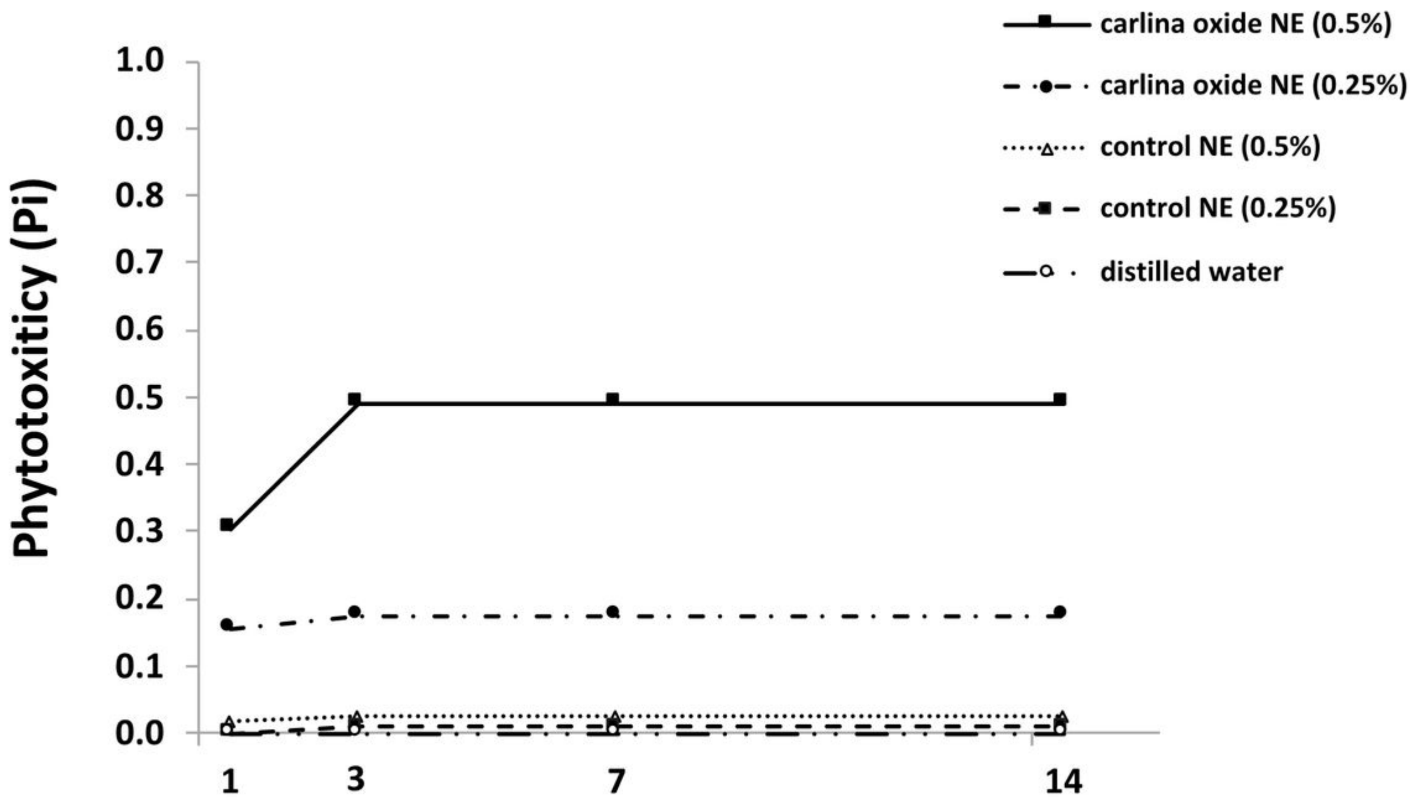


Figure 4

Phytotoxicity index (P_i, mean values) of the two carlina oxide nanoemulsions (NE) and related formulations recorded 1, 3, 7 and 14 days post-treatment

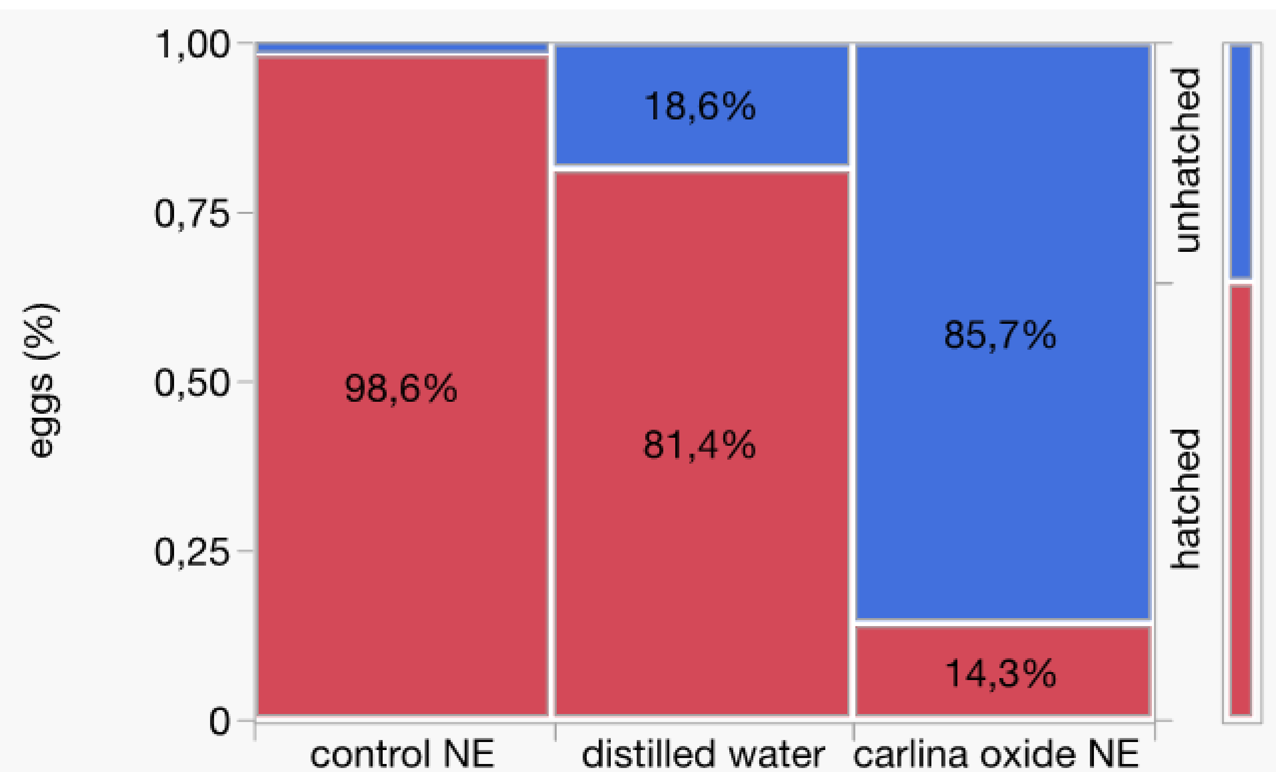


Figure 5

Mosaic diagram showing *Tuta absoluta* eggs hatchability post-treatment with carlina oxide nanoemulsion (NE), control NE, and distilled water. The right bar indicates hatchability rates after 11 days. The value within each mosaic tile indicates the percentage of eggs and the size of each mosaic tile varies according to it

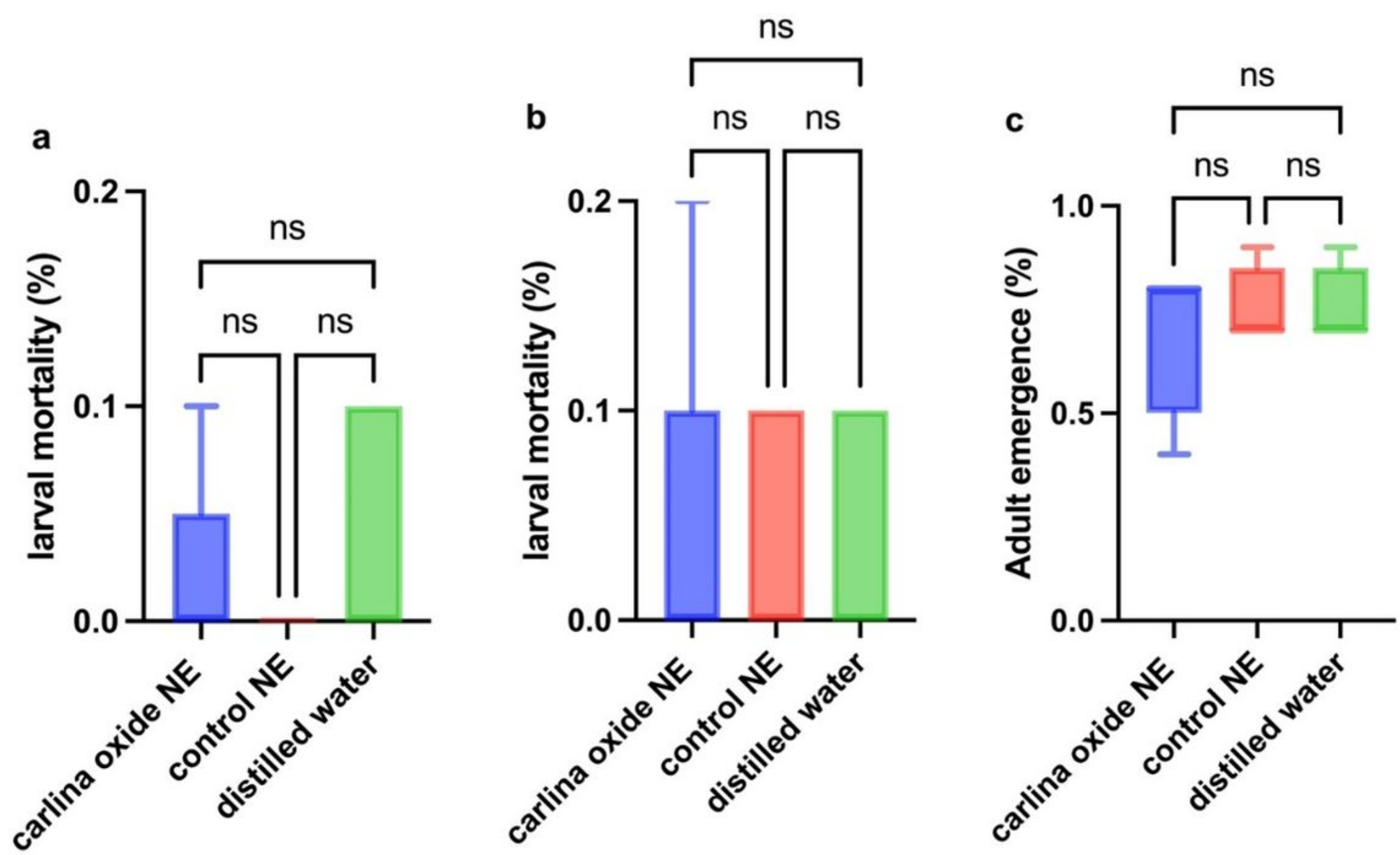
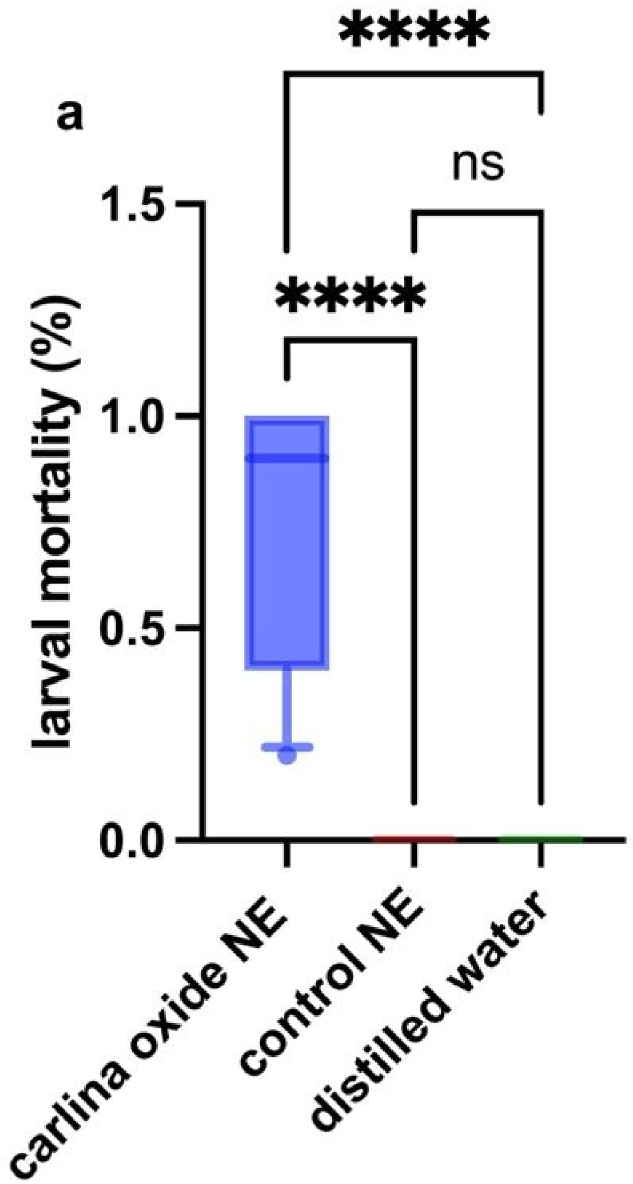


Figure 6

(a) Mortality (%) of 2nd instar larvae of *Tuta absoluta* in topical bioassays testing carlina oxide nanoemulsion (NE) after 24 h; (b) mortality (%) of 2nd instar larvae of *T. absoluta* in topical assays after 72 h; (c) percentage of adult emergence after 12 days from the beginning of the trial. Each box plot indicates the median and its dispersion range (lower, upper quartile, and extreme values, outliers). ns= not significant (Kruskall-Wallis test, $p<0.05$)



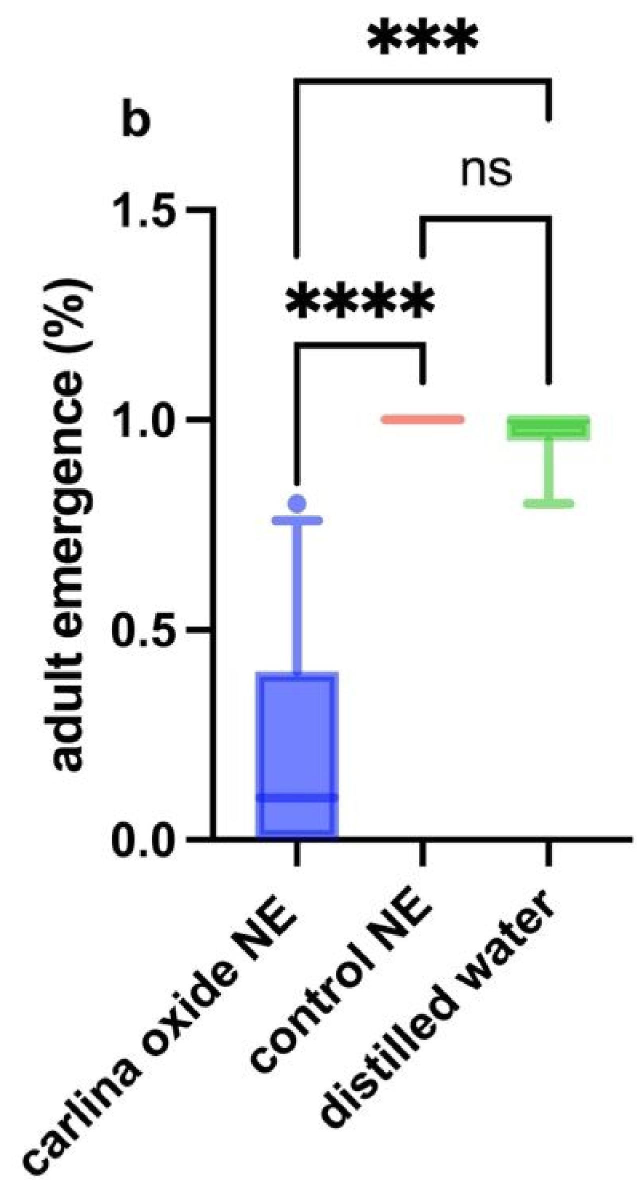


Figure 7

(a) Mortality (%) of 2nd instar larvae of *Tuta absoluta* in ingestion bioassays testing carlina oxide nanoemulsion (NE) after 24 h; the percentage of adult emergence after and 12 days (b) from the beginning of the trial. Each box plot indicates the median and its dispersion range (lower, upper quartile, extreme values, outliers). *=significant, ns= not significant (Kruskall-Wallis test, Steel-Dwass post hoc, $p < 0.05$)

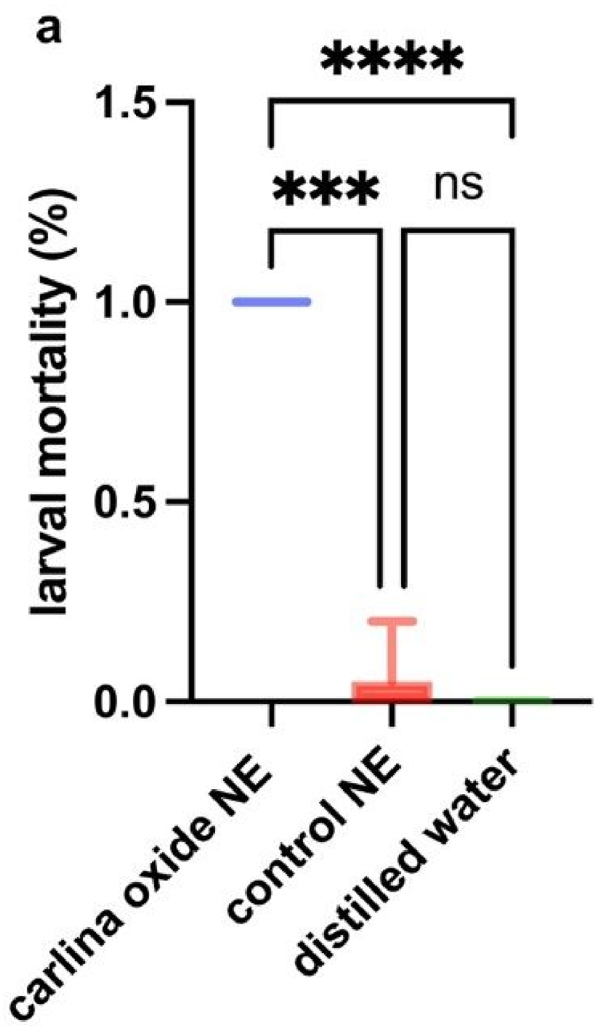


Figure 8

(a) Mortality (%) of 2nd instar larvae of *Tuta absoluta* in translaminar assays testing carlina oxide nanoemulsion (NE) after 24 h; (b) mortality (%) of 2nd instar larvae of *T. absoluta* in translaminar assays after 72 h. Each box plot indicates the median (red line) and its dispersion range (lower, upper quartile, and extreme values, outliers). * = significant, ns= not significant (Kruskall-Wallis test, Steel-Dwass post hoc, $p < 0.05$)

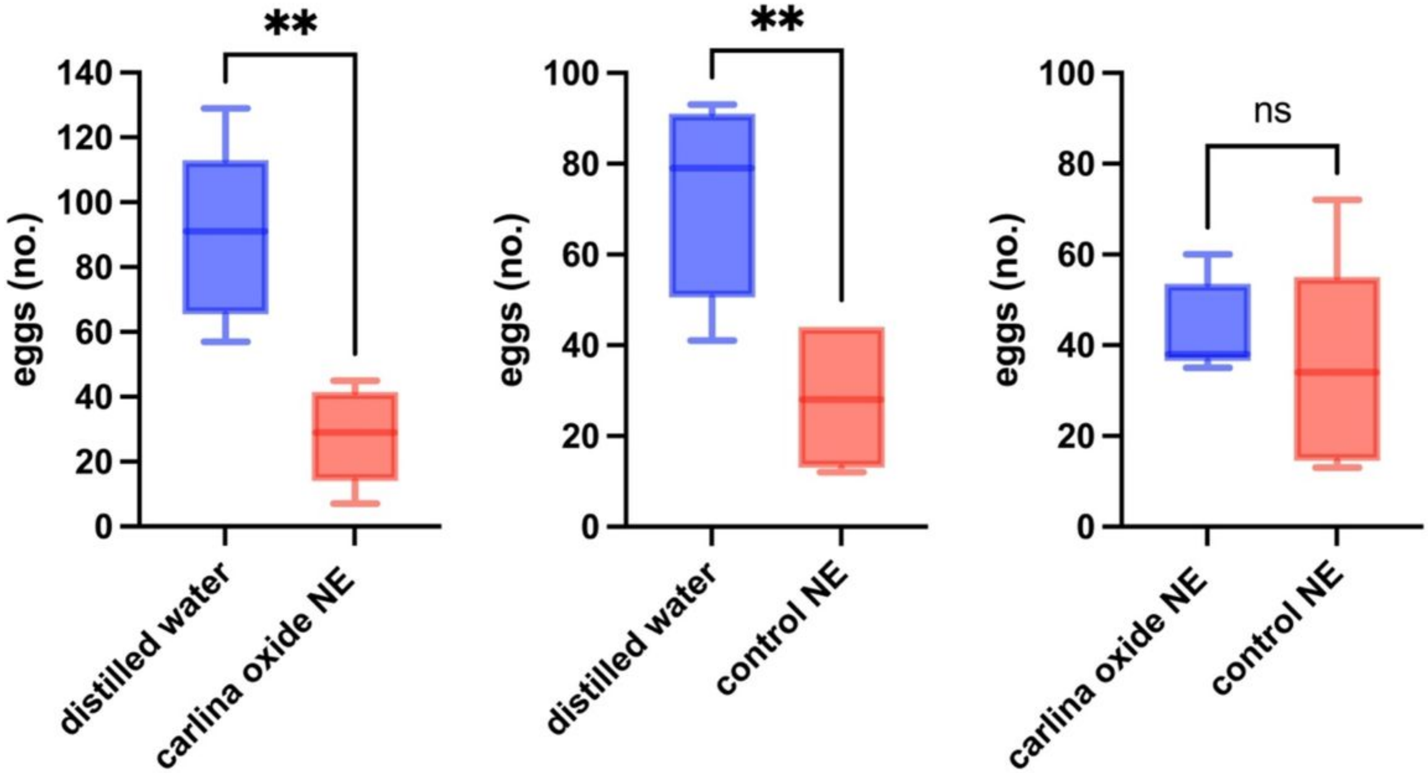


Figure 9

Number of *Tuta absoluta* eggs laid on tomato sprouts in ovi-deterrence two-choice assays testing carlina oxide nanoemulsion (NE). Each box plot indicates the median and its dispersion range (lower, upper quartile, extreme values, outliers). * = significant, ns= not significant (*t*-test, p<0.05)