

Targeting *Xylella fastidiosa*: Sustainable management of *Philaenus spumarius* using carlina oxide

Simona Tortorici^a, Stefano Bedini^b, Anita Casadei^b, Marco Onofrio Pistillo^c, Fabrizio Lapenda^c, Ilaria D'Isita^c, Riccardo Petrelli^d, Giulia Bonacucina^d, Diego Romano Perinelli^d, Marta Ferrati^d, Eleonora Spinuzzi^d, Angelo Canale^b, Salvatore Giacinto Germinara^c, Filippo Maggi^{d,*}, Giovanni Benelli^b, Roberto Rizzo^a

^a CREA - Research Centre for Plant Protection and Certification, Palermo 90145, Italy

^b Department of Agriculture, Food and Environment, University of Pisa, Pisa 56124, Italy

^c Department of Agricultural Sciences, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Foggia 71122, Italy

^d Chemistry Interdisciplinary Project (ChIP) research center, School of Pharmacy, University of Camerino, Via Madonna delle Carceri, Camerino 62032, Italy

ARTICLE INFO

Keywords:

Meadow spittlebug
Aphrophoridae
Botanical insecticides
EAG
Toxicity
Antifeedant

ABSTRACT

Xylella fastidiosa Wells, a bacterial plant pathogen, represents a threat to many crops all around the world. Unfortunately, no effective treatments are available to reduce the infection and, to date, the most promising strategy relies on controlling the meadow spittlebug *Philaenus spumarius* L. one of the main vectors of *X. fastidiosa*. Among insecticides and repellents, botanical insecticides represent valuable candidates. Their encapsulation into stable formulations, like nanoemulsions (NEs), can boost the efficacy and stability. We investigated the toxicity, repellent, and antifeedant activity of carlina oxide, a polyacetylene isolated from the roots of *Carlina acaulis* L. (Asteraceae), and its NEs, against *P. spumarius* adults. In addition, we carried out electroantennographic (EAG) tests to evaluate the capability of the male and female antennae to perceive carlina oxide. EAG assays demonstrated that *P. spumarius* antennal olfactory system of both sexes is capable of perceiving carlina oxide over a wide range of doses and that females, at the lowest doses, have significantly greater olfactory sensitivity than males (0.01 and 0.1 µg, respectively ($p < 0.001$)). In toxicity experiments, the percentage of adult survival in topical and fumigant trials was high, while it resulted significantly lower in ingestion trials. In olfactory tests, *P. spumarius* showed a slight preference for green beans treated with 1 % carlina oxide. However, no significant differences were observed between treatments when the concentration of carlina oxide was increased to 3 %. On the contrary, feeding tests showed a significant repellent effect of carlina oxide against *P. spumarius* adults up to 24 h after the treatment and a reduction in *P. spumarius* presence on treated green beans ranging from 51.2 % to 94.7 %, if compared with control green beans. Overall, this study sheds light on the possible development of effective and environmentally friendly formulations of carlina oxide to manage *P. spumarius*. Further studies are needed to evaluate the efficacy of this natural compound in field conditions, and to investigate its potential side effects on non-target species.

1. Introduction

Xylella fastidiosa Wells, Raju, Hung, Weisburg, Parl & Beemer (Xanthomonadaceae) is a gram-negative bacterium encompassing many subspecies, widespread all around the world in more than 40 countries (EPPO, 2024) and transported by many insect vectors (Cornara et al., 2018). In Italy, the first report was recorded in Salento, Apulia region, in 2013 (Saponari et al., 2013) and the bacterium was first identified

thanks to leaf scorch exhibited in oleander, almond, and olive trees. This pathogen is known to cause severe direct damage to host plants until their death (Saponari et al., 2017). In fact, there are more than 550 plant species all over the world, commercial and wild, susceptible to this bacterium (EFSA, 2024). Due to the high severity of the plant damage it causes and the large number of host species, this pathogen represents a threat to economically important crops and to the environment (Ali et al., 2021; Schneider et al., 2021). Unfortunately, no treatments are

* Corresponding author.

E-mail address: filippo.maggi@unicam.it (F. Maggi).

<https://doi.org/10.1016/j.indcrop.2024.119923>

Received 31 July 2024; Received in revised form 18 October 2024; Accepted 24 October 2024

0926-6690/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

available against this bacterium. However, control strategies targeting the insect vector population can limit the insect from accessing the infective plants and prevent subsequent pathogen inoculation (Lago et al., 2022). The meadow spittlebug *Philaenus spumarius* L. (1758) (order Hemiptera, superfamily Cercopoidea, family Aphrophoridae) is one of the main vectors of *X. fastidiosa* (Cornara et al., 2018) and it is widely distributed all around the world, covering most of the Palearctic regions until Nearctic, especially in the most temperate areas (Godefroid et al., 2021). *Medicago sativa* L. (1753), *Trifolium* spp. L., *Vicia* spp. L., and *Xanthium strumarium* L. (1753) are the main host plants (Thompson et al., 2023). However, the meadow spittlebug has a wide range of hosts belonging to more than 100 families, which probably represents the reason why this pest has a high world distribution (Cornara et al., 2018; Santoiemma et al., 2019). This insect, as nymph and adult, ingests a high amount of xylem sap by feeding on the young parts of the plant and contemporarily acquires *X. fastidiosa*, becoming a harmful vector. Purcell (1981) asserted that the probability of transmission of the bacterium depends on four factors: the vector infectivity, the transmission efficiency, the number of vectors, and the time they spent on the host. Interfering and decreasing the number of vectors and the time that they spend on the host, represent effective strategies to control this insect and, consequently, the bacterium.

In this framework, the control of *P. spumarius* has been focused on synthetic insecticides. In particular, the main effective insecticides employed against *P. spumarius* belong to the pyrethroid and neonicotinoid classes. However, the recent restrictions on the use of neonicotinoids and the poor effectiveness of pyrethroids along with the resistance insurgence make the available control methods against this pest extremely weak (Lago et al., 2022). Therefore, the control of this highly polyphagous insect requires an integrated pest management (IPM) strategy. The use of tools based on alternative and effective substances with repellent or toxic activity, for instance those based on botanical derivatives, is extremely encouraged for their promising effectiveness (Bedini et al., 2024; Dongiovanni et al., 2018). Specifically, the use of essential oils (EOs) or botanically derived compounds may contribute to the development of innovative tools and alternative approaches to the use of synthetic pesticides (Ganassi et al., 2020; Gondwal et al., 2024; Rizzo et al., 2020). However, these products usually need to be formulated to be efficiently delivered and to reduce the health and environmental impact (An et al., 2022; 2017). In this regard, nanoemulsions (NEs) play a crucial role in agriculture and they are considered the most promising nano-systems for pest control purposes given their particle nanometric size and simplicity of formulation and handling (Rizzo et al., 2021). According, a highly promising NE is that based on the polyacetylene carlina oxide. This compound is extracted from *Carlina acaulis* L. (Asteraceae) plant roots, whose concentration overcomes 97 % of the overall chemical composition and is the primary cause of the EO efficacy (Benelli et al., 2021; Spinozzi et al., 2023). Carlina oxide exhibited good antimicrobial and antiviral potential as reported in previous studies (Rosato et al., 2021; Saraliev et al., 2022; Wnorowska et al., 2022). In addition, *C. acaulis* EO and extracts showed a reduced toxicity on fibroblasts, enterocytes, and zebrafish embryos, a limited effect on non-target terrestrial and aquatic organisms, and low toxicity on rats by oral administration (Benelli et al., 2022; Wnorowska et al., 2024). Based on the above, the present study was focused on the evaluation of carlina oxide and its formulated form (NE) as toxic, repellent, attractive and antifeedant against *P. spumarius* adults. The use of the NE was evaluated in the bioassays with direct toxicity (ingestion, topical and fumigant) to better understand the stability of encapsulated carlina oxide, exposed to external factors (e.g. plant and insect body contact), so that it could have more adaptability as potential field bioinsecticide.

2. Materials and methods

2.1. Plant laboratory rearing

The fava bean plants (*Vicia faba* L. var. 'Reina mora' (Semillas Fitó Italia®)) used in the ingestion, topical and fumigant experiments were cultivated at CREA - Research Centre for Plant Protection and Certification (CREA-DC), Palermo, Italy. Seeds were sown and grown in 0.5 L pots (10 × 10 × 10 cm) with peat, topsoil (OmniaVitaflor®, VitaFlor, Italy), and expanded vermiculite (VIC, Italiana®) and were placed in fine mesh net cage (90 × 70 × 200 cm) to prevent infestations by undesirable insect species. The cage was placed at 25 ± 2°C and 50 ± 10 % relative humidity (RH) and 15:9 (L:D) photoperiod. Water fertilization was done once every two weeks with Foliar Gold® (Hydro Fert, Italy) and no pesticides were used. Plants of about 60 cm in height and with at least 7 true sprouts were used for experiments and *P. spumarius* rearing.

2.2. Insect laboratory rearing

The insect laboratory colony for ingestion, topical and fumigant experiments was started with the collection of foams with young instars of *P. spumarius* from March to June in the eastern Sicilian wild field (Catania, Italy). The stock culture was maintained at the CREA-DC in screened cages with fine mesh (45 × 45 × 45 cm) and placed in a chamber with controlled conditions of temperature (24 ± 1°C) and relative humidity (50 ± 10 %) with 16:8 (L:D) of photoperiod until adult emergence. Once a week, fresh new green beans and fava bean plants were replaced as feeding substrate. The newly emerged coetaneous adults were used for the experimental activities.

2.3. Extraction of carlina oxide

Carlina acaulis dry roots, obtained by Minardi & Figli S.r.l. (Bagnacavallo, Ravenna, Italy; <https://www.minardierbe.it>; batch no C-230223–31), were employed for the isolation of carlina oxide through hydrodistillation, according to a procedure already reported (Rizzo et al., 2024). Once extracted, carlina oxide was stored at - 20°C until bioassays. Carlina oxide was obtained with a yield of 0.82 % (w/w) and a purity of 99.2 %, as determined by GC-MS analysis (Fig. 1). Its structure was confirmed by the combination of MS and NMR analyses that were in line with those previously reported (Benelli et al., 2019).

2.4. Preparation and characterization of tested carlina oxide nanoemulsions

Carlina oxide was encapsulated into NE by following the same procedure reported by Tortorici et al. (2024). The formulation included a wetting agent (Agrozoofarma S.r.l.), Polysorbate 80, as surfactant, and ethyl oleate, as oil-cosolvent, in the amount required to have final concentrations of 0.1, 2, and 1 % (w/w), respectively. The required amount of carlina oxide to reach the concentration of 3 % (w/w) in the final formulations was solubilized in ethyl oleate. Afterward, the oil phase was emulsified with an aqueous solution of surfactant and the wetting agent by high-speed stirring (Ultraturrax T25 basic purchased from IKA® Werke GmbH and Co.KG, Staufen, Germany) at 13,500 rpm for 5 min. The obtained emulsion was processed in a 2L-Ultrasound Extractor U2020 (170 W, 230 V, 50 Hz), purchased from Albrigi Luigi S.r.l. (Verona, Italy) for 40 min. The high power + homogenization (H + M) program was selected to reduce the droplet size of the oil phase. A control NE was prepared by substituting the amount of carlina oxide with water and following the same procedure. The particle size of the dispersed phases of 3 % carlina oxide and control NE was assessed through a dynamic light scattering device (Zetasizer nanoS, Malvern Instrument, Malvern, UK) by using the same procedure of Benelli et al. (2020). The formulations were stored at 4°C protected from light in tight closed vials. The stability was evaluated by reading-through the mean

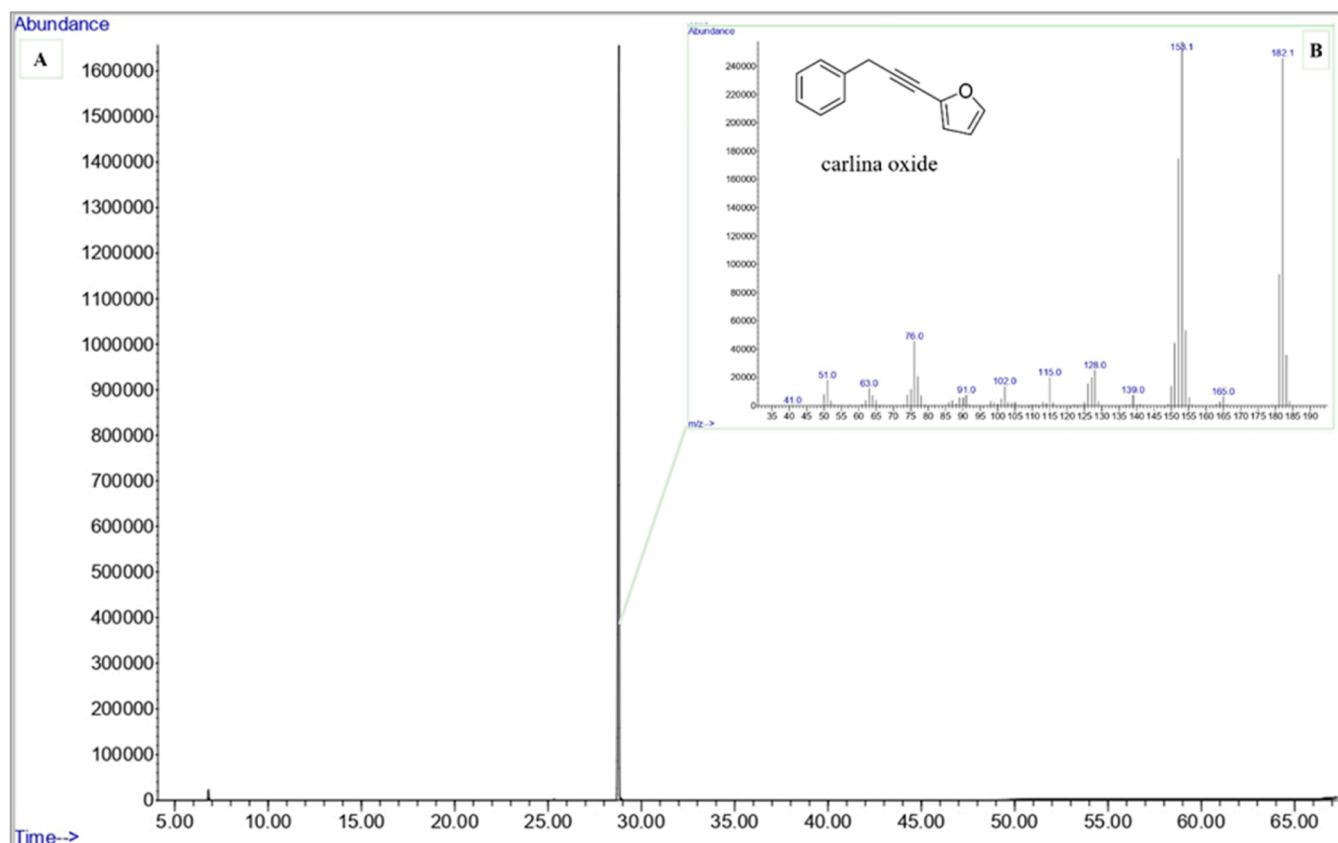


Fig. 1. GC-MS chromatogram (A) and mass spectrum (B) of carlina oxide.

droplet size (Z-average) and polydispersity index (PDI) over a period of 3 months.

2.5. Electroantennographic (EAG) recordings

The antennal chemoreceptivity of *P. spumarius* males and females to increasing concentrations of carlina oxide was evaluated using the EAG technique described in previous studies (Ganassi et al., 2020; Germinara et al., 2017). Briefly, the head of the insects was dissected and the distal half of the arista was removed. A glass pipette filled with Kaissling saline (Kaissling and Thorson, 1980) was inserted into the base of the head as the indifferent electrode. The tip of the amputated arista was put in contact with the end of a similar pipette (0.1 mm diameter) serving as the recording electrode. AgCl-Coated silver wires were used to maintain the electrical continuity between the antennal preparation and an AC/DC UN-6 amplifier in DC mode connected to a PC equipped with the EAG 2.0 program (Syntech Laboratories, Hilversum, The Netherlands). Stimuli were puffed by a disposable syringe into a constant stream (500 mL/min) of charcoal-filtered humidified air flowing in a stainless-steel delivery tube (1 cm diameter) with the outlet positioned approximately 1 cm from the adaxial surface of the antenna. During the experiment, 2.5 cm³ of vapor from an odour cartridge was added over 1 sec. Stimuli were 10 μ L of decimal mineral oil (Sigma-Aldrich, Milan, Italy) solutions of carlina oxide presented at increasing concentrations from 0.001 to 100 μ g/ μ L providing the 0.01, 0.1, 1.0, 10, 100, 1000 μ g dose, respectively. Control (10 μ L of mineral oil) and standard stimuli (10 μ L of a 1 μ g/ μ L (Z)-3-hexenol solution) were applied at the start of the experiment and after every 3 carlina oxide test solutions. There was a 30 s interval between stimuli. EAG responses were recorded from 5 antennae of different males and females.

2.6. Two-choice behavioral response bioassay

The olfactory responses of *P. spumarius* adults to carlina oxide were evaluated in the still-air olfactometer described by Carpita et al. (2012). A chamber contained a segment of organic green bean (Almaverde bio Italia S.r.l. Cesena, Italy) (1 cm) treated with *n*-hexane (control) and the other chamber contained a similar segment of green bean treated with carlina oxide. The carlina oxide tested concentrations were 1 and 3 %. The beans were dipped for 1 s in a solution of carlina oxide dissolved in *n*-hexane or *n*-hexane only and let drying for 1 min. For each test, the beans (treated and non-treated) were placed into the olfactometer lateral chambers and an unsexed *P. spumarius* adult was gently placed on the floor of the release chamber using tweezers. Then, the top of the arena was closed. The choice was considered valid when *P. spumarius* entered one of the later chambers after at least 20 s and stayed in it for at least 30 s. The total observation time was 360 s. Insects that were not able to choose after 360 s were discarded. Each *P. spumarius* was tested only once. For each concentration of carlina oxide, 40–60 unsexed field-collected adults of *P. spumarius* were tested. The test was replicated until at least 30 valid choices were obtained. All experiments were conducted at 25 \pm 2°C and 65 % RH and lit only with a daylight fluorescent tube (Philips 30 W/33, 10.000 lux) placed over the arena. After each tested insect, the arena was rotated 90° clockwise to avoid positional biases and the green beans were renewed. After four consecutive tests, the arena and the glass panels were wiped with hexane, washed with warm water and mild soap, rinsed first with hot water for about 30 s, then with distilled water, and, eventually, dried. After the evaluation, each insect specimen was placed in individual vials, stored at -20°C and subsequently sexed.

2.7. Antifeeding bioassay

The antifeeding bioassay was performed into nylon net cages measuring 47.5 cm × 47.5 cm × 93 cm (BugDorm-4M4590 Insect Rearing Cage, MegaView Science Co., Ltd., Taiwan). A bunch of ten green beans was placed at each of the four corners of the cage about 10 cm from the edge. The cups (two cups with treated and two with untreated green beans) were placed, in the corners, alternating treated and non-treated green beans cups. The green beans were placed in cups containing water-soaked cotton wool. The surface of the green beans was treated by immersion in a 3 % carlina oxide ethanol solution (treated) or ethanol only (control). The cages were arranged under fluorescent lamps, to provide even lighting (light intensity around 14 lux at the cages) and were maintained at 23°C and approximately 65 % RH. To maintain humidity inside the cage, a beaker (500 mL) containing water-soaked cotton wool was positioned in each cage. The experiment was replicated five times. 40–60 unsexed adults (depending on field captures) were placed in each cage. Specifically, the number of insects was 40, 60, 55, 40, and 44 for cages 1, 2, 3, 4, and 5, respectively. The number of insect specimens on treated and untreated green beans was recorded after 1, 6, 12, and 24 h. The different number of specimens in the cage was normalized expressing the number of insects on the treated and untreated green beans as a percentage of the total number of insects in each cage after checking the effect of the population size ($F_{2,30} = 2.067$, $p = 0.342$).

2.8. Ingestion toxicity bioassay

Ingestion toxicity test was performed to assess the toxic effect of carlina oxide NE on *P. spumarius* adults. Healthy fava bean sprouts were dipped for 3 s in the NE at 3 % and let drying in laboratory conditions for 30 min. The same procedure was performed for the negative control (distilled water) and for the control NE without carlina oxide. The treated sprouts were put in a 2 mL Eppendorf with distilled water and sealed with Parafilm® (Bemis™) to avoid desiccation. In the experimental arena, composed of a ventilated plastic box (175 × 120 × 70 mm) and the treated sprouts, a single unsexed *P. spumarius* adult was placed inside with a mouth aspirator. The experimental arenas were in a climatic chamber with controlled environmental conditions of 24 ± 1°C, 50 ± 10 % relative humidity (RH) and with 16:8 (L:D) of photoperiod. Adult survival (%) was checked at 24, 48 and 72 h after the release of the insects. For each treatment 30 replicates (boxes), for a total number of 30 *P. spumarius* adults, were performed.

2.9. Topical toxicity bioassay

The toxic effect of carlina oxide NE was evaluated also through topical treatment on *P. spumarius*. Untreated adults were collected from laboratory rearing and put at -4°C for 80 s. A total amount of 0.2 µL of NE at 3 % was released on the insect pronoto with a micropipette and was let drying for 30 min. The same treatment was performed with the negative control and the control NE without carlina oxide. Subsequently, the treated insects were moved with a mouth aspirator and placed on fava bean sprouts arranged in the ventilated plastic box as described above. The experimental arenas were in a climatic chamber with controlled environmental conditions of 24 ± 1°C, 50 ± 10 % RH and with 16:8 (L:D) of photoperiod. Adult survival (%) was recorded at 24, 48 and 72 h after the release. For each treatment, three replicates (boxes), for a total number of 30 *P. spumarius* adults, were performed.

2.10. Fumigant activity bioassay

Fumigant effect of carlina oxide NE was assessed on *P. spumarius* adults. NE at 3 % (216 µL) was released on a filter paper square (2 × 2 cm) (Whatman® qualitative filter paper, Grade 1) and let drying for 2 h. The same procedure was followed for the negative control and the

control NE without carlina oxide. The experimental arena, as described above, was a sealed plastic box (175 × 120 × 70 mm) where the treated filter paper was placed inside a closed aerated petri dish, glued to the bottom of the plastic box, to prevent the direct contact with the insect. Inside the box, a fava bean sprout and a single unsexed *P. spumarius* adult were placed. Adult survival (%) was checked every 24, 48, and 72 h after the release. The experimental arenas were in a climatic chamber with controlled environmental conditions of 24 ± 1°C, 50 ± 10 % RH, and with 16:8 (L:D) of photoperiod. For each treatment 30 replicates (boxes), for a total number of 30 *P. spumarius* adults, were performed.

2.11. Data analysis

EAG responses were evaluated by measuring the maximum amplitude of negative polarity deflection (-mV) elicited by a stimulus (Germinara et al., 2019). The absolute value of the EAG amplitude (mV) to each test stimulus was adjusted to compensate for solvent and/or mechanosensory artifacts by subtracting the mean EAG response of the two nearest mineral oil controls (Pistillo et al., 2022). The resulting EAG amplitude was corrected according to the reduction of the EAG response to the standard, to compensate for the diminishing antennal sensitivity throughout the experiment (Den Otter et al., 1991).

The corrected EAG responses to each carlina oxide concentration were compared with a baseline value of “0” using the Wilcoxon rank sum test and considered measurable if significant at $p = 0.05$. For each carlina oxide concentration, the mean male and female EAG responses were compared using the Student’s t-test for independent samples. In dose-response experiments, the first dose at which the mean EAG response was higher than a “0” value using the Shapiro Wilk test for normality followed by the one-sample Student’s t test ($p = 0.05$) was considered as the activation threshold (Germinara et al., 2017); the saturation threshold was defined as the lowest dose at which the mean response was equal to or less than the preceding dose (Germinara et al., 2016).

In the two-choice behavioural response bioassay, the proportion of individuals choosing the carlina oxide-treated chamber was compared using a likelihood chi-square test, assuming a null hypothesis of a 50:50 chance of insects choosing the control chamber versus the carlina oxide-treated chamber.

Antifeeding bioassay data were analyzed by a Repeated Measurement ANOVA (RM-ANOVA). The analysis aimed to assess differences in the abundance of *P. spumarius* feeding on green beans treated with the carlina oxide and those feeding on control beans, with time as the repeated measure, treatment (treated or non-treated) as the between-subjects factor, and percentage of insects on treated and untreated green beans as the dependent variable. The Greenhouse-Geisser correction was applied for sphericity. Differences between estimated marginal means for the treated and control beans were tested using Bonferroni pairwise comparison procedure. For each time of observation, differences between treatments were checked by Student’s t-test.

Data from ingestion, topical, and fumigant toxicity were checked through Levene test for homogeneity and normality of the dependent variables whenever needed. Data were evaluated through GLM univariate followed by Tukey’s HSD post hoc tests when suitable ($p \leq 0.05$) for multiple mean comparisons among treatments.

Statistical analyses were carried out using SPSS 25.0 software (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Characterization of tested carlina oxide nanoemulsion

The DLS measurements of carlina oxide NE revealed that Z-average size of oil droplets was within the nanometric range. Specifically, nanodroplets showed a size distribution centred at 157.7 ± 4.4 nm

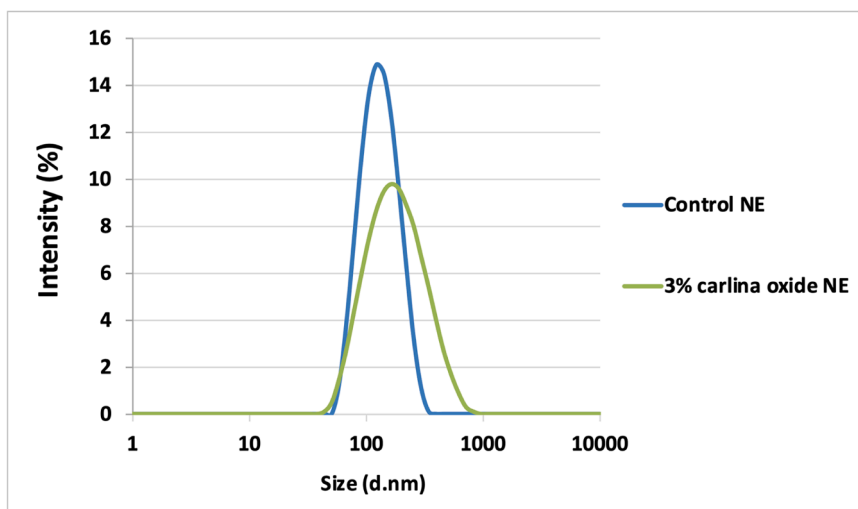


Fig. 2. Particle size distribution (Intensity, %) of 3% (w/w) carlina oxide and control nanoemulsions (NEs), obtained through dynamic light scattering (DLS).

(Fig. 2).

The mean values of Z-average size and PDI obtained from the DLS measurements of 3% (w/w) carlina oxide NE at different time points have been reported in Fig. 3. The grade of polydispersity was determined by checking the PDI and a value of 0.22 ± 0.01 was obtained. The physical stability of the formulation was determined by monitoring their Z-average size and PDI at 0 (T₀), 15 (T₁), 30 (T₂), 60 (T₃), and 90 (T₄) days of storage. Z-average of 3% (w/w) carlina oxide NE remained almost similar over the first 2 months of storage (around 160 nm) and slightly increased by only 20 nm in the T₄ time point (182.3 ± 1.14 nm) (Fig. 3a). Regarding the PDI, values obtained at different time points remained almost unchanged for the formulation, bearing out its physical stability for at least 3 months (Fig. 3b).

3.2. EAG recordings

The EAG responses of *P. spumarius* males and females to increasing concentrations of carlina oxide are shown in Fig. 4. All the concentrations elicited measurable EAG responses in both sexes ($p < 0.05$ in all Wilcoxon rank sum tests). In the range of dose tested, the mean EAG response varied from 0.004 ± 0.001 – 0.672 ± 0.032 mV in males and from 0.064 ± 0.009 – 0.632 ± 0.0729 mV in females. The mean EAG response of females was significantly higher than that of males at doses of 0.01 ($t = 6.794$; $df = 8$; $p < 0.001$), 0.1 ($t = 14.994$; $df = 8$; $p < 0.001$), 10 ($t = 2.655$; $df = 8$; $p < 0.029$) and 100 μg ($t = 2.772$; $df = 8$; $p < 0.024$) of carlina oxide.

In dose-response curves, the antennal activation threshold was

0.01 μg in females and 1 μg in males. The EAG responses of males and females at the highest dose tested (1000 μg) were higher than those at the previous dose (100 μg), indicating that saturation of the antennal olfactory receptors was not reached in the range of doses tested.

3.3. Two-choice behavioural response bioassay

At the concentration of 1% the green beans treated with carlina oxide were slightly more attractive than the non-treated ones ($\chi^2 = 3.920$, $p = 0.048$). Analyzing the responses of males and females separately, it appears that the attractive activity was exerted mainly toward females ($\chi^2 = 4.122$; $p = 0.042$) rather than towards males ($\chi^2 = 0.000$; $p = 1.000$). No significant difference between treatments was observed at 3% of carlina oxide concentration ($\chi^2 = 0.133$; $p = 0.715$) (Fig. 5).

3.4. Antifeeding bioassay

The RM-ANOVA indicated a significant effect of time (1, 6, 12, and 24 h) on the number of insects feeding on the treated or control green beans ($F_{2,15,6} = 7.360$, $p = 0.004$) and a significant antifeeding effect of the carlina oxide treatment ($F_{1,8} = 309.435$, $p < 0.001$), with estimated marginal means of 4.2 and 25.6% of insects feeding on treated and non-treated green beans, respectively (Bonferroni pairwise comparison, $p < 0.001$) (Fig. 6).

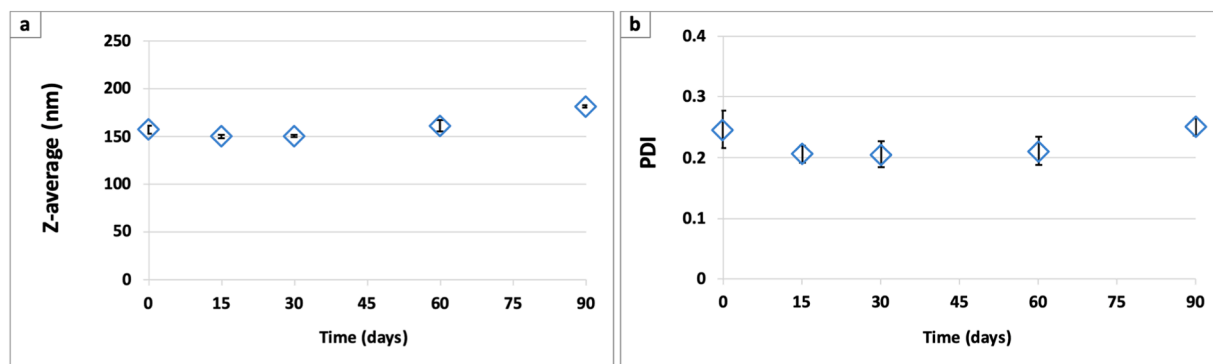


Fig. 3. Z-average and PDI of 3% (w/w) carlina oxide nanoemulsion obtained through dynamic light scattering (DLS) at 0 (T₀), 15 (T₁), 30 (T₂), 60 (T₃), and 90 (T₄) days of storage.

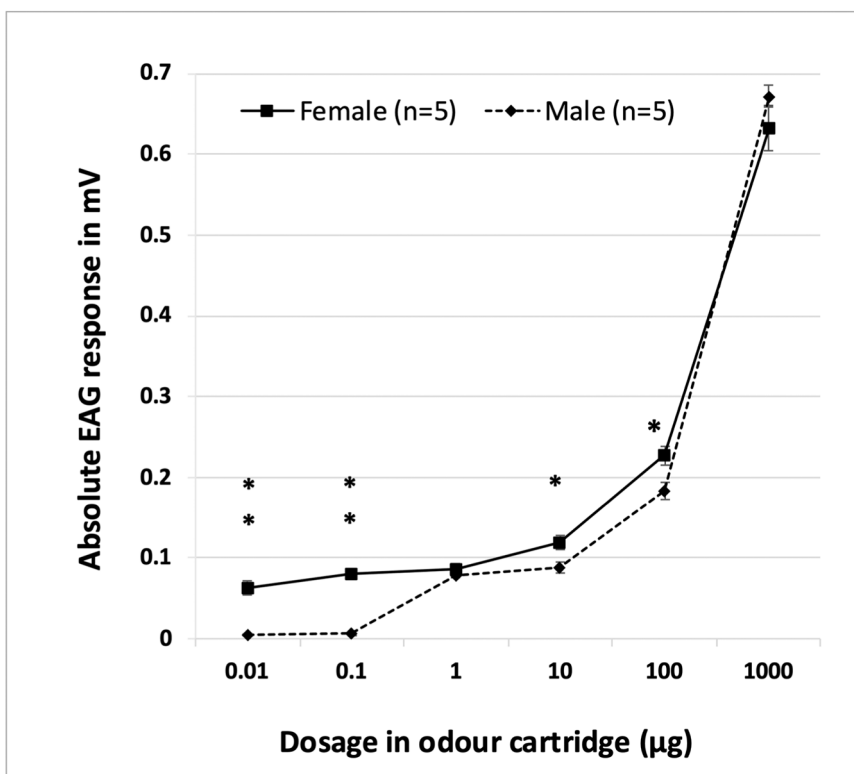


Fig. 4. EAG dose-response curves (Mean ± SE) of male and female of the meadow spittlebug *Philaenus spumarius* to increasing doses of carlina oxide. n = number of insects tested. Asterisks indicate significant differences between sexes. *, $p \leq 0.05$; **, $p \leq 0.01$ according to Student's *t*-test.

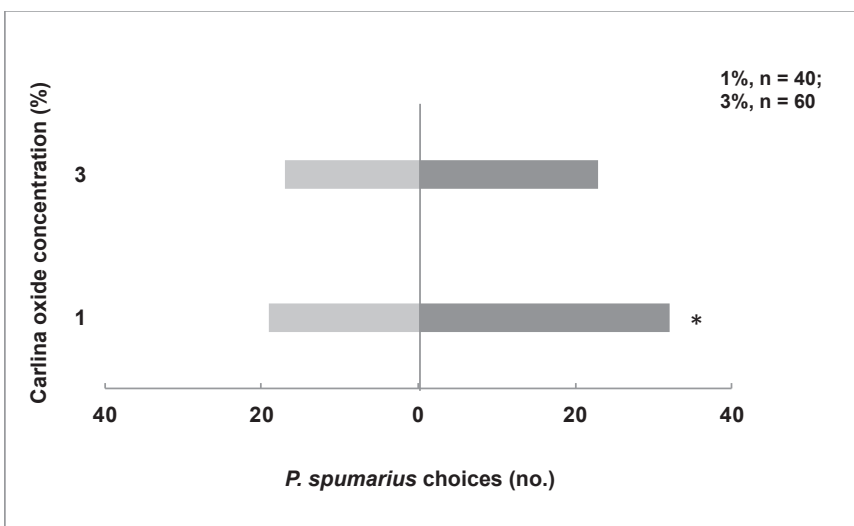


Fig. 5. Behavioral responses of the meadow spittlebug *Philaenus spumarius* adults exposed to green beans treated or not treated with carlina oxide (1 and 3 %) in the two-choice olfactometric tests. Light grey bars: number of insects choosing the control chamber; dark grey bars: number of insects choosing the carlina oxide-treated beans chamber (chi-square test, $p < 0.05$). n = number of tested insects.

3.5. Ingestion toxicity bioassay

Carlina oxide NE at 3 % demonstrated a toxic effect after 72 h ($F_{2,30} = 42.695, p < 0.001$) in an ingestion toxicity experiment (Fig. 7). High adult mortality was observed only in carlina oxide NE treatment.

3.6. Topical toxicity bioassay

Topical toxicity results indicated that 3 % carlina oxide NE was scarcely effective till 72 h post-treatment if compared with positive and

negative controls ($F_{2,30} = 2.071, p = 0.132$) (Fig. 8). No significant reduction in adult survival was recorded after 72 h.

3.7. Fumigant toxicity bioassay

Fumigant toxicity results indicated that 3 % carlina oxide NE was scarcely effective till 72 h post-treatment if compared with positive and negative controls ($F_{2,30} = 1.218, p = 0.301$) (Fig. 9). No significant reduction in adult survival was recorded after 72 h.

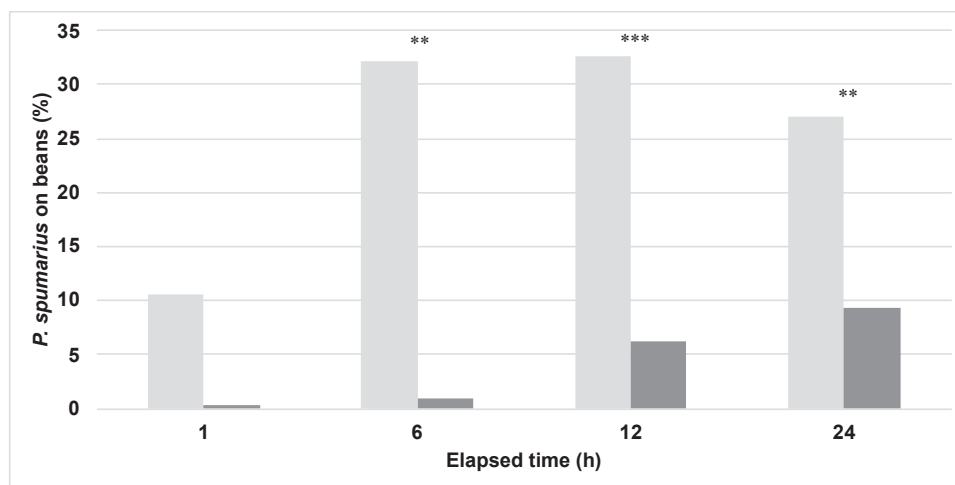


Fig. 6. Percentage of the meadow spittlebug *Philaenus spumarius* adults feeding on non-treated (light gray) and carlina oxide-treated green beans (3%) (dark gray) after 1, 6, 12, and 24 h, $n = 40\text{--}60$ spittlebugs (see Materials and methods for details). Asterisks indicate significant differences between treatments for each time. **, $p \leq 0.01$; ***, $p \leq 0.001$ according to Student's t-test.

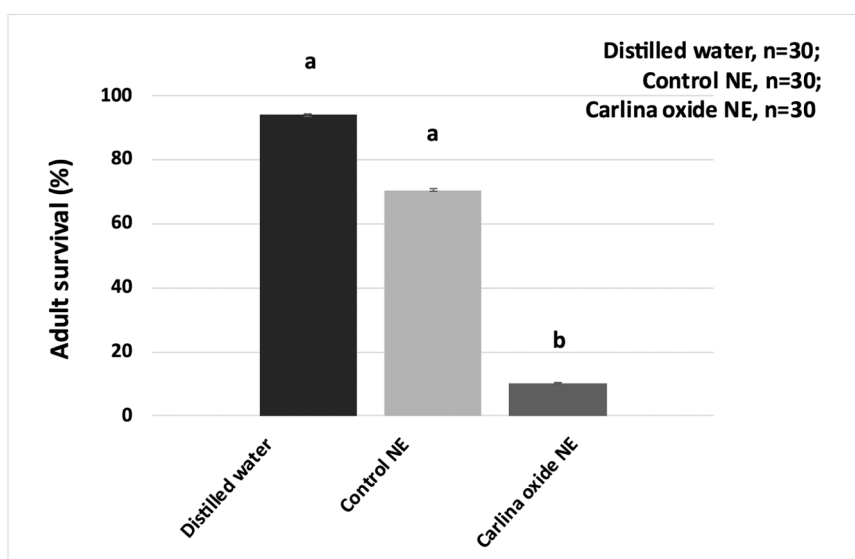


Fig. 7. Meadow spittlebug *Philaenus spumarius* adult survival (%) in ingestion toxicity bioassays after 72 h testing 3% carlina oxide nanoemulsion (NE). $n =$ number of tested insects. Vertical bars indicate SE. Different letters indicate significant differences at $p < 0.05$ (GLM univariate, Tukey's HSD post hoc).

4. Discussion

Nanoemulsions represent one of the most explored encapsulation strategies to incorporate EOs and natural compounds for insecticidal formulations (Badawy et al., 2022; Jesser et al., 2020; Sharma et al., 2020). Indeed, the effectiveness of EO NEs was assessed on many agricultural and stored product pests, such as *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Tortorici et al., 2024), *Xilosandrus compactus* (Eichhoff) (Coleoptera: Curculionidae: Scolytinae) (Gugliuzzo et al., 2023), *Oryzaephilus surinamensis* (Linnaeus) (Coleoptera: Silvanidae) (Gharsan et al., 2022), *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae) (Louni et al., 2018) and *Aphis gossypii* Glover (Homoptera: Aphididae) (Heydari et al., 2020) with encouraging results. The EO NEs guarantee a long-lasting stability and improved efficiency by overcoming the bottleneck of these products such as EO volatility and poor water solubility, as well as favouring crop leaf diffusion and adhesiveness (Pavoni et al., 2020). Carlina oxide has been previously encapsulated into NE through two different high-energy methods, i.e., high-pressure homogenization and sonication (Benelli et al., 2020; Kavallieratos et al., 2022;

Ntalli et al., 2023; Pavela et al., 2021; Tortorici et al., 2024). Both methodologies were demonstrated to be suitable to achieve the encapsulation of these compounds and to obtain a particle size of the dispersed phase within the nanometric range. Herein, ultrasounds were selected to favour the reduction of the oil droplet size of the carlina oxide NE and control NE. In fact, this methodology usually ensures higher stability and smaller size of droplets and demands for a lower energy input with respect to other high energy methods, such as high-pressure homogenization and microfluidification (Kumar et al., 2019). Tortorici et al. (2024) successfully developed 0.25 and 0.50% w/w carlina oxide NEs by using ultrasounds. The higher concentration of the main ingredient employed in this study did not affect the formation and stability of the NE. Moreover, in this case, a wetting agent was included to favour the adhesion of the formulation onto the leaves. Neither this ingredient negatively influenced the formation and the stability of NEs. In fact, values obtained for Z-average and PDI of 3% carlina oxide NE were stable over time for a period at least of three months.

These excellent results obtained in terms of particle size distribution and long-lasting stability of the NE allowed us to evaluate its effect on

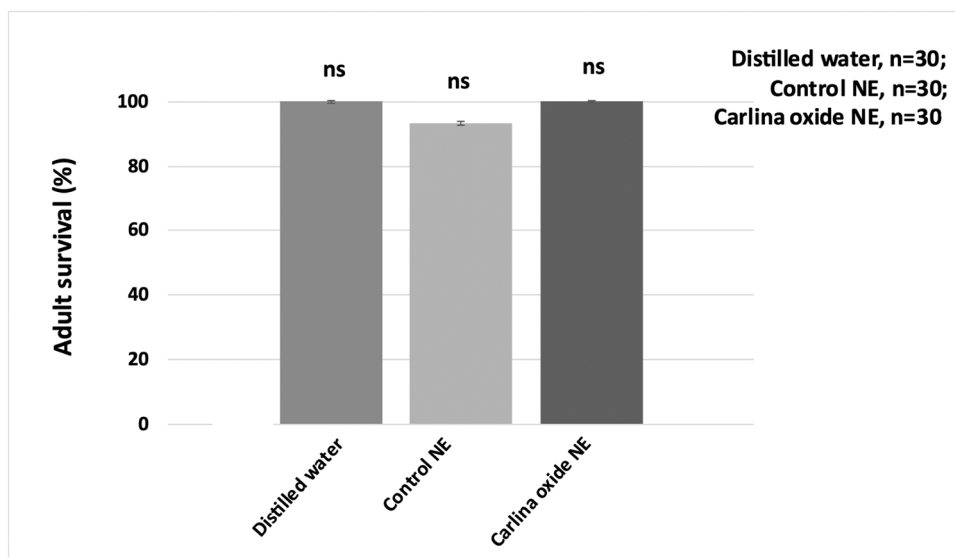


Fig. 8. Meadow spittlebug *Philaenus spumarius* adult survival (%) in topical toxicity bioassays after 72 h testing 3 % carlina oxide nanoemulsion (NE). n = number of tested insects. ns = not significant (GLM univariate). Vertical bars indicate SE.

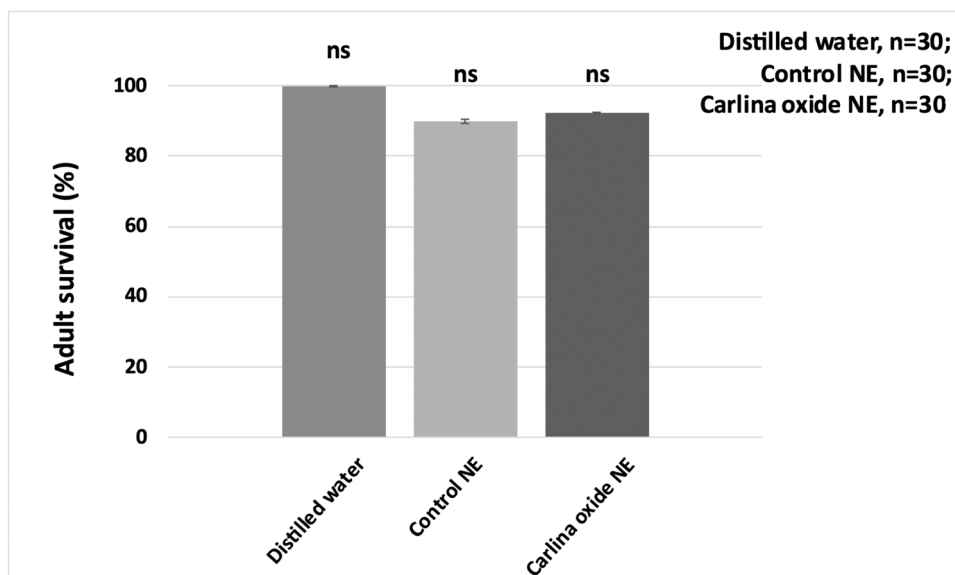


Fig. 9. Meadow spittlebug *Philaenus spumarius* adult survival (%) in fumigant toxicity bioassays after 72 h testing 3 % carlina oxide nanoemulsion (NE). n = number of tested insects. ns = not significant (GLM univariate). Vertical bars indicate SE.

P. spumarius in terms of direct toxicity to decrease the insect population and in terms of repellency/attractiveness. In this context, EAG bioassay showed that both male and female peripheral olfactory systems are capable of perceiving carlina oxide in a wide range of doses supporting the possibility that the compound may act as long-distance cue to *P. spumarius* adults. Moreover, a marked higher sensitivity of females compared with males was found mainly at the lowest doses tested. Sexually dimorphic EAG responses in *P. spumarius* have also been reported in a previous study (Germinara et al., 2017) and this could be due to quantitative and/or to qualitative physiological differences in olfaction (Raguso et al., 1996). This likely reflects differences in the role played by the same volatile organic compounds (VOCs) in the ecology of males and females. Ganassi et al. (2020) evaluated the olfactory responses of *P. spumarius* adults towards different EOs and related plants both at close (Y-tube) and long ranges (wind tunnel), observing differences between males and females to the same odour source. Moreover, the two-choice tests in the static-air olfactometer showed that, at close

range, the behavioural response of *P. spumarius* appears to be dependent on the concentration with a different response between sexes. According to Ganassi et al. (2020), in our two-choice behavioural response bioassay, carlina oxide at 1 % exhibited a rather attractive activity that appears to be due to a differential response of the female but not of the male population. This agrees with the results of the EAG bioassay that showed a higher sensitivity of females compared with males. On the contrary, at 3 %, no repellency or attractiveness was observed. The lack of a clear effect at the higher concentration could be due to the saturation of the confined environment of the olfactometer by the carlina oxide, resulting in a confused perception of the cue by the insect. The attractive effect of carlina oxide observed in the two-choice behavioural response bioassay was, however, in contrast with the results of the antifeeding experiment. In a more open environment of the cages, the presence of *P. spumarius* on green beans was significantly reduced by the carlina oxide treatment (3 %) for up to 24 h. Here we observed a clear repellent effect of carlina oxide against *P. spumarius* adults, with

repellency remaining significant for up to 24 h, and resulting in a reduction of the presence of *P. spumarius* on the green beans. The different effects observed in the two experiments may be due to the different experimental systems utilized and/or the different chemical compositions of the botanicals tested. The results obtained in the bioassays of toxicity by ingestion, topical and fumigant treatments, with a significant toxic effect only in ingestion trials, showed that the mode of action of carlina oxide towards the adults of *P. spumarius* is different depending of the treatment. Indeed, the ingestion treatment seems to be the most effective probably due to the ability of carlina oxide to sink in the leaf cuticle and entering in the lymphatic vessels (Tortorici et al., 2024). Therefore, the high *P. spumarius* mortality could be due to its sup sucking activity (Horsfield, 1978). Unfortunately, the mechanism of action of carlina oxide is not already known, even if some hypotheses have been advanced. For example, Kavallieratos et al. (2022) supposed the ability of carlina oxide to damage the insect cells by forming carbocations. Moreover, Spinozzi et al. (2023) suggested that carlina oxide could contribute as an inhibitor of the acetylcholinesterase, octopamine receptors, and P450 cytochromes. On the other side, the results obtained in topical and fumigant toxicity showed no significant differences in adult mortality. Similarly, Tortorici et al. (2024) observed no significant efficacy of the compound in topical treatment towards larvae of *T. absoluta*. Therefore, our study confirms that carlina oxide does not act as a topical insecticide. Probably this could be due to its low ability to penetrate the insect exoskeleton (Tortorici et al., 2022). Furthermore, the scarce effectiveness of carlina oxide NE in the fumigant test could be due to the low choking effect of the polyacetylene. Probably the encapsulation of carline oxide into a nanoemulsion reduced its volatility, thus also resulting in a lower intensity of the odorous source and consequently in a failed fumigant effect. It could also depend on the concentration of carlina oxide in the NE. Probably higher concentrations could result in a higher fumigant effect. In future research, assessing the interference of carlina oxide with the transmission of *X. fastidiosa* by *P. spumarius* after treatment would be also of crucial importance. In conclusion, carlina oxide could represent a useful botanical ingredient for the developing of new sustainable and alternative control systems to be used in IPM strategies aimed to reduce *X. fastidiosa* diffusion by controlling *P. spumarius*.

Funding sources

This research was granted by the project COVEXY “Contenimento insetti vettori di *Xylella fastidiosa* con metodi a basso impatto ambientale” (DM 664980, 29.12.2022) supported by the Italian Ministry of Agriculture, Food, Sovereignty and Forestry (MASAF), and by the project PRIN 2022 “Bioformulations for controlled release of botanical pesticides for sustainable agriculture” (Prot. 202274BK9L) supported by the Italian Ministry of University and Research (MUR).

CRedit authorship contribution statement

Salvatore Giacinto Germinara: Writing – review & editing, Methodology, Investigation. **Stefano Bedini:** Methodology, Investigation. **Angelo Canale:** Writing – review & editing. **Simona Tortorici:** Writing – original draft, Methodology, Data curation. **Roberto Rizzo:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition. **Marco Onofrio Pistillo:** Writing – original draft, Methodology, Investigation. **Giovanni Benelli:** Writing – review & editing, Supervision. **Anita Casadei:** Methodology, Investigation. **Filippo Maggi:** Writing – review & editing, Supervision. **Marta Ferrati:** Writing – original draft, Methodology, Data curation. **Diego Romano Perinelli:** Methodology, Data curation. **Eleonora Spinozzi:** Writing – original draft, Methodology, Investigation. **Ilaria D’Isita:** Methodology, Data curation. **Fabrizio Lapenda:** Methodology, Investigation, Data curation. **Giulia Bonacucina:** Writing – review & editing. **Riccardo Petrelli:** Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors wish to thank the company Minardi & Figli S.r.l for providing the raw material for the extraction of carlina oxide and Livia De Fazi for her kind statistical advice.

Data Availability

Data will be made available on request.

References

- Ali, B.M., Van Der Werf, W., Lansink, A.O., 2021. Assessment of the environmental impacts of *Xylella fastidiosa* subsp. *paucis* in Puglia. *Crop Prot.* 142, 105519. <https://doi.org/10.1016/j.cropro.2020.105519>.
- 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>.
- Badawy, M.E., Abd-Elnabi, A.D., Saad, A.F.S., 2022. Insecticidal activity of nanoemulsions of organophosphorus insecticides against cotton leafworm (*Spodoptera littoralis*) and molecular docking studies. *Int. J. Trop. Insect Sci.* 42 (1), 293–313. <https://doi.org/10.1007/s42690-021-00545-0>.
- Bamisaye, A., Adegoke, K.A., Alli, Y.A., Bamidele, M.O., Idowu, M.A., Ogunjinmi, O.E., 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>.
- Bedini, S., Djebbi, T., Ascricchi, R., Farina, P., Pieracci, Y., Echeverría, M.C., Flamini, G., Trusendi, F., Ortega, S., Chilibingua, A., Conti, B., 2024. Repellence and attractiveness: the hormetic effect of aromatic plant essential oils on insect behavior. *Ind. Crops Prod.* 210, 118122. <https://doi.org/10.1016/j.indcrop.2024.118122>.
- Benelli, G., Ceccarelli, C., Zeni, V., Rizzo, R., Lo Verde, G., Sinacori, M., Boukouvala, M. C., Kavallieratos, N.G., 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 pest and its safety to mammals. *Chemosphere* 287, 132089. <https://doi.org/10.1016/j.chemosphere.2021.132089>.
- Benelli, G., Pavela, R., Petrelli, R., Nzekoue, F.K., 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>.
- 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*. *Nanomater* 10 (9), 1867. <https://doi.org/10.3390/nano10091867>.
- Benelli, G., Rizzo, R., Zeni, V., Govigli, A., Samková, A., Sinacori, M., Lo Verde, G., Pavela, R., Cappellacci, L., Petrelli, R., Spinozzi, E., Morshedloo, Reza, Maggi, M., Canale, A., F., 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>.
- Carpita, A., Canale, A., Raffaelli, A., Saba, A., Benelli, G., Raspi, A., 2012. Z)-9-tricosene identified in rectal gland extracts of *Bactrocera oleae* males: first evidence of a male produced female attractant in olive fruit fly. *Naturwissenschaften* 99, 77–81. <https://doi.org/10.1007/s00114-011-0868-y>.
- Cornara, D., Bosco, D., Ferreres, A., 2018. *Philaenus spumarius*: when an old acquaintance becomes a new threat to European agriculture. *J. Pest Sci.* 91, 957–972. <https://doi.org/10.1007/s10340-018-0966-0>.
- Dongiovanni, C., Altamura, G., Di Carolo, M., Fumarola, G., Saponari, M., Cavalieri, V., 2018. Evaluation of efficacy of different insecticides against *Philaenus spumarius* L., vector of *Xylella fastidiosa* in olive orchards in southern Italy, 2015–17. *Arthropod Manag. tests.* 43 (1). <https://doi.org/10.1093/amt/tsy034>.
- EFSA (Autorità Europea per la Sicurezza Alimentare). <https://www.efsa.europa.eu/it/topics/topic/xylella-fastidiosa> (accessed in April 2024).
- EPPO (European and Mediterranean Plant Protection Organization), 2024. EPPO Global Database, *Xylella fastidiosa* (<https://gd.eppo.int/taxon/XYLEFA/distribution> (accessed in April)) (<https://gd.eppo.int/taxon/XYLEFA/distribution> (accessed in April)).
- Ganassi, S., Cascone, P., Di Domenico, C., Pistillo, M., Formisano, G., Giorgini, M., Grazioso, P., Germinara, G.S., De Cristofaro, A., Guerrieri, E., 2020. Electrophysiological and behavioural response of *Philaenus spumarius* to essential oils and aromatic plants. *Sci. Rep.* 10, 3114. <https://doi.org/10.1038/s41598-020-59835-1>.

- 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>.
- Germinara, G.S., De Cristofaro, A., Rotundo, G., 2016. Electrophysiological and behavioral responses of *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae) to cereal grain volatiles. *Biomed. Res. Int.*, 5460819 <https://doi.org/10.1155/2016/5460819>.
- Germinara, G.S., Ganassi, S., Pistillo, M.O., Di Domenico, C., De Cristofaro, A., Di Palma, A.M., 2017. Antennal olfactory responses of adult meadow spittlebug, *Philaenus spumarius*, to volatile organic compounds (VOCs). *PLoS ONE* 12, e0190454. <https://doi.org/10.1371/journal.pone.0190454>.
- Germinara, G.S., Pistillo, M., Griffò, R., Garonna, A.P., Di Palma, A., 2019. Electroantennographic responses of *Aromia bungii* (Faldermann, 1835) (Coleoptera, Cerambycidae) to a range of volatile compounds. *Insects* 10, 274. <https://doi.org/10.3390/insects10090274>.
- Gharsan, F.N., Kamel, W.M., Alghamdi, T.S., Alghamdi, A.A., Althagafi, A.O., Aljassim, F. J., Al-Ghamdi, S.N., 2022. Toxicity of citronella essential oil and its nanoemulsion against the sawtoothed grain beetle *Oryzaephilus surinamensis* (Coleoptera: Silvanidae). *Ind. Crops Prod.* 184, 115024. <https://doi.org/10.1016/j.indcrop.2022.115024>.
- Godefroid, M., Morente, M., Schartel, T., Cornara, D., Purcell, A., Gallego, D., Moreno, A., Pereira, J.A., Fereres, A., 2021. Climate tolerances of *Philaenus spumarius* should be considered in risk assessment of disease outbreak related to *Xylella fastidiosa*. *J. Pest Sci.* 1–14. <https://doi.org/10.1007/s10340-021-01413-z>.
- Gondwal, M., Gautam, B.P.S., Shah, G.C., 2024. Essential oils as biorational insecticides. *Biorationals Biopestic. Pest Manag.* 335. <https://doi.org/10.1515/9783111204819-016>.
- Gugliuzzo, A., Francardi, V., Simoni, S., Roversi, P.F., Ferrati, M., Spinozzi, E., Perinelli, D.R., Bonacucina, G., Maggi, F., Tortorici, S., Garzia, Tropea, Biondi, G., 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>.
- 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. R.* 27, 6667–6679. <https://doi.org/10.1007/s11356-019-07332-y>.
- Horsfield, D., 1978. Evidence for xylem feeding by *Philaenus spumarius* (L.) (Homoptera: Cercopidae). *Entomol. Exp. Appl.* 24 (1), 95–99. <https://doi.org/10.1111/j.1570-7458.1978.tb02759.x>.
- Jesser, E., Yeguerman, C., Gili, V., Santillan, G., Murray, A.P., Domini, C., Werdin-González, J.O., 2020. Optimization and characterization of essential oil nanoemulsions using ultrasound for new ecofriendly insecticides. *ACS Sustain Chem. Eng.* 8 (21), 7981–7992. <https://doi.org/10.1021/acsschemeng.0c02224>.
- Kaissling, K.E., Thorson, J.O.H.N., 1980. Insect olfactory sensilla: structural, chemical and electrical aspects of the functional organisation. In *Receptors for Neurotransmitters, Hormones and Pheromones in Insects*. Elsevier/North-Holland Biomedical Press, pp. 261–282 <https://hdl.handle.net/11858/00-001 M-0000-0029-7443-D>.
- Kavallieratos, N.G., Nika, E.P., Skourti, A., Boukouvala, M.C., Ntalaka, C.T., Maggi, F., Spinozzi, E., Petrelli, R., Perinelli, D.R., 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 (6), 2434–2442. <https://doi.org/10.1002/ps.6877>.
- Kumar, R., Soni, P., Siril, P.F., 2019. Engineering the morphology and particle size of high energetic compounds using drop-by-drop and drop-to-drop solvent–antisolvent interaction methods. *ACS Omega* 4 (3), 5424–5433. <https://doi.org/10.1021/acsomega.8b03214>.
- Lago, C., Cornara, D., Minutillo, S.A., Moreno, A., Fereres, A., 2022. Feeding behaviour and mortality of *Philaenus spumarius* exposed to insecticides and their impact on *Xylella fastidiosa* transmission. *Pest Manag. Sci.* 78 (11), 4841–4849. <https://doi.org/10.1002/ps.7105>.
- Louni, M., Shakarami, J., Negahban, M., 2018. Insecticidal efficacy of nanoemulsion containing *Mentha longifolia* essential oil against *Ephesia kuehniella* (Lepidoptera: Pyralidae). *J. Crop Prot.* 7 (2), 171–182 <https://doi.org/10.1007/s10011.1.22519041.2018.7.2.7.2>.
- Ntalli, N., Zochios, G., Nikolaou, P., Winkiel, M., Petrelli, R., Bonacucina, G., Perinelli, D. R., 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>.
- Otter, Den, Tchicaya, C.J., Schutte, T., A.M., 1991. Effects of age, sex and hunger on the antennal olfactory sensitivity of tsetse flies. *Physiol. Entomol.* 16, 173–182. <https://doi.org/10.1111/j.1365-3032.1991.tb00554.x>.
- 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., 2021. 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>.
- Pavoni, L., Perinelli, D.R., Bonacucina, G., Cespi, M., Palmieri, G.F., 2020. An overview of micro-and nanoemulsions as vehicles for essential oils: formulation, preparation and stability. *Nanometer* 10 (1), 135. <https://doi.org/10.3390/nano10010135>.
- Pistillo, M.O., D'Isita, I., Germinara, G.S., 2022. Olfactory response of the spotted asparagus beetle, *Crioceris duodecimpunctata* (L.) to host plant volatiles. *J. Chem. Ecol.* 48, 41–50. <https://doi.org/10.1007/s10886-021-01323-5>.
- Purcell, A.H., 1981. Vector preference and inoculation efficiency as components of resistance to Pierce's disease in European grape cultivars. *Phytopathology* 71, 429–435. <https://doi.org/10.1094/Phyto-71-429>.
- Raguso, R.A., Light, D.M., Pickersky, E., 1996. Electroantennogram responses of *Hyles lineata* (Onagraceae) and other moth-pollinated flowers. *J. Chem. Ecol.* 22, 1735–1766. <https://doi.org/10.1007/BF02028502>.
- Rizzo, R., Pistillo, M., Germinara, G.S., 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 (10), 880. <https://doi.org/10.3390/insects12100880>.
- Rizzo, R., Ragusa, E., Benelli, G., Lo Verde, G., Zeni, V., Maggi, F., Petrelli, R., Spinozzi, E., Ferrati, M., Sinacori, M., Tsolakakis, H., 2024. Lethal and sublethal effects of the polyacetylene carlina oxide on *Tetranychus urticae* (Acari: Tetranychidae) and *Neoseiulus californicus* (Acari: Phytoseiidae). *Pest Manag. Sci.* 80 (3), 967–977. <https://doi.org/10.1002/ps.7827>.
- Rizzo, R., Verde, G.L., Sinacori, M., Maggi, F., Cappellacci, L., Petrelli, R., Vittori, S., Morshedloo, Reza, Fofie, M., Benelli, G., Y.N.'G.B., 2020. Developing green insecticides to manage olive fruit flies? Ingestion toxicity of four essential oils in protein baits on *Bactrocera oleae*. *Ind. Crops Prod.* 143, 111884. <https://doi.org/10.1016/j.indcrop.2019.111884>.
- Rosato, A., Barbarossa, A., Mustafa, A.M., Bonacucina, G., Perinelli, D.R., Petrelli, R., Maggi, F., Spinozzi, E., 2021. Comprehensive evaluation of the antibacterial and antifungal activities of *Carlina acaulis* L. essential oil and its nanoemulsion. *Antibiotics* 10, 1451. <https://doi.org/10.3390/antibiotics10121451>.
- Santoiemma, G., Tamburini, G., Sanna, F., Mori, N., Marini, L., 2019. Landscape composition predicts the distribution of *Philaenus spumarius*, vector of *Xylella fastidiosa*, in olive groves. *J. Pest Sci.* 92, 1101–1109. <https://doi.org/10.1007/s10340-019-01095-8>.
- Saponari, M., Boscia, D., Altamura, G., Loconsole, G., Zicca, S., D'attoma, G., Morelli, M., Palmisano, F., Saponari, A., Tavano, D., Savino, V.N., Dongiovanni, C., Martelli, G. P., 2017. Isolation and pathogenicity of *Xylella fastidiosa* associated to the olive quick decline syndrome in southern Italy. *Sci. Rep.* 7 (1), 17723. <https://doi.org/10.1038/s41598-017-17957-z>.
- Saponari, M., Boscia, D., Nigro, F., Martelli, G.P., 2013. Identification of DNA sequences related to *Xylella fastidiosa* in oleander, almond and olive trees exhibiting leaf scorch symptoms in Apulia (Southern Italy). *Plant Pathol. J.* 95 (3), 668.
- Saraliev, E., Dincheva, I., Tumbarski, Y., Petkova, N., Vilhelmova-Ilieva, N., Nikolova, I., Simeonova, L., Ivanov, I., 2022. Chemical composition, antibacterial, antiviral, antioxidant, and acetylcholinesterase inhibitory properties of essential oils from *Carlina acanthifolia* All. roots. *J. Essent. Oil Bear. Plants* 25, 976–986. <https://doi.org/10.1080/0972060X.2022.2133973>.
- Schneider, K., Mourits, M., van der Werf, W., Lansink, A.O., 2021. On consumer impact from *Xylella fastidiosa* subspecies *pauca*. *Ecol. Econ.* 185, 107024. <https://doi.org/10.1016/j.ecolecon.2021.107024>.
- Sharma, S., Loach, N., Gupta, S., Mohan, L., 2020. Phyto-nanoemulsion: an emerging nano-insecticidal formulation. *Environ. Nanotechnol. Monit. Manag.* 14, 100331. <https://doi.org/10.1016/j.enmm.2020.100331>.
- Spinozzi, E., Ferrati, M., Cappellacci, L., Caselli, A., Perinelli, D.R., Bonacucina, G., Maggi, F., Strzemeski, M., Petrelli, R., Pavela, R., Desneux, N., Benelli, G., 2023. *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>.
- Thompson, V., Harkin, C., Stewart, A.J., 2023. The most polyphagous insect herbivore? Host plant associations of the meadow spittlebug, *Philaenus spumarius* (L.). *PLoS One* 18 (10), e0291734. <https://doi.org/10.1371/journal.pone.0291734>.
- 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>.
- Tortorici, S., Zeni, V., Perinelli, D.R., Ferrati, M., Spinozzi, E., Maggi, F., Benelli, G., Rizzo, R., 2024. Toxicity and repellent activity of a carlina oxide nanoemulsion toward the South American tomato pinworm, *Tuta absoluta*. *J. Pest Sci.* 1–12. <https://doi.org/10.1007/s10340-024-01785-y>.
- Wnorowska, S., Grzegorzczak, A., Kurzepa, J., Maggi, F., Strzemeski, M., 2024. Fractionation of *Carlina acaulis* L. Root Methanolic Extract as a Promising Path towards New Formulations against *Bacillus cereus* and Methicillin-Resistant *Staphylococcus aureus*. *Molecules* 29, 1939. <https://doi.org/10.3390/molecules29091939>.
- Wnorowska, S., Targowska-Duda, K., Kurzepa, J., Wnorowski, A., Strzemeski, M., 2022. *Carlina* oxide inhibits the interaction of SARS-CoV-2 S glycoprotein with angiotensin-converting enzyme 2. *Ind. Crops Prod.* 187, 115338. <https://doi.org/10.1016/j.indcrop.2022.115338>.