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ORIGINAL PAPER

Lethal and sublethal effects of carlina oxide on the aphid *Metopolophium dirhodum* and its non-target impact on two biological control agents

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Abstract

This study was designed to investigate the acute toxicity (mortality) and sublethal effects (fertility and potential natality) of carlina oxide, the main constituent of *Carlina acaulis* essential oil (EO), against adults of *Metopolophium dirhodum* (Walker) (Hemiptera: Aphididae). Moreover, its toxicity was evaluated against two aphid natural enemies, i.e., *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) and *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae). The highest tested concentration (3.0 mL L^{-1}) resulted in 96.7% mortality of adults of the target pest, highlighting that this concentration of carlina oxide had a similar effectiveness as the positive control we used. Furthermore, probit analysis allowed the estimation of a LC_{50} of 1.06 mL L^{-1} and a LC_{90} of 2.58 mL L^{-1} for the target pest, which resulted in a much higher mortality rate than that found on natural enemies, i.e., *A. aphidimyza* ($6.7 \pm 4.7\% \pm$ SD when exposed to the aphid LC_{90}) and *C. carnea* ($7.0 \pm 5.5\% \pm$ SD when exposed to the aphid LC_{90}), showing the limited non-target impact of carlina oxide. The use of LC_{30} and LC_{50} of this compound allowed the fertility inhibition of the target pest by $35.68 \pm 6.21\%$ and $23.66 \pm 10.58\%$, respectively, and potential natality inhibition of the target pest by $52.78 \pm 4.48\%$ and $59.69 \pm 5.60\%$, respectively. Of note, carlina oxide showed excellent insecticidal activity against *M. dirhodum,* comparable to the commercial insecticide considered. Overall, the low toxicity of carlina oxide toward *A. aphidimyza* and *C. carnea* makes it a safe compound for non-target organisms as well as suitable for developing a green insecticide for the management of *M. dirhodum* and perhaps other insects of agricultural or medical and veterinary interest.

Keywords Biological control agent · Botanical insecticide · Cecidomyiidae · Chrysopidae · Integrated pest management · Non-target predator

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Key message

- The aphicidal activity of carlina oxide, the main component of *C. acaulis* root oil, was studied.
- Lethal and sub-lethal effects of carlina oxide were investigated on *M. dirhodum*.
- Carlina oxide LC_{50} was 1.06 mL L^{-1} and the LC90 was 2.58 mL L^{-1}.
- Being exposed to the aphid LC_{90} showed little toxicity on *A. aphidimyza* and *C. carnea*.

Introduction

The aphid *Metopolophium dirhodum (*Walker) (Hemiptera: Aphididae) is an important pest of cereals, especially wheat and barley (Honěk 1994). In addition to the injury to plant tissue caused by sucking plant sap, which reduces grain quality, this insect pest is also an important vector of viral diseases such as barley yellow dwarf virus (BYDW) (Holt et al. 1984). Protection against aphids is based on the application of synthetic insecticides, mainly pyrethroids, organophosphates, and neonicotinoids (Gong et al. 2021a). However, the frequent application of synthetic insecticides leads, similarly to other pests, to the emergence of resistant populations. For example, Gong et al. (2021b) reported resistant populations of *M. dirhodum* against the insecticides thiamethoxam, imidacloprid, abamectin, and omethoate. In addition, the use of non-selective pesticides has a negative effect on aphid predators (*Aphidoletes* spp., *Chrysoperla* spp., *Syrphus* spp. *Episyrphus* spp., and *Epistrophe* spp.) and parasitoids (*Praon* spp. *Aphidius* spp., *Aphelinus* spp., etc.) (Honěk and Kocourek 1988; Takada 2002; Wojciechowicz-Zytko 2009).

For these reasons, it is necessary to search for new active substances characterized by new mechanisms of action (MoA) and, at the same time, tolerable to non-target organisms. Botanical insecticides also belong to promising products replacing synthetic insecticides. These plant protection preparations use secondary metabolites as active substances, which plants synthesize as part of their natural defense against pathogens and pests (Pavela and Benelli 2016). These metabolites also include essential oils (EOs) which are partially responsible for the taste and aroma of plants. In addition to many health benefits, they also exhibit bactericidal, fungicidal, and insecticidal effects, which have been proven in a wide number of studies (Isman and Grieneisen 2014; Pavela 2018; Benelli et al. 2020b).

Carlina acaulis L., a plant that naturally grows in the calcareous soils of southern and central Europe, belongs to the Asteraceae (Compositae) family (Tutin et al. 1976). It is well-known for its traditional medicinal use and possesses various beneficial health effects (Herrmann et al. 2011; Stojanović-Radić et al. 2012; Strzemski et al. 2019; Belabbes et al. 2020). The EO extracted from the roots of *C. acaulis* is primarily composed of the polyacetylene 2-(3-phenylprop-1-ynyl) furan, commonly known as carlina oxide. Polyacetylenes are a class of plant secondary metabolites involved in defense against insults and attacks of fungal, viral, and insecticidal origin (Spinozzi et al. 2023b).

Researchers have conducted experiments using *C. acaulis* EO, carlina oxide, and formulations containing these substances to test their efficacy against arthropods and nematodes. These include vectors of pathogens such as *Culex quinquefasciatus* Say and *Musca domestica* L., agricultural pests such as *Lobesia botrana* (Denis & Schiffermüller), *Bactrocera oleae* (Rossi), *Ceratitis capitata* (Wiedemann), *Meloidogyne incognita* (Kofoid & White), and stored-products pests such as *Acarus siro* L., *Alphitobius diaperinus* (Panzer), *Oryzaephilus surinamensis* L., *Prostephanus truncatus* (Horn), *Rhyzopertha dominica* (F.), *Sitophilus oryzae* L., *Tribolium confusum* Jacquelin du Val, *T. castaneum* (Herbst), *Tenebrio molitor* L., and *Trogoderma granarium* Everts (Pavela et al. 2020; Rizzo et al. 2021; Kavallieratos et al. 2022; Spinozzi et al. 2023a). These studies have also demonstrated that carlina oxide shows limited toxicity to non-target species and holds promise for being safe based on LD_{50} and LC_{50} values determined on rats and human cells, respectively (Pavela et al. 2020, 2021; Benelli et al. 2022).

Considering these findings, herein we evaluated the acute toxicity of carlina oxide on adults of the aphid *M. dirhodum.* Furthermore, sublethal effects caused by being exposed to selected concentrations of carlina oxide on aphid fertility and potential natality were investigated. At the same time, to better estimate the environmental safety of this compound, its effectiveness was tested on two important natural enemies of aphids, i.e., *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) and *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae).

Materials and methods

Chemicals

Carlina oxide was obtained by hydrodistillation of *C. acaulis* roots (Minardi & Figli S.r.l., Bagnacavallo, Ravenna, Italy) (yield of 0.75%, w/w); it was a yellowish oil with a density of 1.063 g/mL. Specifically, 1 kg of dry roots and 10 L of distilled water were inserted in a 20 L round-bottom flask and carlina oxide was collected after 6 h of hydrodistillation with a Clevenger-type apparatus. Once obtained, the compound was stored at—20 °C until chemical analysis and biological assays. GC–MS analysis was performed to assess the purity of the compound (98.1%, Fig. 1), adopting the same method by Spinozzi et al. (2023a). The chemical structure was confirmed by MS and NMR analyses and comparing the results with those of a chemical standard obtained in the authors' laboratory (Benelli et al. 2019).

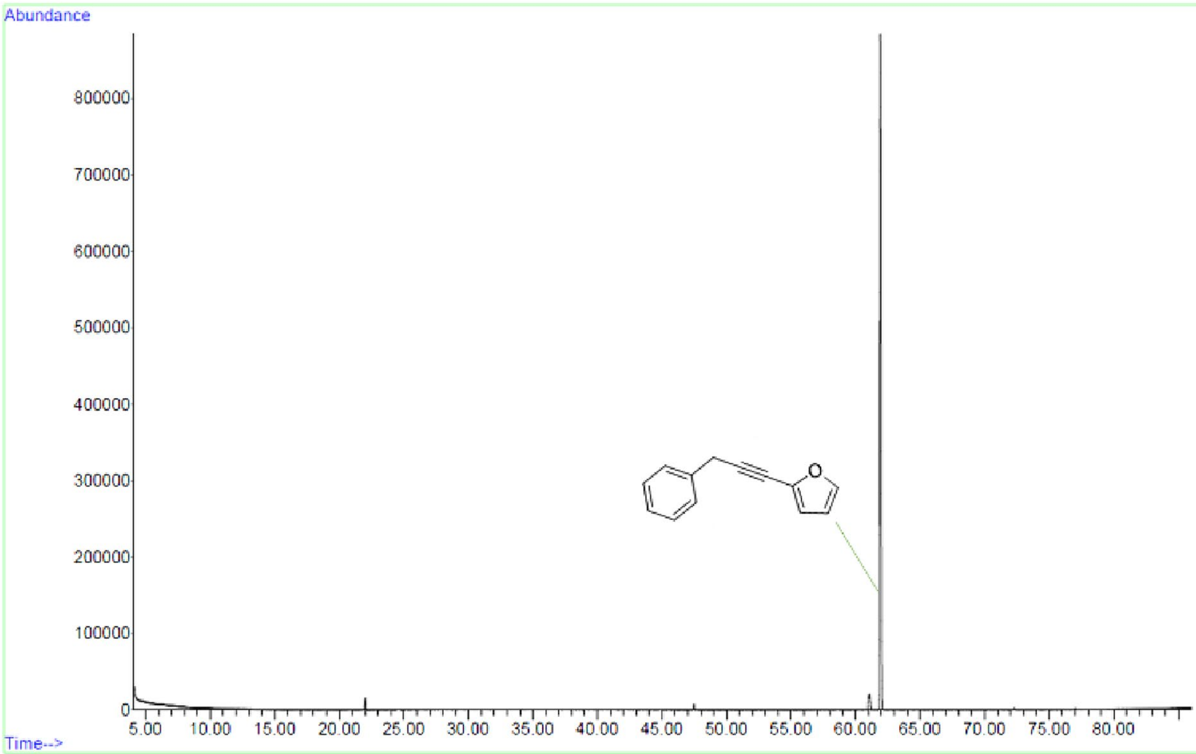

Fig. 1 Carlina oxide chromatogram obtained by GC–MS analysis displaying a purity of 98.1% based on total peak areas

Insects

Metopolophium dirhodum

M. dirhodum adults (wingless females, 1–2 days old) were obtained from laboratory mass-rearing (Crop Research Institute, Czech Republic). Colonies of *M. dirhodum* aphids were maintained for > 20 generations on wheat plants (*Triticum aestivum* L.) at a temperature of 21 ± 3 °C, 65 ± 5% R.H., and a16:8 (L:D) photoperiod.

Aphidoletes aphidimyza

Third instar larvae were obtained from established laboratory breeding (Crop Research Institute, Czech Republic). Adults were placed in insect cages of dimensions 35 × 35 × 60 cm where they were allowed to oviposit on leaves near *Myzus persicae* (Sulzer) aphids that were on *Brassica oleracea* var. *gongylodes* L. Predatory larvae fed on aphids developing on kohlrabi plants ad libitum until reaching the 3rd instar, when they were used for experiments. Breeding was maintained at a temperature of 21 ± 3°C, 65 ± 5% R.H. and a 16:8 (L:D) photoperiod.

Chrysoperla carnea

Second instar larvae were purchased from a commercial biofactory (Koppert, Holland). Larvae were used in experiments immediately after delivery.

Bioassays

Acute toxicity against *Metopolophium dirhodum*

Aphid adults were transferred with a fine paintbrush to sown wheat plants (BBCH scale 11, 5 planted in a standard peat substrate, pots with a diameter of 9 cm) at the rate of 15 adults/pot. Between the transfer and application was a 3 h time gap, when aphids were allowed to freely settle on plant leaves and feed. Carlina oxide was emulsified using Tween 20 (Sigma Aldrich, Czech Republic), when stock emulsions were subsequently prepared using a Witeg HG15A homogenizer (5000 revolutions/min) in a concentration range of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL L^{-1}. The concentrations used were estimated based on preliminary tests. To reduce the surface tension of the spray liquid, Tween 20 was used as a surfactant (3.0 mL L^{-1}). The emulsions were applied to the plants using a laboratory Sprayer Sge1 (Biostep, Fisher, Czech Republic) at a dose of 5 mL/pot (corresponding to the equivalent of 500 L ha^{-1}). Only water with Tween 20 (3.0 mL L^{-1}) was used as a negative control. The commercially available product Neudosan (Neudorff W.GmbH. KG, Germany, also in potassium salts of fatty acids 515 g kg^{-1}) was chosen as a positive control at the concentration recommended by the manufacturer (20 mL L^{-1}).

The treated plants were placed in a greenhouse where the temperature was maintained at 21 ± 3°C, 65 ± 5% R.H., and a 16:8 (L:D) photoperiod. Each treatment was replicated 5 times. Mortality was assessed 48 h after application.

Inhibition of fertility and potential natality of *Metopolophium dirhodum*

The experiment was performed using the same method as for acute toxicity, except that adult females were treated with concentrations corresponding to the estimated lethal concentration that kills 30% (LC_{30}) and 50% (LC_{50}) of the population (i.e., 0.7 and 1.1 mL L^{-1}, respectively). After 48 h, the surviving individuals were placed on new *T. aestivum* plants and the number of newly born nymphs was recorded for 7 days. Every each day the newborn nymphs were removed from the plants with a fine paintbrush to avoid issues on the following days. The experiment was located at a temperature of 21 ± 3°C, 65 ± 5% R.H. and a 16:8 (L:D) photoperiod. The experiment was repeated 5 times.

Fertility was expressed as the number of newly hatched nymphs per surviving treated female per day. Fertility inhibition then expresses the percentage by which the number of laid nymphs was reduced compared to the control.

Potential birth rates are then expressed by the number of hatched nymphs that a population of 100 treated females will produce in one day, assuming that their 30% or 50% mortality occurs within 24 h (for females treated with LC_{30} or LC_{50}, respectively).

Potential natality was calculated according to the following formula: Nat = average number of nymphs laid by 100 treated females * predicted mortality coefficient, and mortality coefficient = 0.7 for aphids treated with concentrations corresponding to the estimated LC_{30} or by the coefficient 0.5 for aphids treated with concentrations corresponding to the estimated LC_{50}.

Acute toxicity against *Aphidoletes aphidimyza* and *Chrysoperla carnea*

Third instar larvae of *A. aphidimyza* and 2nd instar larvae of *C. carnea* were treated with carlina oxide at concentrations corresponding to the LC_{50} and LC_{90} estimated for *M. dirhodum* (i.e., 1.1 and 2.6 mL L^{-1}, respectively). Larvae (15 insects per replicate) were immersed in prepared stock solutions for 3 s and then placed in plastic cups (10 cm diameter) containing filter paper and covered with perforated lids. Larvae of *C. carnea* were kept individually in cups because of their cannibalistic tendencies. Different species of aphids were added to the cup as food for the test larvae in an ad libitum amount. The experiment was maintained at a temperature of 21 ± 3°C, 65 ± 5% R.H., and a 16:8 (L:D) photoperiod. The experiment was repeated 5 times. Mortality was assessed 48 h after application.

Data analysis

Metopolophium dirhodum mortality rates observed in acute toxicity experiments were adjusted according to Abbott (1925); then, LC_{50} and LC_{90} with 95% confidence interval (Cl_{95}) were estimated through probit analysis (Finney 1971). Percentage data on the inhibition of aphid fertility and potential natality as well as mortality data of *A. aphidimyza* and *C. carnea* were transformed through arcsine square root transformation before being analyzed by ANOVA followed by Tukey's HSD test ($P \leq 0.05$). For all statistical analyses, software Biostat 5.9.8 was used.

Results

From an extraction and purification perspective, carlina oxide can be easily prepared by simple hydrodistillation, leading to a 98% purity (Fig. 1).

The effectiveness of carlina oxide from *C. acaulis* on the acute toxicity of *M. dirhodum* aphids is shown in Table 1. At the highest tested concentration of 3.0 mL L^{-1}, a mortality of 96.7% was found, and it is evident that this concentration of carlina oxide had a similar effectiveness as the positive control we used. Probit analysis allowed the estimation of an LC_{50} of 1.06 mL L^{-1} and LC_{90} of 2.58 mL L^{-1}.

The effects of being exposed to different lethal concentrations of carlina oxide on *M. dirhodum* fertility and potential natality are shown in Table 2. Wingless adults were exposed to LC_{30} and LC_{50} estimated in acute toxicity tests. Tween-formulated emulsion applied at lethal concentrations reduced potential natality compared to the untreated control by more than 50%, with no significant difference observed between LC_{30} and LC_{50} application; on the other hand, the use of LC_{30} and LC_{50} of this compound allowed the fertility inhibition of the target pest by 35.68 ± 6.21% and 23.66 ± 10.58%.

From a non-target point of view, the effect of the application of carlina oxide concentrations corresponding to LC_{50} and LC_{90} on aphid predators *A. aphidimyza* and *C. carnea* is reported in Table 3. For carlina oxide, only low mortality (less than 10%) was observed for both non-target species, and this mortality was not higher than the negative control.

Table 1 Acute toxicity of carlina oxide isolated from *Carlina acaulis* roots against *Metopololphium dirhodum* adults

Concentration of carlina oxide (mL L^{-1})	Mortality* (% ± SD)	LC_{30}** (CI_{95})	LC_{50}** (CI_{95})	LC_{90}** (CI_{95})	χ^2 (df = 4)	*P*-value
0.5	11.2 ± 4.6	0.68 (0.37–0.83)	1.06 (0.95–1.23)	2.58 (2.12–3.07)	4.511	0.341 ns
1.0	24.2 ± 9.9					
1.5	58.1 ± 8.8					
2.0	64.5 ± 7.9					
2.5	75.1 ± 4.6					
3.0	96.7 ± 3.6					
Positive control	100.0 ± 0.0					

*Mortality was corrected using Abbott; positive control = 20 mL L^{-1} Neudosan (active ingredient: potassium salts of fatty acids)

**Concentration—LC_{30}($_{50,90}$) in mL L^{-1} causing 30, (50, 90%) mortality of aphids 48 h after application. SD = standard deviation. CI95—95% confidence intervals, activities of extract and compounds are considered significantly different when the 95% CI fails to overlap. Chi-square value, not significant (ns)

Table 2 Sublethal effect of carlina oxide on fertility and potential natality of *Metopolophium dirhodum* aphids

Treatment	Fertility		Potential natality	
	No. nymphs/female/day	Inhibition (%) compared to control	No. nymphs/100 females/day	Inhibition (%) compared to control
LC_{50}	2.96 ± 0.41[a]	23.66 ± 10.58	147.76 ± 20.54[a]	59.69 ± 5.60
LC_{30}	2.61 ± 0.40[a]	35.68 ± 6.21	173.09 ± 16.42[a]	52.78 ± 4.48
Negative control	3.88 ± 0.47[b]	–	366.61 ± 16.42[b]	–
ANOVA $F_{2,12}$; P	9.98; 0.002	ns	118.71; < 0.001	ns

Within a column, different letters indicate significant differences among means (ANOVA, Tukey's HSD test, $p < 0.05$). Negative control = water; *ns* not significant

Table 3 Acute toxicity of carlina oxide LC estimated on *Metopolophium dirhodum* against two natural enemies of aphids

Treatment	*A. aphidimyza* mortality (% ± SD)	*C. carnea* mortality (% ± SD)
Aphid LC_{50}	5.0 ± 5.5	7.0 ± 5.5[a]
Aphid LC_{90}	6.7 ± 4.7	2.4 ± 3.4[a]
Positive control	1.7 ± 2.8	84.0 ± 8.1[b]
Negative control	3.3 ± 3.3	2.2 ± 3.1[a]
ANOVA $F_{3,16}$; P	ns	58.68; < 0.001

Negative control = water; positive control = 20 mL L^{-1} Neudosan (active ingredient: potassium salts of fatty acids). Within a column, different letters indicate significant differences among means (ANOVA, Tukey's HSD test, $p < 0.05$), *SD* standard deviation, *ns* not significant

Discussion

The control of aphids represents a major challenge due to their fast reproduction capacity and the significant crop losses they cause (Ikbal and Pavela 2019). In fact, they can damage many crops worldwide and carry dangerous pathogenic viruses (Luo et al. 2022). Pyrethroids, neonicotinoids and carbamates are currently used to protect crops from these pests. However, aphids are becoming resistant to traditional products due to different mechanisms which avoid the toxic effect of insecticides (Bass and Nauen 2023). Specifically, *M. dirhodum* resistance phenomena mainly rely on detoxification enzymes genes expression (Gao et al. 2021). In this regard, we evaluated the insecticidal potential of carlina oxide, a natural product with well-demonstrated efficacy against other target insects, to find an eco-friendly alternative for the treatment of this dangerous pest.

Herein, carlina oxide was prepared by simple hydrodistillation, leading to a > 98% purity. This is certainly a factor favoring its future industrial application as ingredient of botanical insecticides (see also Spinozzi et al. 2023c). When carlina oxide was tested for on *M. dirhodum* at 3.0 mL L^{-1}, it was able to cause > 96% mortality, with a similar effectiveness comparable to the positive control; the LC_{50} and LC_{90} were 1.06 and 2.58 mL L^{-1}. Despite the negative impact of *M. dirhodum* adults on many crops worldwide, only few EOs and related botanical constituents have been evaluated for their insecticidal potential against this important pest (Ikbal and Pavela 2019). As a general trend, the tested botanicals showed lower toxicity with respect to carlina oxide. For example, phytol, (*E*)-nerolidol and spathulenol isolated from *Stevia rebaudiana* Bertoni showed LC_{50} values of 1.4, 3.5, and 4.3 mL L^{-1}, respectively, which were higher with respect to those estimated for carlina oxide (Benelli et al. 2020a). Chopa and Descamps (2012) determined even higher LC_{50} values of 76.2 mL L^{-1} and 15.2 mL L^{-1} for *Tagetes terniflora* Kunth and *Salvia officinalis* L. EOs, respectively.

Carlina oxide has been proven to be highly effective against a relatively wide range of insect species (Spinozzi et al. 2023a). However, this is the first study exploring its insecticidal activity against aphids. The low LC_{50} values obtained depend probably on the contact toxicity exerted by carlina oxide. The exact mode of action related to the insecticidal action has not been determined yet, but it is probably linked to the formation of reactive oxygen species and free radicals following its reaction with sunlight. The propynyl chain of the molecule is embedded with a triple bond moiety which could be the main responsible for radicals' production (Spinozzi et al. 2023a). The latter are highly reactive and can cause oxidative damages and insect death (McLachlan et al. 1984). The photosensitization is typical of polyacetylenes, and the class of compound carlina oxide belongs to (Gommers and Geerligs 1973; Konovalov 2014). Moreover, it has been demonstrated that this compound can inhibit the acetylcholinesterase (AChE) of insects (Benelli et al. 2019).

As reported in earlier research, low doses or concentrations of EOs can significantly reduce insect fertility (Benelli et al. 2018), among other sublethal effects (Giunti et al. 2022). This phenomenon is of practical importance. Indeed, applying a smaller amount of the active ingredients (AIs) makes the application of botanical insecticides cheaper in practice, and also thanks to the ability of EOs to inhibit fertility, the number of pests can be reduced below the threshold of economic harm. We previously found that carlina oxide from *C. acaulis* inhibits the fecundity of *Musca domestica* L. (Diptera: Muscidae) (Pavela et al. 2020) and *Tetranychus urticae* Koch (Acari: Tetranychidae) adults (Rizzo et al. 2024). However, information on the effectiveness of this EO on aphid fertility is still lacking. Therefore, we studied this phenomenon in *M. dirhodum*. In fact, parthenogenetic reproduction of this aphid, which typically develops from unfertilized eggs, combined with viviparity causes a rapid grow of their population.

When wingless *M. dirhodum* adults were exposed to the LC_{30} and LC_{50} estimated in acute toxicity tests, sublethal effects on aphid fertility and potential natality were noted. Tween-formulated emulsion applied at both LC_{30} and LC_{50} reduced potential natality compared to the untreated control by more than 50%. Therefore, we showed that being exposed to the above-mentioned concentrations can reduce the abundance of aphid colonies on plants. That means that weakened colonies can then be further reduced by aphid natural enemies. This approach fully matches the Integrated Pest Management (IPM) concept, given that green insecticides and biological control can be used simultaneously to better manage the pest (Ehler 2006). Therefore, it is important that carlina oxide, as the active ingredient (AI) of potential botanical insecticides, is also friendly to non-target organisms (Giunti et al. 2022). Studying the effect of insecticides AIs on non-target organisms is important for estimating their environmental safety. Indeed, the preference for a selective insecticide or selective application are key decisions for the preservation of natural enemies. Where, for some reason, the insecticide cannot show enough effect, preserved natural enemies can significantly reduce the outbreak and resurgence of a given pest (Torres and Bueno 2018).

Based on these considerations, we evaluated the non-target effect of carlina oxide against the aphid natural enemies *A. aphidimyza* and *C. carnea*. When exposing non-target predators to carlina oxide concentrations corresponding to aphid LC_{50} and LC_{90}, only low mortality (i.e., $< 10\%$) was observed on both species of non-target biocontrol agents, and this mortality was not higher than the negative control. The product we used, which was applied as part of the positive control, contained a salt of fatty acids as active substance. This product is commonly used in organic farming and is therefore generally considered friendly to non-target organisms. However, as was evident from our experiments, this product was friendly to *A. aphidimyza*, but it showed toxicity $> 80\%$ on *C. carnea* larvae. Overall, it can be concluded that carlina oxide was friendly for the aphid predators *A. aphidimyza* and *C. carnea*. This is also outlined by our previous findings, when non-target impact was evaluated through experiments on *Daphnia magna* Straus (Cladocera: Crustacea) adults. Carlina EO and carlina oxide showed lower toxicity if compared to cypermethrin (Benelli et al. 2019).

However, we are fully aware that further tests on non-target organisms and on the behavior of carlina oxide in the environment (e.g., adhesion/absorption to soil and organic matter, and bioaccumulation capacity) should be carried out to clarify the possible environmental impacts of the applications of botanical insecticides based on carlina oxide and their synthetic analogs (Spinozzi et al. 2023a), which are currently the subject of our further research.

Conclusions

This work represents the first evidence for the aphicidal activity of carlina oxide. This compound showed excellent efficacy against *M. dirhodum,* in a comparable manner to that of a commercial insecticide. Furthermore, minimal toxicity for natural enemies of aphids *A. aphidimyza* and

C. carnea was showed. Therefore, it is possible to conclude that carlina oxide is safe for these insects in concentrations effective against aphids. The agrochemical exploitation of this compound will be assured by in field cultivation and/or synthetic procedures that have been recently developed by our research group.

Author contributions

RPa, RPe, FM, and GB conceived and designed the research. MN, RP, ES, MF, RP, FM, and RR conducted the experiments and/or analyzed the data. MN, RP, ES, MF, FM, and GB wrote the original draft. MN, RP, and RR contributed to writing, review, and editing. All authors approved the final version of the manuscript.

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Declarations

Competing interests The authors declare no competing interests.

References

Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18:265–267

Bass C, Nauen R (2023) The molecular mechanisms of insecticide resistance in aphid crop pests. Insect Biochem Mol Biol 156:103937. https://doi.org/10.1016/j.ibmb.2023.103937

Belabbes R, Mami IR, Dib MEA et al (2020) Chemical composition and biological activities of essential oils of *Echinops spinosus* and *Carlina vulgaris* rich in polyacetylene compounds. Curr Nutr Food Sci 16(4):563–570. https://doi.org/10.2174/1573401315666190206142929

Benelli G, Pavela R, Giordani C et al (2018) Acute and sub-lethal toxicity of eight essential oils of commercial interest against the filariasis mosquito *Culex quinquefasciatus* and the housefly *Musca domestica*. Ind Crops Prod 112:668–680. https://doi.org/10.1016/j.indcrop.2017.12.062

Benelli G, Pavela R, Petrelli R et al (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, Pavela R, Drenaggi E et al (2020a) Phytol, (E)-nerolidol and spathulenol from *Stevia rebaudiana* leaf essential oil as effective and eco-friendly botanical insecticides against *Metopolophium dirhodum*. Ind Crops Prod 155:112844. https://doi.org/10.1016/j.indcrop.2020.112844

Benelli G, Pavoni L, Zeni V et al (2020b) Developing a highly stable *Carlina acaulis* essential oil nanoemulsion for managing *Lobesia botrana*. Nanomaterials 10:1–15. https://doi.org/10.3390/nano10091867

Benelli G, Ceccarelli C, Zeni V et al (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

Ehler LE (2006) Integrated pest management (IPM): definition, historical development and implementation, and the other IPM. Pest Manag Sci 62:787–789. https://doi.org/10.1002/ps.1247

Finney DJ (1971) Probit analysis. Cambridge University Press, Cambridge

Gao H, Zhu X, Li G et al (2021) RNA Sequencing Analysis of *Metopolophium dirhodum* (Walker) (Hemiptera: Aphididae) Reveals the Mechanism Underlying Insecticide Resistance. Front Sustain Food Syst 5:1–9. https://doi.org/10.3389/fsufs.2021.639841

Giunti G, Benelli G, Palmeri V et al (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

Gommers FJ, Geerligs JWG (1973) Lethal effect of near ultraviolet light on *Pratylenchus penetrans* from roots of Tagetes. Nematologica. https://doi.org/10.5555/19740809853

Gong P, Chen D, Wang C et al (2021a) Susceptibility of four species of aphids in wheat to seven insecticides and its relationship to detoxifying enzymes. Front Physiol 11:1–8. https://doi.org/10.3389/fphys.2020.623612

Gong P, Li X, Wang C et al (2021b) The sensitivity of field populations of *Metopolophium dirhodum* (Walker) (Hemiptera: Aphididae) to seven insecticides in Northern China. Agronomy 11:1–12. https://doi.org/10.3390/agronomy11081556

Herrmann F, Hamoud R, Sporer F et al (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

Holt J, Griffiths E, Wratten SD (1984) The influence of wheat growth stage on yield reductions caused by the rose-grain aphid, *Metopolophium dirhodum*. Ann Appl Biol 105:7–14

Honěk A (1994) The effect of plant quality on the abundance of *Metopolophium dirhodum* (Homoptera: Aphididae) on maize. Eur J Entomol 91:227–236

Honěk A, Kocourek F (1988) Thermal requirements for development of aphidophagous Coccinellidae (Coleoptera), Chrysopidae, Hemerobiidae (Neuroptera), and Syrphidae (Diptera): some general trends. Oecologia 76:455–460. https://doi.org/10.1007/BF00377042

Ikbal C, Pavela R (2019) Essential oils as active ingredients of botanical insecticides against aphids. J Pest Sci 92:971–986. https://doi.org/10.1007/s10340-019-01089-6

Isman MB, Grieneisen ML (2014) Botanical insecticide research: many publications, limited useful data. Trends Plant Sci 19:140–145. https://doi.org/10.1016/j.tplants.2013.11.005

Kavallieratos NG, Nika EP, Skourti A et al (2022) *Carlina acaulis* essential oil: a candidate product for agrochemical industry due to its pesticidal capacity. Ind Crops Prod 188:115572. https://doi.org/10.1016/j.indcrop.2022.115572

Konovalov DA (2014) Polyacetylene Compounds of Plants of the asteraceae family (review). Pharm Chem J 48:613–631. https://doi.org/10.1007/s11094-014-1159-7

Luo K, Zhao H, Wang X, Kang Z (2022) Prevalent pest management strategies for grain aphids: opportunities and challenges. Front Plant Sci 12:1–12. https://doi.org/10.3389/fpls.2021.790919

McLachlan D, Arnason T, Lam J (1984) The role of oxygen in photosensitizations with polyacetylenes and thiophene derivatives. Photochem Photobiol 39:177–182. https://doi.org/10.1111/j.1751-1097.1984.tb03425.x

Pavela R (2018) Essential oils from *Foeniculum vulgare* Miller as a safe environmental insecticide against the aphid *Myzus persicae* Sulzer. Environ Sci Pollut Res 25:10904–10910. https://doi.org/10.1007/s11356-018-1398-3

Pavela R, Benelli G (2016) Essential oils as ecofriendly biopesticides? Challenges and constraints. Trends Plant Sci 21:1000–1007. https://doi.org/10.1016/j.tplants.2016.10.005

Pavela R, Maggi F, Petrelli R et al (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

Pavela R, Pavoni L, Bonacucina G et al (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

Rizzo R, Pistillo M, Germinara GS et al (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. https://doi.org/10.3390/insects12100880

Rizzo R, Ragusa E, Benelli G et al (2024) Lethal and sublethal effects of 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

Sánchez Chopa C, Descamps LR (2012) Composition and biological activity of essential oils against *Metopolophium dirhodum* (Hemiptera: Aphididae) cereal crop pest. Pest Manag Sci 68:1492–1500. https://doi.org/10.1002/ps.3334

Spinozzi E, Ferrati M, Baldassarri C et al (2023a) 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

Spinozzi E, Ferrati M, Cappellacci L et al (2023b) *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

Spinozzi E, Ferrati M, Lo GD et al (2023c) Microwave-assisted hydrodistillation of the insecticidal essential oil from *Carlina acaulis*: a fractional factorial design optimization study. Plants. https://doi.org/10.3390/plants12030622

Stojanović-Radić Z, Čomić L, Radulović N et al (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

Strzemski M, Wójciak-Kosior M, Sowa I et al (2019) Historical and traditional medical applications of Carlina acaulis L.—a critical ethnopharmacological review. J Ethnopharmacol. https://doi.org/10.1016/j.jep.2019.111842

Takada H (2002) Parasitoids (Hymenoptera: Braconidae, Aphidiinae; Aphelinidae) of four principal pest aphids (Homoptera: Aphididae) on greenhouse vegetable crops in Japan. Appl Entomol Zool 37:237–249. https://doi.org/10.1303/aez.2002.237

Torres JB, de Bueno AF (2018) Conservation biological control using selective insecticides—a valuable tool for IPM. Biol Control 126:53–64. https://doi.org/10.1016/j.biocontrol.2018.07.012

Tutin F, Heywood V, Burges N et al (1976) Plantaginaceae to Compositae (and Rubiaceae). Cambridge University Press, Cambridge, Flora Europea

Wojciechowicz-Zytko E (2009) Predatory syrhpids (Diptera, Syrphidae) and ladybird beetles (Coleoptera, Coccinellidae) in the colonies of *Aphis fabae* Scopoli, 1763 (Hemiptera, Aphidoidea) on *Philadelphus coronarius* L. Monogr Aphids Other Hemipterous inSects 15(15):169–181