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Original article

Mortality and sublethal effects of *Solanum elaeagnifolium* L. (Solanales: Solanaceae) extract, imidacloprid and chlorpyrifos on *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) larvae

Mortalidad y efectos subletales del extracto de *Solanum elaeagnifolium* L. (Solanales: Solanaceae), imidacloprid y clorpirifos en larvas de *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae)

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ABSTRACT. In this study, the toxicity and sublethal effects of an aqueous extract of *Solanum elaeagnifolium* on *Chrysoperla carnea* larvae, and of the insecticides imidacloprid and chlorpyrifos, were evaluated. Third-instar larvae were exposed to the treatments through ingestion using a modified feeding method under laboratory conditions. The results showed that chlorpyrifos had the highest toxicity, with a 50% lethal concentration (LC_{50}) of 10.9 ppm and 100% mortality at 500 ppm. Imidacloprid had an intermediate effect, with an LC_{50} of 3.6 ppm (which was lower than the lowest concentration tested) and a maximum of 94.4% mortality at 500 ppm, while the *S. elaeagnifolium* extract had a lower mortality effect, with an LC_{50} that exceeded the highest concentration tested (>500 ppm) resulting in a maximum mortality of 42.2% at 500 ppm. However, at low concentrations (5-50 ppm), the effect of the botanical extract was similar to the control group, suggesting greater compatibility with the predator compared to broad-range synthetic insecticides. In terms of sublethal effects, *S. elaeagnifolium* extract caused pupal malformations, delayed adult emergence and caused abdominal necrosis, effects that were not observed with the same magnitude in the synthetic insecticide treatments. These results suggest that *S. elaeagnifolium* extract at moderate concentrations may be compatible with the natural enemy evaluated, facilitating its integration into integrated pest management (IPM) strategies.

Key words: aqueous extract; predators; Chrysopidae; insecticides; toxicity

RESUMEN. En este estudio se evaluó la toxicidad y efectos subletales del extracto acuoso de *Solanum elaeagnifolium* en larvas de *Chrysoperla carnea*, y de los insecticidas imidacloprid y clorpirifos. Las larvas del tercer instar fueron expuestas a los tratamientos mediante el método de ingestión modificado, bajo condiciones de laboratorio. Los resultados mostraron que el clorpirifos tuvo la mayor toxicidad, con una concentración letal 50% (CL_{50}) de 10.9 ppm y 100% de mortalidad a 500 ppm. El imidacloprid presentó un efecto intermedio, con una CL_{50} de 3.6 ppm (menor que la concentración más baja del bioensayo) y un máximo de 94.4% de mortalidad a 500 ppm, en tanto que el extracto de *S. elaeagnifolium* tuvo un efecto de mortalidad más bajo, con una CL_{50} mayor a 500 ppm y un máximo de 42.2% de mortalidad a 500 ppm. Sin embargo, a bajas concentraciones (5-50 ppm), el efecto del extracto botánico fue similar al grupo control, lo que sugiere una mayor compatibilidad con el depredador en comparación a los insecticidas sintéticos. En cuanto a los efectos subletales, el extracto de *S. elaeagnifolium* ocasionó malformaciones en las pupas, retrasos en la emergencia de adultos y necrosis abdominal, efectos que no se observaron con la misma magnitud en los tratamientos con los insecticidas sintéticos. Estos resultados sugieren que el extracto de *S. elaeagnifolium* a concentraciones moderadas podría ser más compatible con el enemigo natural evaluado, facilitando su integración en estrategias de manejo integrado de plagas (MIP).

Palabras clave: extracto acuoso; depredadores; Chrysopidae; insecticidas; toxicidad

INTRODUCTION

Agricultural pest control has historically been based on using synthetic insecticides. However, despite their widespread use, these products have a significant negative impact on the environment. In addition, they generate resistance in target pests, encourage the emergence of secondary pests, and adversely affect natural enemies present in the agroecosystem (Baker *et al.*,

2020). To address this situation, alternatives for the control of pest arthropods have been developed, which represent less harmful approaches for agricultural ecosystems. One of these alternatives is Integrated Pest Management (IPM), which includes various techniques and practices, considering economic, environmental and social aspects that promote sustainable agriculture (FAO, 2023). IPM aims to reduce dependence on synthetic pesticides and encourage the balanced use of different control strategies. These include crop rotation, constant monitoring of pest populations, and the use of biological control agents, such as parasitoids or predatory insects, as well as the use of bioinsecticides of botanical origin, which are currently considered a promising alternative to the use of synthetic pesticides.

Solanum elaeagnifolium, is an herbaceous plant, that contains secondary metabolites with insecticidal properties such as quinones, alkaloids, saponins and tannins (Feki *et al*., 2014). The larvicidal activity of this plant on *Culiseta longiareolata* (Macquart, 1838) (Belkhiri *et al*., 2021) has been evaluated, as well as its effect as a feeding deterrent on larvae and adults of *Tribolium castaneum* (Herbst, 1797) and *Sitophilus oryzae* (Linnaeus, 1763) (Descamps & Sánchez, 2013) exposed to various extracts of *S. elaeagnifolium*.

However, further research is essential to gain a more complete understanding of the compatibility and effects of botanicals on natural enemies of pests (Kalita & Hazarika, 2018; Sayed *et al*., 2020). These studies are necessary to develop effective IPM strategies and to rule out those extracts that may harm the beneficial entomological fauna. The objective of this study was to evaluate the toxicity and sublethal effects of an aqueous extract of *S elaeagnifolium* and the synthetic insecticides imidacloprid and chlorpyrifos on larvae of the predator *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), under laboratory conditions.

MATERIALS AND METHODS

Study location. The study was conducted in the entomology laboratory of the Regional University Unit for Arid Zones of the Autonomous University of Chapingo (UACh), located in Bermejillo, Durango, Mexico.

Experimental Design. A completely randomized factorial experimental design was used. The larval bioassay involved the factorial combination of 3x5 treatments, which generated 15 combinations. The first factor involved three toxicants (one of natural origin and two synthetic) with five concentrations, including the controls; each treatment was performed three times (replicates).

Experimental Unit. The experimental unit consisted of one larva placed in a cell of the larval rearing unit. These cells were made of hard plastic with a thickness of 0.3 cm. Each larval rearing unit consisted of 625 cells of 0.16 cm^2 per cell and a space of 0.2 cm between them. To avoid cross contamination between the products evaluated, the rearing units were cut into smaller units, ensuring had a complete treatment with its respective replicates. Two pieces of 15x15 cm organza were used as the base and lid of each larval rearing unit. To guarantee the emergence of one larva per cell, five *C. carnea* eggs sourced from a laboratory specializing in the mass-production of beneficial insects were placed in each larval rearing unit. For each bioassay, 30 first instar larvae (L1) with 24 hours of emergence were used.

Median Lethal Concentration (**LC_{50}**). The concentrations used were determined by using a biological response window. This method consists of identifying the range of concentrations that

generate mortality from zero to 100%, to select the concentrations to be tested. Four concentrations and a control were selected within this range, which were: 0 ppm (control with distilled water), 5, 50, 100 and 500 parts per million (ppm) of the formulated products. In the case of the plant extract, the concentrations were calculated based on the active compounds extracted from the plant material. Three replicates were performed for each concentration.

The maximum acceptable mortality value for absolute control was established as 10%. In the case of the bioassay with larvae, a mortality of 9.2% was observed, complying with the established criterion.

Botanical extract and synthetic insecticides. To prepare the botanical extract, *S. elaeagnifolium* was collected at the fruiting stage at the El Carmen ranch, belonging to the UACh-URUZA University during October 2023. The collected plants were cut into small pieces to facilitate their processing by grinding. The extract was prepared using the entire plant, including leaves, stems, fruits, flowers, and roots.

A stock solution was prepared using a 1:2 m/v ratio. In an airtight container, 500 g of plant material was placed in 1000 ml of distilled water. Subsequently, the pressing method described by García-González *et al*. (2019) was used to obtain the stock solution. The extracted liquid was filtered using Whatman No. 1 paper and stored in an airtight container at a temperature of 4°C.

In the case of the insecticides imidacloprid and chlorpyrifos, the corresponding formulated products were used as a stock solution, from which the previously mentioned concentrations (5-500 ppm) were prepared. For imidacloprid, the commercial product Singular 350 SC (Agro Lucava, Celaya, Mexico) was used, and for chlorpyrifos, the product Velban 480 EC (Velsimex, S.A. de C.V., Mexico City, Mexico) was used.

The ingestion method described by Amarasekare and Shearer (2013) was used, with certain modifications. A 15ml volume of *Sitotroga cerealella* (Oliver, 1789) eggs were taken and treated with 6 ml of the aqueous solutions corresponding to each of the different treatments. They were then left to dry at room temperature for a period of 30 minutes. Once dry, a thin layer of the treated eggs was applied to the external surface of the organza covering the larval rearing units.

The larval rearing units were placed in an incubator and maintained at a constant temperature between 23.9 and 25°C throughout the biological cycle. The development was monitored for approximately 35 days. At the end of the mortality bioassay, the larvae that survived the treatments were transferred to gel capsules containing uncontaminated *S. cerealella* eggs and then returned to the incubator. This allowed the evaluation of the possible sublethal effects of the treatments.

Variables Evaluated. Mortality assessments were carried out at 24, 48 and 72 hours, to obtain cumulative mortality during this period. A 10X magnifying lens was used to assist the observation tool. After each assessment, a new treated egg was placed in each cell every 24 hours. Larvae that showed no response to the touch of a camel-hair brush were considered dead. The median lethal concentration (LC_{50}) of each of the toxins was then determined.

After assessing mortality, the development of the surviving larvae was monitored through the pupal stage to the adult phase. During a 20-day observation period, periodic monitoring was carried out every 5 days for the larvae that reached the pupal stage and those that reached adulthood. In addition, a detailed record was made of pupae that showed malformations or dark

coloration. Similarly, adults that showed any type of malformation were monitored during this same period.

Statistical Analysis. The data were analyzed with a factorial random linear model and the separation of treatment means was performed by Tukey test at a significant level of 95%, using InfoStat software version 2020. In addition, the LC_{50} 2022 calculator software was used for the probit analysis (Mekapogu, 2021).

RESULTS

The different concentrations of the *S. elaeagnifolium* extract resulted in the highest mortality of 42.2% at the 500ppm concentration (Table 1). The concentrations of 50 and 100 ppm did not differ significantly from one another and both exhibited intermediate mortality effects. In contrast, the concentrations of 5 ppm and the control group (0 ppm) resulted in no mortality.

Table 1. Effect of an aqueous extract of *Solanum elaeagnifolium*, imidacloprid and chlorpyrifos on mortality of *Chrysoperla carnea* larvae under *in vitro* conditions at 72 h.

Source of the toxic	Concentration (ppm)	Mortality of larvae (%)	Standard Error
Absolute control	0	9.3 f	0.62
Extract of *S. elaeagnifolium*	5	13.3 f	0.62
Imidacloprid	5	55.6 c	0.62
Chlorpyrifos	5	40 d	0.62
Extract of *S. elaeagnifolium*	50	30 e	0.62
Imidacloprid	50	86.6 b	0.62
Chlorpyrifos	50	83.7 b	0.62
Extract of *S. elaeagnifolium*	100	36.6 de	0.62
Imidacloprid	100	86.6 b	0.62
Chlorpyrifos	100	94.4 ab	0.62
Extract of *S. elaeagnifolium*	500	42.2 d	0.62
Imidacloprid	500	94.4 ab	0.62
Chlorpyrifos	500	100 a	0.62

Averages with the same letters within the same column are statistically equal according to Tukey's least significant difference ($p \leq 0.05$).

Imidacloprid caused high mortality at concentrations of 50, 100, and 500 ppm, ranging from 86.6% to 94.4% (Table 1). No statistically significant differences were detected among these concentrations. In contrast, the 5ppm concentration resulted in 55.6% mortality which was significantly lower than observed at the higher concentrations.

For chlorpyrifos, concentrations of 100 and 500 ppm resulted in mortality values of 94.4 and 100%, respectively, with no significant difference between the two concentrations. At 50 ppm, mortality reached 83.7%, which, was lower than at 100 and 500 ppm. At 5 ppm, mortality was 40%, much higher than the control group (6.7%).

The median lethal concentrations (LC_{50}) determined in this study for *C. carnea* larvae, showed that the aqueous extract of *S. elaeagnifolium* had a lower toxicity than either of the synthetic insecticides. The value of the LC_{50} of the extract was greater than 500 ppm, which means that concentrations equal to or greater than this concentration would result in 50% mortality. Regarding chlorpyrifos, the estimated LC_{50} was 10.9 ppm (Table 2). Imidacloprid had the lowest LC_{50} with an estimated value of 3.6 ppm, which was lower than the lowest concentration tested (5 ppm). The accuracy of the LC_{50} estimate should therefore be viewed with caution.

Table 2. Median lethal concentration (LC_{50}) of chlorpyrifos on *Chrysoperla carnea* larvae

Insecticide	**LC_{50} (ppm)**	**LC_{50} fiducial limits at 95%**	
		Lower	**Upper**
Chlorpyrifos	10.9	3.8	31.8

The probit regression had a slope of 0.98, an intercept of 3.98 and an R^2 value of 0.97.

Adverse effects: After assessing larval mortality, the development of the surviving individuals was monitored through the pupal and adult stages of *C. carnea*. Several morphological changes were seen throughout the period of insect development.

The botanical extract of *S. elaeagnifolium* was associated with an elevated prevalence of *C. carnea* deformities or incomplete pupation. These morphological anomalies seriously impaired the normal development and transition of the pupae to the adult stage, significantly affecting the life cycle of the predator. Additionally, pupae that completed this stage showed a marked delay in their development. There were other instances where adults attempted to emerge from pupae but failed to finish their development. Observations revealed that these non-viable pupae eventually underwent necrosis and death. Even among the pupae that managed to emerge as adults, various adverse effects were observed. Some of these adults did not fully develop their bodies, including antennae and wings. As a result, they presented a dark abnormal coloration. Some adults that successfully formed wings and antennae had additional physical defects. These insects developed folded wings (in some cases) as well as dark and aberrant abdominal coloring (Fig. 1a). The morphological changes caused by imidacloprid (Fig. 1b.) and chlorpyrifos (Fig. 1c.) were identical to those observed with the botanical extract of *S. elaeagnifolium*. In the case of pupae, incomplete individuals or those with abnormal dark pigmentation were observed. In contrast, some adults had compressed bodies and/or an atypical dark coloration and some completely folded adults were observed with unextended wings.

The observed negative effects on pupal morphology reveal the developmental impacts of the botanical extract and the insecticides examined. These effects could result in a significant reduction in the emergence and survival of *C. carnea* adults. The current results are relevant to understanding the potential long-term effects of this biopesticide and insecticides on predator populations.

DISCUSION

When the findings on larval mortality following treatment with extracts of *S. elaeagnifolium* were compared to those from other plant extract studies, it was found that *S. elaeagnifolium* extracts obtained from fruits may exhibit the highest insecticidal capacity. For example, Markouk *et al*. (2000) found 50% mortality in the mosquito *Anopheles labranchiae* (Falleroni, 1926), at a fruit

extract concentration of 59 ppm. Similarly, Hamouda *et al*. (2015c) observed 34% mortality in *T. castaneum* and 23.6% mortality in *Myzus persicae* (Sulzer, 1776) after treating them with the *S. elaeagnifolium* fruit extract. Another study done by Hamouda *et al*. (2015a) found that the methanolic extract of berries had a 94% lethal antifeedant effect on *T. castaneum*. Hamouda *et al*. (2015b) reported that the same methanolic extract of fruit also had an antifeedant effect on *Spodoptera littoralis* larvae (Boisdual, 1833), resulting in 100% mortality.

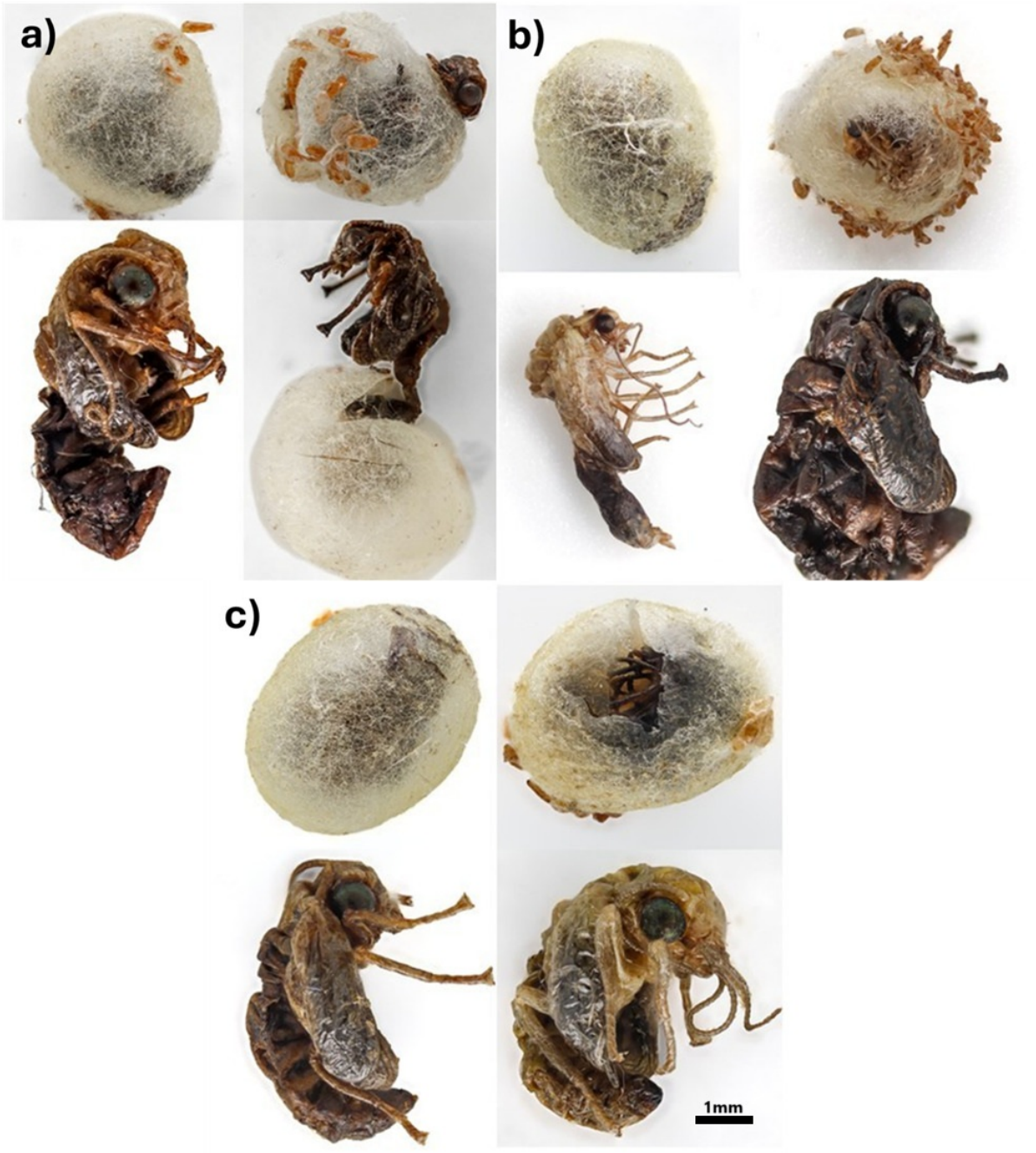

Figure 1. Adverse effects of different treatments on the development of *Chrysoperla carnea*. a) *S. elaeagnifolium* extract: induced atypical dark coloration in pupae, morphological malformations, and incomplete adult emergence; b) Imidacloprid: caused darkened pupae, deformities, and adults with folded wings and dark abdominal coloration; c) Chlorpyrifos: resulted in abnormal pupal pigmentation, pupal malformations, and undeveloped or darkly pigmented adults.

These results suggest that both the chemical composition of the extract and the specific plant part used may have a significant impact on the insecticidal activity of *S. elaeagnifolium* extracts. The observation that berry extracts appear more powerful than whole plant extracts is most likely attributable to changes in the concentration and profile of the bioactive compounds present. Weissenberg *et al.* (1998) reported that glycoalkaloids such as solamargine, solasonine, and solasodine are responsible for the toxic effects on insects and can inhibit larval development.

The results of imidacloprid are consistent with those of earlier research. Serratos-Tejeda *et al.* (2023) reported 100% mortality in third-stage *C. carnea* larvae after 72 hours of ingesting food contaminated with 0.35 g/L of imidacloprid. Similarly, Huerta *et al.* (2003) demonstrated that this insecticide has a highly toxic effect on this organism. In another study, Preetha *et al.* (2009) observed that a 100ppm concentration in an artificial diet caused 60% mortality in *C. carnea* larvae within 48 hours.

These results indicate that imidacloprid has a potent insecticidal effect on *C. carnea* larvae, especially at moderate to high concentrations (≥50 ppm). Lower concentrations of imidacloprid, such as 5 ppm, still caused notable mortality, but the effect was significantly lower. Furthermore, the mortality response varies depending on the concentration and exposure period.

The observed toxic effects of imidacloprid on *C. carnea* suggest that this compound could act through mechanisms of action like those of other neonicotinoids, such as interference with nicotinic acetylcholine receptors. However, further research is needed to understand the precise mechanisms of action of this substance and other variables that influence this insect.

The findings from this investigation on the insecticidal effect of chlorpyrifos are congruent with those published in the literature. Maia *et al*. (2016) observed 100% mortality after 24 hours in third instars of *C. carnea* exposed to 960 ppm of chlorpyrifos. Likewise, Abd-Ella *et al*. (2022) demonstrated a 50% lethal effect on second instars of this species at a very low concentration of 0.201 ppm. Hussain *et al*. (2012) also observed that chlorpyrifos was toxic to all three larval stages of *C. carnea*, resulting in 32-92% mortality after 48 hours of exposure to a very high concentration (4000 ppm), which suggests that their predator population may have been pesticide resistant.

Chlorpyrifos has a potent insecticidal effect on *C. carnea* larvae, even at relatively low exposure levels and over short periods of time. Furthermore, its lethal effect is observed across all instars. The mortality response of *C. carnea* was concentration dependent.

The findings on median lethal concentrations of *S. elaeagnifolium* differed from those reported by Markouk *et al*. (2000), who determined an LC_{50} of 59.8 ppm for an extract of *S. elaeagnifolium* fruits. This discrepancy might be attributed to a variety of factors that influence the composition and concentration of the metabolites present in the extracts of this plant species. In addition, the geographical origin of the plants is a key factor, since environmental conditions affect the accumulation of secondary metabolites with insecticidal properties. Similarly, the extraction procedures used, such as temperature, time and solvent, can favor the selective solubilization of certain compounds (Bitwell *et al*., 2023), influencing the insecticidal efficiency of the final extract. In addition, factors derived from the biology of the insect, such as the development stage of the *C. carnea* larvae or the environmental conditions of the bioassay, can also influence its susceptibility to *S. elaeagnifolium* extracts, contributing to the differences observed in the LC_{50} value. To reduce bias, we choose the L1 larvae, the most sensitive stage to demonstrate that the synthetic insecticides were significantly more toxic to *C. carnea* larvae than the botanical extract.

The median lethal concentration of imidacloprid in the present study differed from those published by Mohammadi *et al.* (2009), who recorded an LC_{50} of 24.6 ppm using direct contact exposure, and Ail-Catzim *et al.* (2015), who reported an LC_{50} of 165 ppm using the residual method. These discrepancies are likely due to differences in the exposure routes employed. While the present study assessed toxicity through ingestion, the other studies used contact or residual exposure, which can greatly influence larval susceptibility. Variations in experimental conditions, insect developmental stages, or environmental factors may also contribute to the observed differences in LC_{50} values. Nevertheless, imidacloprid was highly toxic to *C. carnea* larvae under all conditions.

The findings on the median lethal concentration of chlorpyrifos contrast with the findings of Abd-Ella *et al.* (2022), who reported a much lower LC_{50} of 0.201 ppm in second-instar larvae, using an immersion method. This again highlights the influence of exposure methods and developmental stages on insecticide toxicity. As with imidacloprid, these discrepancies are probably due to the exposure route used, which may significantly influence the susceptibility of *C. carnea* larvae to insecticides, in addition to other experimental conditions.

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