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EVALUATION OF CHLOROGENIC ACID EFFICACY AGAINST *FRANKLINIELLA OCCIDENTALIS* (PERGANDE)

Short communication

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ABSTRACT

The western flower thrips (*Frankliniella occidentalis* Pergande), a global agricultural pest, poses a serious crop threat to crops. Chlorogenic acid is a bioactive phytochemical pesticide that controls various insect pests. This study determined the susceptibility of western flower thrips to chlorogenic acid and other common insecticides, as well as the susceptibility of several field-collected populations to chlorogenic acid. Cross-resistance to four common insecticides was also measured. Chlorogenic acid had the highest toxicity of all insecticides tested. All field-collected populations were susceptible to chlorogenic acid, and little cross-resistance was detected between chlorogenic acid and other tested insecticides. Furthermore, analysis of cytochrome P450 enzyme activity in western flower thrips following treatment with LC_{50} of chlorogenic acid suggests that increased cytochrome P450 activity could be involved in chlorogenic acid detoxification. The sublethal effects of chlorogenic acid were then assessed, and it was found that treatment with LC_{25} of chlorogenic acid prolonged the duration of two developmental stages, reduced fecundity, and decreased the survival rate of treated western flower thrips compared to untreated insects. Overall, these findings demonstrate substantial toxicity and significant sublethal effects of chlorogenic acid on western flower thrips, suggesting that overexpression of cytochrome P450 genes may be associated with chlorogenic acid detoxification.

Key words: botanical insecticide, sublethal effects, western flower thrips

INTRODUCTION

Pest management is an essential component of agricultural production. Synthetic chemical pesticides are the dominant pests control agent and have been widely used for decades to combat insect pests, usually with effective results. However, long-term use of chemical pesticides has resulted in undesirable side effects, including ecosystem destruction, high levels of chemical residues in the environment and food chain, and sublethal impacts on nontarget insects (Woodcock et al. 2017; Zhou et al. 2025). Due to their high biodegradability, exceptional specificity of action, low toxicity, and limited adverse effects on nontarget organisms, bioinsecticides have been proposed as a suitable alternative to pesticides (Ayilara et al. 2023). To survive herbivorous attacks, plants have evolved various environmental adaptations, including physiological adaptations. Specific natural plant components, known as specialized metabolites, have been evaluated for potential use as commercial pest control compounds that protect plants from insect pests (Al-Khayri et al. 2023).

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Herbivore resistance is facilitated by various key phytochemicals, notably phenolics and flavonoids (Kumar et al. 2023; Ahlawat et al. 2024). Chlorogenic acid, tannic acid, and methyl jasmonate are examples of plant phenolic compounds that exhibit toxicity to insect pests and negatively impact key physiological processes (Tan et al. 2022; Chen et al. 2023; Wang et al. 2023). Over the past decade, researchers have focused on plant-derived pesticides due to their safety and low overall environmental toxicity. Among plant-based insecticides, chlorogenic acid is relatively environmentally friendly compared to conventional synthetic pesticides.

Cytochrome P450 monooxygenases (P450s) are multifunctional biocatalysts with extensive enzymatic activity on several substrates that are part of insect oxidase systems. One of the standard mechanisms of resistance to various xenobiotics is metabolic detoxification, in which P450s play a key role in the detoxification of synthetic and natural toxins (Lu et al. 2021). P450 genes can be highly expressed when insects are exposed to xenobiotics, such as pesticides and plant metabolites. Treatment of *Helicoverpa armigera* with allelochemicals increases the expression of the P450 genes CYP6B7, CYP6B6, and CYP6B2, thereby reducing the susceptibility of the bollworms to methomyl (Chen et al. 2019).

In recent years, threats from exotic, invasive pest species have been significant. Some ornamental and agricultural plants worldwide are threatened by the invasion of the polyphagous western flower thrips (WFT) (Reitz et al. 2020). The life cycle of WFT encompasses egg, instars, prepupa, pupa, and adult (Lewis 1973). Gerbera is one of the most valuable ornamental plants, ranking fifth in the global cut flower market (Deng & Bhattarai 2018). WFT deforms the leaves and flowers of gerberas, causing silvery lesions that reduce their aesthetic value and make them unsuitable for sale in the global market (Madam et al. 2022).

The widespread and long-term use of various synthetic pesticides to suppress WFT has led to increased insect resistance to these pesticides (Bass et al. 2015; Demiröz & Kumral 2022). Therefore, finding an environmentally friendly alternative that can help delay the emergence of insecticide resistance is crucial. A previous study conducted in our laboratory examined the biochemical and metabolite levels of 'Latara' gerbera resistance to WFT (Talla & Vidyavardhini 2024). Preliminary evaluation of WFT mortality rates on gerbera 'Latara' and 'Faith' flower buds revealed that the former exhibited strong resistance to WFT shortly after infestation. The results of profiles obtained by gas chromatography-mass spectrometry revealed three significant characteristics, including increased antioxidant enzyme activity and concentrations of various secondary metabolites – alkaloids and flavonoids etc., in the WFT-resistant gerbera cultivar after infestation.

The objective of this study was to assess cross-resistance to nine pesticides approved for use in India and chlorogenic acid (CA). The next objective was to assess the expression of genes involved in resistance to CA in the WFT, including glutathione S-transferase (GST), esterase (EST), and cytochrome P450, as well as the effect of CA on the survival and development of the WFT.

MATERIALS AND METHODS

Insects

WFT were initially collected from damaged gerbera flowers. WFT were tested as part of a basic susceptibility study as described by Guillén et al. (2014). Insects were initially fed gerbera plants (without pesticides) under a 14/10 h light/dark photoperiod, at a temperature of 26 ± 2 °C, and a relative humidity (RH) of 55 ± 5%. Adults 3-day-old or younger were randomly sampled for all assays, and their numbers were approximately equal to those of males and females.

Chlorogenic acid (CA) toxicity to western flower thrips (WFT)

In this experiment (Table 1), nine products registered for use against WFT in India were tested along with CA (Sigma-Aldrich), a bioactive polyphenolic compound found in many plants (Olthof et al. 2001).

CA dosage was based on experimental studies. All bioassays were conducted on adult WFT as described by Jahani et al. (2018). Each chemical was prepared at five different working concentrations with four replicates. One bioassay replication consisted of 30–40 adult WFT that were randomly sampled and placed in a bioassay tube containing either a pesticide or an artificial food solution without insecticides (control). WFT were considered dead after 96 hours in the tube if they remained immobile when brushed with a fine-bristle brush. Survival and mortality rates were then determined and documented.

Detoxifying enzyme activity assays

GST, EST, and cytochrome P450 activity were measured using the method previously described by Chen et al. (2011) with minor modifications. The control group consisted of adult WFT treated with distilled water for the same duration as the treatment groups. The LC_{50} treatment group consisted of individuals that survived exposure to the median lethal concentration for 96 hours. Each experimental group contained one replicate of 200 thrips of each sex. For biochemical enzymatic assays, preparations were made in triplicate for each of the three classes of detoxification enzymes. protein content was determined by measuring bovine serum albumin (BSA) as a reference, according to the method of Bradford (1976).

Sublethal effect of chlorogenic acid (CA) on western flower thrips (WFT)

In this study of CA toxicity to WFT, sublethal concentrations (LC_{10}, LC_{25}, and LC_{50}) were determined and used to assess various fitness parameters. Two insect-proof cages (one for the LC_{10}, LC_{25},and LC_{50} treatments and the other for the control) were set up with 12 clean cotton plants each. Following a 96-hour feeding period with either LC_{25} or the control, 120 adult WFT treated with LC_{25} were placed on gerbera buds to observe egg-laying behavior, with a control group for comparison. The plants were removed after the 12-hour oviposition period, and eggs were marked on leaves in clamp cages. These cages were maintained at room temperature, and newly emerged adults were evaluated for fecundity. Egg hatch rates were monitored until all test subjects died. This methodology was adapted from Gong et al. (2009) with minor adjustments.

Statistical analysis

To assess the mortality of insects exposed to CA at different concentrations, probit analysis was performed using PoloPlus (Le Ora Software 2002). Resistance levels were determined based on a published method, and the resistance ratio (RR) was calculated as the ratio of LC_{50} (field-collected population) to LC_{50} (Wang et al. 2023). Resistance levels were classified as low (RR 5–10-fold), moderate (RR 10–40-fold), high (RR 40–160-fold), and very high (RR > 160-fold). Student's t-test was used to analyze significant differences in growth time, viability, fecundity, oviposition time, and hatching rate of WFT between the control and treated groups, as well as in detoxifying enzyme activity. All statistical analyses, such as the chi-squared test, Student's t-test, and one-way ANOVA (Tukey's HSD tests), were performed using SigmaPlot 12.

Table 1. List of insecticides against western flower thrips (WFT) approved in India and their recommended dose concentrations

Insecticide	Active ingredient content	Recommended dose	Formulation	Trade name in India
Fipronil	5% SC	0.5–1.0 ml·L^{-1} water	suspension concentrate	Regent
Imidacloprid	17.8% SL	0.25–0.5 ml·L^{-1} water	soluble liquid	Confidor
Chlorpyrifos	20% EC	2.0–2.5 ml·L^{-1} water	emulsifiable concentrate	Dursban
Thiamethoxam	25% WG	0.2–0.3 g·L^{-1} water	water-dispersible granules	Actara
Spinosad	45% SC	0.15–0.3 ml·L^{-1} water	suspension concentrate	Tracer
Malathion	50% EC	2.0 ml·L^{-1} water	emulsifiable concentrate	Cythion
Abamectin	1.9% EC	0.3–0.5 ml·L^{-1} water	emulsifiable concentrate	Vertimec
Cypermethrin	10% EC	1.0–1.5 ml·L^{-1} water	emulsifiable concentrate	Superkiller
Acephate	75% SP	1.0–1.5 g·L^{-1} water	soluble powder	Acemain
Chlorogenic acid*	95% pure	1.0–1.5 g·L^{-1} water	crystalline powder	Sigma-Aldrich, cat. no. C3878

* not registered as a commercial insecticide in India

RESULTS

Effectiveness of synthetic pesticides and chlorogenic acid (CA) against western flower thrips (WFT)

Table 2 presents the lethal concentration (LC_{50}) values of CA and nine synthetic insecticides on adult WFT. The LC_{50} represents the concentration of each insecticide required to kill 50% of the WFT population, and the slope of the curve indicates the steepness of the dose-response. Key findings from this study revealed that CA had an LC_{50} of 0.910 mg·L^{-1}, with a lower slope (1.110), indicating moderate toxicity. Fipronil had the lowest LC_{50} value (0.014 mg·L^{-1}), indicating its highest toxicity. Imidacloprid and cypermethrin exhibited much higher LC_{50} values (17.882 mg·L^{-1} and 74.798 mg·L^{-1}, respectively), indicating the lowest toxicity. Malathion and chlorpyrifos LC_{50} values (0.869 mg·L^{-1} and 0.170 mg·L^{-1}) indicate higher potency than imidacloprid and cypermethrin but lower than fipronil. Acephate was the least toxic (101.800 mg·L^{-1}). Thiamethoxam, spinosad, and abamectin had medium LC_{50} values, indicating moderate toxicity. The results highlight that fipronil is the most effective insecticide, while acephate and cypermethrin are among the least effective.

Effect of chlorogenic acid (CA) on detoxification enzymes of western flower thrips (WFT)

Table 3 illustrates the activity of metabolic enzymes in WFT individuals under control conditions and those treated with the LC_{50} of CA. P450s activity was significantly different from the control (0.61 pmol·min^{-1}·mg^{-1} and the LC_{50} 1.41 pmol·min^{-1}·mg^{-1}, 2.3-fold). EST and GST activity did not differ significantly between the control and samples treated with CA at the LC_{50}.

Effect of chlorogenic acid (CA) on the length of the duration of developmental stages from egg to adult of the western flower thrips (WFT)

The developmental stages of WFT exposed to sublethal concentrations of CA (LC_{10}, LC_{25}, LC_{50}) compared to the control differed significantly with increasing CA concentration (Table 4), showing that the time to egg development increased with CA concentration, from 64.3 hours in the control to 69.1 hours at LC_{50}. A similar trend was observed for the first and second instar stages, where development times increased by 6.8 and 2.7 hours at LC_{50} compared with the control. For the prepupal and pupal stages, the differences were 7.2 and 4.8 hours. The total time from egg to adult showed an apparent increase at higher CA concentrations, ranging from 256.3 hours in the control to 270.5 hours at the LC_{50} (difference of 24.2 hours).

Effect of chlorogenic acid (CA) on the survival of western flower thrips (WFT) at different developmental stages

The survival rates (%) of different developmental stages of WFT after exposure to sublethal concentrations of CA (LC_{10}, LC_{25}, LC_{50}) compared to the control are shown in Figure 1. In the control, survival rates ranged from 90% to 100%. The LC_{10} concentration was insignificantly lower. The LC_{25} concentration did not reduce survival, only in the prepupal and adult stages. The LC_{50} concentration reduced survival to 50–60% in all stages. Overall, increased CA concentrations progressively reduced survival in all stages, particularly in adults.

Effect of chlorogenic acid (CA) on longevity, oviposition period, fecundity, oviposition rate and sex ratio

Table 5 highlighted how sublethal concentrations of CA negatively affected reproductive and longevity parameters of WFT. Female longevity decreased from 676 hours in the control to 412 hours at the LC_{50}, and male longevity decreased from 387 hours to 267 hours. Oviposition period significantly decreased from 604 hours to 329 hours at the LC_{50}. Fecundity and oviposition rate decreased, with fecundity decreasing from 88.65 to 70.20 and oviposition rate from 4.41 to 3.19 offspring per day at the LC_{50}. These results indicate that exposure to CA considerably affected reproductive and longevity parameters of WFT, particularly at higher sublethal doses.

Table 2. Average lethal concentration (LC_{50}) of nine synthetic insecticides and chlorogenic acid on adult western flower thrips (WFT)

Insecticide solution	LC_{50} (95% [a]CL) (mg L^{-1})	Slope ± SE	X^2
1 Fipronil	0.014 (0.012–0.017)	3.709 ± 0.636	1.695
2 Imidacloprid	17.882 (14.331–22.119)	1.739 ± 0.186	2.926
3 Chlorpyrifos	0.170 (0.152–0.188)	3.910 ± 0.413	2.414
4 Thiamethoxam	4.803 (2.694–7.285)	1.245 ± 0.138	6.452
5 Spinosad	0.456 (0.266–0.779)	2.035 ± 0.239	9.966
6 Malathion	0.869 (0.775–0.956)	5.040 ± 0.628	2.870
7 Abamectin	1.802 (1.253–2.405)	2.005 ± 0.217	4.116
8 Cypermethrin	74.798 (58.977–94.058)	1.620 ± 0.181	1.583
9 Acephate	101.800 (66.163–42.475)	0.974 ± 0.123	2.700
10 Chlorogenic Acid	0.910 (0.621–1.150)	1.110 ± 0.191	3.241

[a]CL – confidence limit; Number of insects (*n*) per each treatment is 200; X^2 – chi-squared test

Table 3. Metabolic enzyme activity (mean ± SE) in control western flower thrips (WFT) individuals and those treated with the median lethal concentration (LC_{50}) of chlorogenic acid

Treatment	P450s activity (pmol·min^{-1}·mg^{-1})	EST activity (nmol·min^{-1}·mg^{-1})	GST activity (nmol·min^{-1}·mg^{-1})
Control	0.61 ± 0.04 b	321.21 ± 30.13 a	42.18 ± 27.31 a
LC_{50}	1.41 ± 0.08 a	341.05 ± 29.18 a	51.07 ± 31.76 a

Mean in a single column followed by the same letter do not differ significantly according to Student's t-test ($p < 0.05$)

Table 4. Developmental time (hours; mean ± SE) from egg to adult of western flower thrips (WFT) following exposure to sublethal concentrations of chlorogenic acid

Stage	Control	LC_{10}	LC_{25}	LC_{50}
Egg	64.3 ± 0.7 a	66.2 ± 0.5 a	67.0 ± 0.7 b	69.1 ± 0.7 c
First instar	56.6 ± 1.0 a	58.3 ± 0.5 b	61.2 ± 0.2 c	63.4 ± 1.7 d
Second instar	69.1 ± 1.9 a	69.4± 0.5 b	70.6 ± 0.5 c	71.8 ± 1.4 d
Prepupa	32.9 ± 0.2 a	35.8 ± 0.7 a	38.6 ± 0.0 b	40.1 ± 0.5 b
Pupa	33.4 ± 0.5 a	34.6 ± 0.0 a	36.5 ± 0.7 b	38.2 ± 0.2 b
Egg to adult	256.3 ± 3.2 a	257.0 ± 2.65 a	262. 2 ± 1.9 b	270.5 ± 2.2 c

Different letters in the same row indicate significant differences (one-way ANOVA followed by Tukey's HSD tests, $p < 0.05$)

Table 5. Longevity, fecundity, oviposition, and sex ratios in western flower thrips (WFT) after exposure to sub-lethal concentrations of chlorogenic acid

Parameters	Control	LC_{10}	LC_{25}	LC_{50}
Longevity – female (hours)	675.8 ± 5.3 a	643.2 ± 6.7 a (−5%)	554.9 ± 5.3 b (−18%)	411.6 ± 11.0 c (−39%)
Longevity – male (hours)	386.9 ± 3.8 a	348.2 ± 7.2 b (−10%)	312.5 ± 13.2 b (−19%)	266.9 ± 1.7 b (−31%)
Oviposition period (hours)	604.3 ± 8.0 a	569.0 ± 9.6 a (−6%)	457.2 ± 21.4 b (−24%)	328.6 ± 21.1 c (−46%)
Fecundity (first instars to female)	88.7 ± 0.4 a	82.0 ± 0.4 b (−8%)	78.2 ± 0.6 b (−12%)	70.2 ± 0.6 c (−21%)
Oviposition rate (first instars to female per day)	4.4 ± 0.1 a	4.2 ± 0.1 a (−5%)	4.1 ± 0.1 b (−8%)	3.2 ± 0.0 c (−28%)

Values in parenthesis indicate percentage decrease; Different letters in the same row indicate significant differences (one-way ANOVA followed by Tukey's HSD tests, $p < 0.05$)

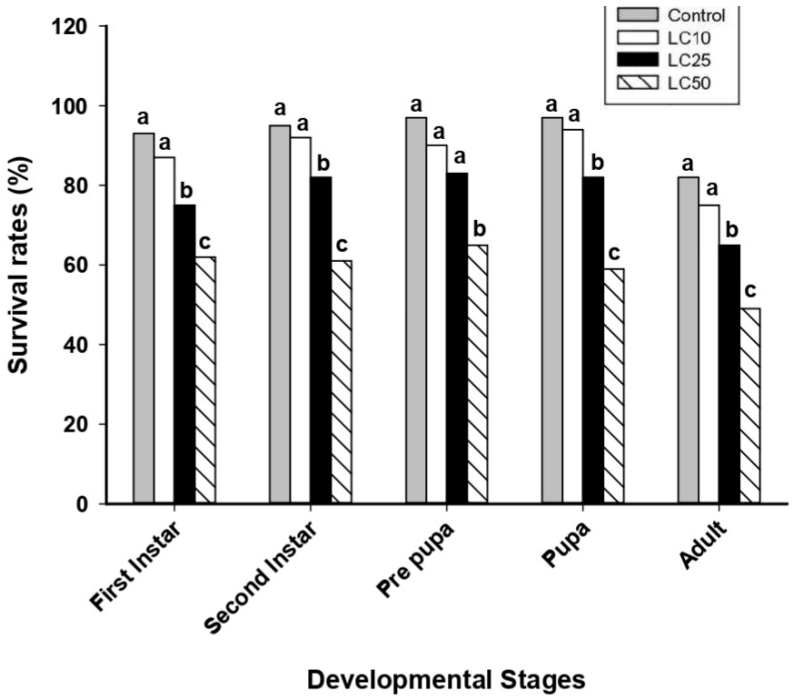


Figure 1. Survival rates (%) of western flower thrips (WFT) developmental stages after exposure to sublethal concentrations of chlorogenic acid

Different letters in the same row indicate significant differences (one-way ANOVA followed by Tukey's HSD tests, $p < 0.05$)

DISCUSSION

Plant metabolites show promise as botanical insecticides, providing alternatives to synthetic chemicals. However, there is limited information on their effectiveness against WFT. This study found that CA exhibited substantial toxicity against WFT, comparable to commercial insecticides, aligning with studies that demonstrated its lethal effects on pests such as *Mythimna separata* and *Bemisia tabaci* (Lin et al. 2022; Wang et al. 2023). These findings suggest that CA could be a potential botanical pesticide for field use, offering a more environmentally friendly alternative to synthetic chemicals.

Our results indicate that P450s play a primary role in the metabolism and detoxification of CA in WFT. The observed 2.3-fold increase in P450s activity in response to CA reflects a strong enzymatic response, aligning with the known function of P450s in breaking down plant allelochemicals and pesticides, preventing their accumulation in the organism (Zhang & Yang 2021). In contrast, the increase in EST and GST activity was moderate and not statistically significant, suggesting a lesser role for these enzymes in the regulation of CA. Minor changes in their activity may indicate that other metabolic pathways, particularly those mediated by P450s, are more efficient or better adapted to detoxify this compound (Bosch-Serra et al. 2021).

The significant upregulation of P450s underscores their crucial role in detoxification, which may have potential implications for understanding the mechanisms of resistance in WFT to plant-derived chemicals and insecticides (Lu et al. 2021). The selective enzyme activity suggests that WFT utilize specific detoxification pathways depending on the xenobiotic involved (Amezian et al. 2021). This selective response is crucial for pest survival and may influence pest management strategies by guiding the design of chemicals that bypass P450-dependent detoxification. Sublethal exposure to CA prolongs development at each stage, potentially delaying reproductive cycles, reducing the number of seasonal generations, and slowing population growth. Prolonged residence time in sensitive stages, such as eggs or pupae, may increase the risks of predation or parasitism. This delay likely stems from metabolic stress, as CA disrupts physiological processes,

directing resources towards detoxification rather than growth. Continued exposure may exert selective pressure, leading to the development of resistance by enhancing detoxification pathways (Andreazza et al. 2021; Wu et al. 2022; Yuan et al. 2023).

CA may affect multiple pathways, as reduced survival across developmental stages suggests interference with key metabolic functions such as molting, energy production, and detoxification. This is consistent with studies that CA exposure increases the activity of metabolic enzymes (e.g., P450s), indicating a potentially overloaded detoxification system (Lin et al. 2022; Wang et al. 2023). Notable declines in survival in the prepupal and pupal stages suggest possible hormonal disruptions necessary for metamorphosis (Tlak Gajger & Dar 2021). Given these effects, CA or its derivatives may be effective biological agents as part of an Integrated Pest Management strategy for controlling WFT populations. Our findings showed that exposure to CA prolongs egg development, suggesting it may impair embryo growth and delay hatching. This compound may also disrupt physiological processes necessary for the transition from the first to the second instar, causing developmental delays that persist into later stages. The prolonged duration of the second instar may be due to the cumulative effects of the initial exposure. In the prepupal stage, CA may interfere with hormonal or metabolic processes required for pupation. Delays in the pupal stage, however, suggest an effect on metamorphosis, potentially affecting energy metabolism or hormonal regulation. Overall, CA at sublethal concentrations slows the development of WFT across various developmental stages, prolonging the time to adulthood, which may impact population dynamics and fitness.

This study reveals a significant effect of CA on the survival of WFT at various developmental stages at sublethal concentrations (LC_{10}, LC_{25}, LC_{50}) compared to the control. The progressive decline in survival rates from the first instar stage to adulthood indicates cumulative toxic effects. Although individuals may survive the early stages, their probability of reaching adulthood decreases rapidly with increasing exposure, especially at LC_{50}, where adult survival is reduced to 49%. High adult susceptibility suggests that accumulated physiological stress and disrupted metabolic pathways are key factors. The observed substantial mortality, particularly among adults, suggests that exposure to CA may reduce reproductive capacity and contribute to population declines over time. The study revealed that increasing concentrations of CA significantly reduce several life cycle traits, indicating toxicity even at sublethal levels. WFT females showed notably reduced longevity, likely due to physiological stress or metabolic disruption, similar to males (Ramaroson et al. 2022). Although males naturally have shorter lifespans, CA further reduces their survival, potentially limiting reproductive opportunities and affecting population sustainability.

CA exposure significantly shortened the oviposition period, indicating a disruption of the reproductive cycle, likely due to physiological stress (Yousuf et al. 2024). Fecundity and oviposition rate also decreased with increasing CA concentrations, reflecting toxic effects that impair effective reproduction (Ramaroson et al. 2022). Furthermore, the decrease in the female-to-total offspring sex ratio suggests a potential future impact on population growth due to reduced reproductive capacity. The novelty of this study lies in demonstrating that CA not only affects reproductive parameters in WFT but also creates a cascade of effects that could affect overall population dynamics. The finding that sex ratio shifts toward fewer females is particularly significant, as it suggests that the effects of the compound extend beyond immediate toxicity and potentially threaten long-term population sustainability.

CONCLUSION

In conclusion, chlorogenic acid has been shown to have a significant lethal effect on western flower thrips. Furthermore, as part of a comprehensive evaluation of the suitability of an insecticide for field use, elucidating its sublethal effects is crucial. This study provides new understanding of the sublethal effects of chlorogenic acid on western flower thrips, enabling more effective use of this compound while minimizing the expenses associated with western flower thrips management and yield losses due to herbivory.

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