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Adverse effects of chlorfenapyr and chlorantraniliprole on silkworm *Bombyx mori* L. parameters and reduction of their effects using ascorbic acid

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ABSTRACT

Pesticides used to control pests challenge silk production because they contaminate mulberry leaves. We determined the toxicity of chlorfenapyr and chlorantraniliprole on the 5th larval instar of the silkworm *Bombyx mori* L by feeding it on sprayed mulberry leaves (*Morus alba* var. *indicia*), as well as approaches to protect silkworms from their effects using ascorbic acid. The LC_{50} values were 10.32 and 13.17 ppm for chlorfenapyr, and chlorantraniliprole, respectively, after 72 h. The larval weight, cocoon weights, cocooning percentages, and silk productivity parameters of individuals fed on leaves treated with 1% ascorbic acid solution were 2.21 g, 1.18 g, 93.05%, and 2.42 cg/d, respectively. While feeding on leaves treated with chlorfenapyr, they were 1.87 g, 1.01 g, 90.0%, and 1.24 cg/d, respectively, and when feeding on leaves treated with chlorantraniliprole, they were 1.91 g, 1.01 g, 90.14%, and 1.36 cg/d, respectively. Furthermore, in this work, we proved the potential of ascorbic acid to protect silkworms from the adverse effects of the examined pesticides. Feeding larvae on mulberry leaves treated with 1% ascorbic acid in the first day before pesticide application resulted in enhanced cocoon weights, cocooning percentages, and silk productivity (1.06 g, 91.95%, and 1.53 cg/d, in the chlorfenapyr with 1% ascorbic acid), and (1.08 g, 91.25 %, and 1.66 cg/d, in the chlorantraniliprole with 1% ascorbic acid).

Keywords: Ascorbic acid, *Bombyx mori*, Chlorantraniliprole, Chlorfenapyr, Non-target organism

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1. INTRODUCTION

The domestic silk moth, *Bombyx mori* (Linnaeus, 1758), (*Lepidoptera*; *Bombycidae*) is an important economic insect since it is the producer of silk. it is dependent on humans for reproduction **(Wu *et al.*, 2010).** Considering the worldwide interest in producing high-quality silk, research on the development of silkworm and mulberry crops is extremely important. The use of pesticides to control pests is a major challenge for silk production. Due to the difficulty in preventing pesticide drifting when applied, the insect is exposed to pesticides by contaminating mulberry leaves with pesticide residues. Several negative effects of pesticides on silkworms, including toxicological, physiological, and biological effects, have been demonstrated in different studies on various groups of pesticides. Additionally, the effect on the silkworm spinning process and the cocoon quality ***(Gao et al.*, 2018)**. The use of pyrethroid, neonicotinoid, and organophosphate insecticides resulted in the appearance of symptoms of silkworm poisoning, which included stiffness of the body, vomiting, tremors, and a shortened body with lethal effects. **(Zhang *et al*., 2008; Yu *et al*., 2016)**. The survival rate, body weight and cocoon quality could be affected by the residue of different pesticides in mulberry leaves among these, indoxcarb, acetamiprid, dinotefuron, flonicamid, azadiractin and chlorantraniliprole **(Munhoz *et al*., 2013; Yeshika *et al*., 2019; Lu *et al*., 2021; Wang *et al*., 2023).** There are also some physiological effects associated with the use of some pesticides, pyriproxyfen led to the apoptosis and separation of posterior silk glands and the decreased activity of cholesterol and alanine aminotransferase in hemoglobin **(Etebari *et al*., 2007; Li *et al*., 2023).** Similarly, the use of chlorantraniliprole caused *B. mori* to develop abnormal glands, apoptosis, disrupt energy homeostasis, and oxidative stress, as well as impact on chitin synthesis. **(Liu *et al*., 2022; Mao *et al*., 2022; Gu *et al*., 2023).** In another study, chlorantraniliprole had a negative effect on *B. mori* by decreasing its biological defense system, thus increasing its susceptibility to diseases. **(Zhu *et al*., 2023).** The metabolic activity of silkworms is significantly altered after exposure to chlorfenapyr **(Shao *et al*., 2021).** The damage caused to silkworms by pesticides was a testament to their high sensitivity to them **(Bora *et al*., 2012)**. Subsequently**,** it is necessary to use certain measures to overcome this problem. Antioxidants are one of the strategies that provide silkworms with protection during larval development from the reactive oxygen species (ROS) formed **(Sahoo *et al*., 2016)**. Proteins, DNA, and carbohydrates are harmed by the production of reactive oxygen species (ROS) during metabolism. In the cells of living organisms, ascorbic acid and glutathione (GSH) act as defenses against (ROS) **(Gao *et al*., 2018; Jena *et al*., 2014; Sahoo *et al*., 2016).** Nevertheless, several pesticides can increase ROS production **(Gao *et al*., 2018)**. The focus of our current study was on two unique systemic pesticides, chlorfenapyr and chlorantraniliprole, which are commonly utilized in agriculture to control several pests **(Shao *et al*., 2021; Gu *et al*., 2023)**. Chlorfenapyr is a pyrroles class acaricide and insecticide that is activated by monooxygenases to oxidize the N-ethyl methyl group. It causes target organisms' death through uncoupling oxidative phosphorylation in mitochondria, thus disrupting cellular respiration and ATP production **(Shao *et al*., 2021; Yoon and Tak, 2022; Huang *et al*., 2023).**

Chlorantraniliprole is an ingredient of the anthranilic diamide class. It is an agonist for the insect ryanodine receptor (RyR), which releases calcium from the intracellular calcium channels. It occurs as an abnormal release of calcium into muscle cells, which can cause death and paralysis **(Munhoz *et al*., 2013; Kumar *et al*., 2017; Gu *et al.*, 2023).** The aim of this investigation is to investigate the harmful impacts of chlorfenapyr and chlorantraniliprole on the survival rate, pupae and larvae weight, silk productivity, and cocoon quality. Additionally, examine whether ascorbic acid may reduce the negative effects of the tested pesticides when provided to *B. mori* larvae before chlorfenapyr and chlorantraniliprole exposure.

2. MATERIALS AND METHODS

2.1. The chemicals used

Chlorantraniliprole **(**Coragen, 20% Sc) was purchased from Agrimatco Ltd – Egypt. Chlorfenapyr (Concord, 24 % SC) was purchased from Egyptchem international. Ascorbic acid 99% in powder form was purchased from Sigma-Aldrich.

2.2. Silkworms rearing

The egg box of silkworm, *B. mori* (local hybrid) was obtained from the Sericulture Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, then it was kept at laboratory conditions in the dark until hatching. The larvae of *B. mori* were reared on fresh mulberry leaves (*Morus alba* var. *indicia*) under laboratory conditions of 26 ± 2 ºC, 75 ± 5 % RH.

All experiments were carried out during the spring season of 2023 in the entomology laboratory, plant protection department at the Faculty of Agriculture, Fayoum University, Egypt.

2.3. Lethal effect experiment

The 5th silkworm larval instar was used to assess the tested pesticides. Chlorfenapyr and chlorantraniliprole were prepared in the following five concentrations: 5, 10, 20, 30, and 40 ppm, in addition to untreated control, it was only with distilled water. Each treatment was replicated three times, with thirty larvae per replicate used. Mulberry leaves were divided into groups and sprayed each group with one of the previous concentrations, then were left to air dry at laboratory conditions for 30 min. The 5th larval instar of silkworms was allowed to feed on treated mulberry leaves. The mortality percentages were recorded after 24, 48 and 72 hours of treatment and corrected according to Abbott's formula **(Abbott, 1925)**. The toxicity line was determined using Finney analysis **(Finney, 1971).**

2.4. Experiment of reducing the effect of pesticides by ascorbic acid

In this experiment, the LC_{25} values of pesticides after 72 hours of treatment were utilized. The 5th Larval instar individuals were divided into six groups. Each group contained three replicates (30 larvae/replicate). The first group of larvae were provided with mulberry leaves sprayed with a 1% solution of ascorbic acid on the first and second days of their 5th larval instar. On the first and second days, the second and third groups were fed on mulberry leaves treated with low concentrations (the LC_{25} values) of chlorfenapyr and chlorantraniliprole, respectively. The fourth and fifth groups were fed on mulberry leaves treated with 1% solution of ascorbic acid on the first day, then on the second day feeding at low concentrations (LC_{25}) of chlorfenapyr and chlorantraniliprole. On the third day of the 5th larval instar, all groups were fed on mulberry leaves sprayed with double distilled water. The sixth group was the control, where larvae were fed on mulberry leaves sprayed with double distilled water, for three days, this method

according to **(Gao *et al.*, 2018)**. Biological parameters of *B. mori*, including fifth larval instar and pupal weights, mortality rate and fifth instar larval durations were recorded. Also, the cocooning percentages, cocoon weights, cocoon shell weights, cocoon shell ratio and silk productivity were calculated as economical parameters.

2.5. Statistical analysis

Data was analyzed using One Way ANOVA through the statistical package for social science (SPSS 16.0) to find out the significance between treatments and control **(Berkowitz and Allaway, 1998).** The least significant differences test (L.S.D at 0.05%) was applied.

3. RESULTS

3.1. Lethal effect of pesticides

Chlorfenapyr and chlorantraniliprole were examined on the 5th silkworm larval instar after 24, 48 and 72 hours of spraying. Results in Table 1 showed the toxicity of both chlorfenapyr and chlorantraniliprole on *B. mori* larvae. Chlorfenapyr was more toxic than chlorantraniliprole at LC_{50} and LC_{90} values. After 24, 48, and 72 hours, the LC_{50} values for chlorfenapyr were 13.87, 12.17, and 10.32 ppm, while for chlorantraniliprole, they were 19.36, 17.33, and 13.17 ppm. Consequently, chlorfenapyr and chlorantraniliprole were highly harmful to the 5th instar silkworm larvae which represents an issue to silkworm rearing.

Table 1. Lethal effect of chlorfenapyr and chlorantraniliprole on the 5th silkworm larval instar, i.e., 24, 48 and 72 h post treatment

Pesticide	Time (hour)	LC_{25} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)	Slope±SE*
Chlorfenapyr	24	5.89	13.87	70.99	1.81±0.25
	48	5.69	12.17	51.81	2.04±0.29
	72	5.35	10.32	36.21	2.35±0.31
Chlorantraniliprole	24	8.29	19.36	97.80	1.82±0.27
	48	7.24	17.33	92.99	1.75±0.29
	72	5.50	13.17	69.99	1.77±0.28

SE: Standard error; LC; Lethal Concentration.

3.2. The impact of tested pesticides on biological parameters of *B. mori*

3.2.1. Larval weights

Data in Table 2 demonstrated that the 5th larval instar of the first group, which was fed on mulberry leaves treated with 1 % ascorbic acid solution, had the highest weight (2.21 g). The larvae weights of the fourth and fifth groups were 2.05 g and 2.05 g, respectively, which were fed mulberry leaves treated with 5.35 ppm of chlorfenapyr and 5.50 ppm of chlorantraniliprole after initially feeding on a 1 % ascorbic acid solution. On the contrary, the larvae weights of the second and third groups (larvae exposure to chlorfenapyr and chlorantraniliprole) were recorded at 1.87 g and 1.91 g, respectively. Thus, the larvae consuming leaves contaminated with chlorfenapyr and chlorantraniliprole were detrimentally affected, as they had the least weight.

3.2.2. Pupal weights

The pupal weight of the first group, which was fed on mulberry leaves sprayed with 1 % ascorbic acid solution for the first two days, was the highest among all other groups. It exhibited 0.9 g, however, pupal weights were recorded 0.85 g, 0.81

g, 0.79 g, 0.71 g and 0.70 g, for the control, chlorfenapyr+1% ascorbic acid, chlorantraniliprole+1% ascorbic acid, chlorantraniliprole and chlorfenapyr groups, respectively, as shown in Table 2.

3.2.3. Larval durations

According to Table 2, larval duration ranged between 9.00 and 10.50 days for 1% ascorbic acid solution and chlorfenapyr+1% ascorbic acid, respectively**.** The differences between the treated groups were not significant.

3.2.4. Larval mortality percentage

The mortality percentages in the treated and control groups were shown in Table 2. It is noted that the mortality percentages in the 5th larval instar of silkworms were reduced (7.50 % of the fourth group and 7.75% of the fifth group) when they were fed on mulberry leaves treated with ascorbic acid before exposure them to chlorfenapyr and chlorantraniliprole, compared to feeding them with pesticides only (8.50 % and 8.00 %, respectively). The mortality percentage was recorded at 6.50 % in larvae fed on mulberry leaves treated with a 1 % ascorbic acid solution. While it was recorded 7.00 % in the control group. These results demonstrate that ascorbic acid confers resistance to larvae from pesticide poisoning. Where the mortality rate of larvae decreased in contrast to the second and third groups that were only exposed to pesticides.

Table 2. Effect of chlorfenapyr and chlorantraniliprole on biological parameters of *B. mori,* with and without adding ascorbic acid

Groups	Fifth instar larval weight (g)	Pupal weight (g)	Fifth instar larval duration (d)	Fifth instar mortality (%)
First group (1 % ascorbic acid)	2.21±0.034	0.9±0.057	9.00±0.153	6.50±0.065
Second group (5.35 ppm Chlorfenapyr)	1.87±0.056	0.70±0.050	10.38±0.146	8.50±0.048
Third group (5.50 ppm Chlorantraniliprole)	1.91±0.050	0.71±0.042	10.48±0.156	8.00±0.186
Fourth group (5.35 ppm Chlorfenapyr+1% ascorbic acid)	2.05±0.033	0.81±0.021	10.50±0.166	7.50±0.142
Fifth group (5.50 ppm Chlorantraniliprole+1% ascorbic acid)	2.05±0.044	0.79±0.024	10.38±0.130	7.75±0.129
Control group	2.10±0.060	0.85±0.023	10.00±0.123	7.00±0.065
Significance	**	**	*	**
LSD at 0.05 %	0.013	0.017	0.535	0.380

3.3. The impact of tested pesticides on cocooning percentages and silk productivity of *B. mori*

Data in Table 3 showed the harmful effects of chlorfenapyr and chlorantraniliprole on cocooning percentages and silk productivity of *B. mori,* as well as the reduction of these effects by ascorbic acid. The highest cocooning percentages and silk productivity appeared in the first group (93.05 % and 2.42 cg/d), followed by the untreated control (92.00 % and 1.97 cg/d), respectively. On the contrary, the lowest percentages of cocooning and silk productivity were (90.00 % and 1.24 cg/d)

in the second group (larvae exposure to chlorfenapyr), and also in the third group (larvae exposure to chlorantraniliprole) were (90.14 % and 1.36 cg/d), respectively. While the cocooning percentages and silk productivity increased to 91.95 % and 1.53 cg/d in the fourth group and 91.25 % and 1.66 cg/d in the fifth group of larvae that were initially treated with 1 % ascorbic acid before exposure to tested pesticides.

Table 3. Effect of chlorfenapyr and chlorantraniliprole with and without adding ascorbic acid, on cocooning percentage and silk productivity of silkworm

Group	Cocooning percentage (%)	Silk productivity (cg/d)
First group (1% ascorbic acid)	93.05±0.012	2.42±0.133
Second group (5.35 ppm Chlorfenapyr)	90.00±0.021	1.24±0.156
Third group (5.50 ppm Chlorantraniliprole)	90.14±0.033	1.36±0.142
Fourth group (5.53 Chlorfenapyr+1% ascorbic acid)	91.95±0.017	1.53±0.156
Fifth group (5.50 ppm Chlorantraniliprole+1% ascorbic acid)	91.25±0.020	1.66±0.200
Control group	92.00±0.013	1.97±0.134
Significance	**	**
LSD at 0.05 %	1.600	0.070

cg/d: centigram per day

3.4. The impact of tested pesticides on economical parameters of *B. mori*

Results in Table 4 revealed that exposure the 5th instar larvae of *B. mori* to chlorfenapyr and chlorantraniliprole decreased cocoon weights, cocoon shell weights, and cocoon shell ratio when compared to the control. However, exposure to 1% ascorbic acid before application of these pesticides decreased the adverse effects. The cocoon weights were 1.18 g, 1.01 g, 1.01 g, 1.06 g, 1.08 g and 1.1 g, in the first, second, third, fourth, fifth and sixth groups, respectively. Thus, chlorfenapyr and chlorantraniliprole showed a significant effect on cocoon weights of 5th instar.

The larvae treated with chlorfenapyr exhibited cocoon shell weights and ratios of 0.13 g and 12.78 %, respectively, whereas larvae treated with chlorantraniliprole had 0.14 g and 14.16 %. Feeding larvae on mulberry leaves sprayed with 1 % ascorbic acid solution before exposure to the tested pesticides resulted in (0.16 g and 15.22 %) and (0.17 g and 16.00 %) for chlorfenapyr and chlorantraniliprole, respectively.

Table 4. Effect of chlorfenapyr and chlorantraniliprole with and without adding ascorbic acid on cocoon weight, cocoon shell weight and cocoon shell ratio

Groups	Cocoon weight (g)	Cocoon shell weight (g)	Cocoon shell ratio (%)
First group (1 % ascorbic acid)	1.18±0.032	0.22±0.002	18.41 ± 0.077
Second group (5.35 ppm Chlorfenapyr)	1.01±0.021	0.13±0.001	12.78±0.1
Third group (5.50 ppm Chlorantraniliprole)	1.01±0.044	0.14±0.003	14.16±0.054
Fourth group (5.35 ppm Chlorfenapyr + 1 % ascorbic acid)	1.06±0.071	0.16±0.003	15.22±0.1
Fifth group (5.50 ppm Chlorantraniliprole + 1 % ascorbic acid)	1.08±0.023	0.17±0.002	16.00±0.077
Control group	1.1±0.023	0.2±0.002	17.85±0.068
Significance	**	**	**
LSD at 0.05 %	0.010	0.008	0.410

4. DISCUSSION

The silkworm is an economically important insect silk-producing insect. The quality and quantity of silk require optimum feeding of the silkworm larvae. Pesticide drift during use cannot be controlled, therefore silkworms will surely be exposed to pesticides by consuming pesticide-contaminated mulberry leaves. The intake of pesticide-contaminated mulberry leaves by silkworms reduces the efficiency of silk production. This investigation demonstrated the toxic effect of chlorfenapyr and chlorantraniliprole on the fifth larval instar of *B. mori*. Also, lambdacyhalothrin and emamectin benzoate were extremely harmful to silkworms after 10 days of treatment, while chlorofenapyr showed the lowest mortality rate **(Kumar *et al*., 2017).** Organophosphorus and pyrethroid insecticides were extremely toxic to silkworms **(Zhang *et al*., 2008).** In another study conducted by **(Munhoz *et al*., 2013)**, fifth-instar silkworm larvae were treated to 0.05 ppm and 0.025 ppm of chlorantraniliprole, resulting in a mortality rate of 100% and 98.33%, respectively. Furthermore, our investigation revealed that the effect of chlorantraniliprole on the larval duration of silkworms was 10.38 days when fed on mulberry leaves treated with 5.50 ppm chlorantraniliprole, compared with 9.66 days with 0.02% chlorantraniliprole. Also, silkworm larval durations were 9.71 and 9.2 days, when treated with 0.05% flubendiamide and imidacloprid, respectively **(Kumar *et al*., 2017)**. In this study, larval exposure to chlorfenapyr and chlorantraniliprole caused lower cocoon weights, cocoon shell weights, and cocoon shell ratios. In the same text, cocoon weight and cocoon shell weight were significantly decreased after indoxacarb exposure, but there was no significant change in the cocoon shell ratio **(Wang *et al*., 2023)**. Not only insecticides but also

fungicides have proven to be harmful on the growth, development, quality cocoon, and silk of silkworms **(Manjunatha *et al*., 2017).** It is hard to avoid being completely exposed to pesticides, hence a system for protecting silkworms from pesticide toxicity must be developed. Feeding is essential for the growth and development of silkworms. The nutritional value of the food influences the health of silkworm larvae as well as the production of high-quality silk **(Tantray and Trivedy, 2011)**. As a result, adding vitamins and other nutrients to silkworm food improves growth of silkworms and its tolerance to toxins and heat stress **(Udayan and Kumar, 2022)**. Antioxidants are one of the strategies that provide silkworms with protection during larval development from oxidative damage **(Sahoo *et al*., 2016)**. The pesticides caused an increase in the reactive oxygen species (ROS) production **(Gao *et al*., 2018).** Therefore, adding vitamins is necessary to protect the silkworms and improve their breeding. the ability of ascorbic acid to produce good quality cocoons from larvae exposed to heat stress has also been demonstrated **(Aneesha and Kumar, 2022; Udayan and Kumar, 2022).** Our findings showed that mulberry leaves treated with ascorbic acid protected silkworms against the negative effects of chlorfenapyr and chlorantraniliprole during exposure to larvae. Furthermore, increased larval weights, cocooning percentages, and silk productivity. Similarly, adding N-acetyl-L-cysteine (NAC) to *B. mori* feeding resulted in protection and recovery of antioxidant equilibrium in imidacloprid-exposed larvae **(Gao *et al.*, 2018).** On the other hand, the current results at the same line with **(Tantray and Trivedy, 2011)** when 5th instar larvae were fed on mulberry leaves treated with vitamin C orally, their larval weight, cocoon weight, and shell weight all improved. In another report by **(Gad and Fathy, 2021),** utilizing 7000 and 10000 ppm of vitamin C was shown to increase larval development rate, cocoon weight, and larval immunity.

5. CONCLUSION

In summary, mulberry leaves treated with 1% ascorbic acid and fed to silkworms improves silkworm larvae growth, cocoon weight and quality, and silk production the quantity when compared to the control group. Furthermore, including ascorbic acid in the silkworm's food defends it from the harmful effects of chlorfenapyr and chlorantraniliprole when exposed to them. As a result, it is recommended to treat mulberry leaves with ascorbic acid for improved silkworm rearing considering their exposure to environmental toxins.

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