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Sublethal effects of chlorfenapyr on the life table parameters, nutritional physiology and enzymatic properties of *Bradysia odoriphaga* (Diptera: Sciaridae)

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ABSTRACT

Bradysia odoriphaga (Diptera: Sciaridae) is the major pest affecting Chinese chive production. Chlorfenapyr is a halogenated pyrrole-based pro-insecticide that is currently used to control insects and mites on a variety of crops. In the present study, fourth-instar larvae of *B. odoriphaga* were exposed to chlorfenapyr at LC_1, LC_{20} and LC_{50} concentrations. The developmental duration of the treated larvae was not significantly different, but fecundity was significantly increased in the LC_1 and LC_{20} treatment groups compared with the control group. The population parameters of the LC_1 treatment group were increased significantly, whereas those of the LC_{50} treatment group were reduced significantly compared with the control. The food consumption by larvae and pupal weight were significantly increased under the LC_1 treatment and decreased under the LC_{50} treatment compared with the control. Moreover, chlorfenapyr decreased the lipid, carbohydrate and trehalose contents significantly, whereas the total protein content was increased compared with the control. Additionally, the activities of protease, lipase and trehalase were significantly decreased. Chlorfenapyr treatment for 24 h also induced the activities of glutathione S-transferase (GST), carboxylesterase (CarE) and *O*-demethylation. The results of this study suggest that low lethal concentrations of chlorfenapyr can affect oviposition, population development, the activities of digestion and detoxification enzymes, and nutrient accumulation in *B. odoriphaga*. This study provides valuable information for the assessment and rational application of chlorfenapyr for effective control of this pest.

1. Introduction

Bradysia odoriphaga (Diptera: Sciaridae) is a pest that causes damage to > 7 plant families and 30 types of vegetables, especially Chinese chives [1,2]. It is widely distributed in Northern China, and its larvae attack Chinese chives by feeding on the plant's bulbs, leading to production losses of between 30% and 80% [3]. One of the most prevalent management practices to combat *B. odoriphaga* is the application of synthetic insecticides, such as organophosphates and neonicotinoid insecticides [3,4]. However, the long-term continuous use of such insecticides has led to widespread resistance in *B. odoriphaga* [5,6]. Therefore, new alternative insecticides that are more effective and exhibit different modes of action are required to control this pest.

Chlorfenapyr, which is currently registered for the control of various insects and mites on cotton, ornamentals and a number of vegetable crops [7,8], is a halogenated pyrrole that disrupts mitochondrial oxidative phosphorylation [9,10]. Chlorfenapyr has a broad spectrum of activity and shows favorable contact and stomach toxicity in *Spodoptera exigua* and *Anopheles gambiae* [11,12]. The substance itself was demonstrated to exhibit no apparent bioactivity. However, as a pro-insecticide, its metabolite shows significantly more activity after activation by mixed function oxidase systems (MFOs) [9]. Previous studies have shown that chlorfenapyr exhibits high bioactivity even in *Culex quinquefasciatus* (Diptera: Culicidae), *Anopheles gambiae* and *Anopheles funestus* (Diptera: Culicidae), which have shown resistance to pyrethroids [12–14]. Moreover, our previous study showed that chlorfenapyr exhibits high bioactivity in each larval instar of *B. odoriphaga*, and no significant differences in susceptibility are observed between

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phoxim-resistant and phoxim-susceptible populations [15]. Thus, chlorfenapyr presents potential as a potent alternative pesticide against *B. odoriphaga*.

Insecticides not only show direct toxicity to insects but also influence population dynamics by affecting insect longevity, fecundity and physiological traits [16–19]. Following a field application of insecticides, pests may be exposed to different doses of insecticides, and such varied doses result in different biological and ecological outcomes [16,20]. For example, low lethal concentrations of thiamethoxam and benzothiazole can decrease the developmental rate of laboratory populations of *B. odoriphaga* [21,22]. Moreover, Zhang et al. [23] found that a low lethal concentration of chlorfluazuron (LC_{10}) can stimulate rapid population growth of *B. odoriphaga*. Therefore, it is important to verify the sublethal effects of insecticides on pests by undertaking a complete assessment.

The objective of present study was to obtain a comprehensive understanding of the sublethal effects of chlorfenapyr on *B. odoriphaga*, including its developmental time, life table parameters, consumption of food, accumulation of energy substances, and activity of digestive and detoxifying enzymes. The results may be employed to understand the sublethal effects of chlorfenapyr on *B. odoriphaga*, which will contribute to the assessment and rational application of chlorfenapyr for controlling this pest.

2. Materials and methods

2.1. Insects and chemicals

The strain of *B. odoriphaga* used in this study was originally collected from a Chinese chive field in Tai'an, Shandong Provence, China (site: 36.19°N, 116.88°E) on September 2013. The insects were reared with fresh rhizomes of Chinese chive under laboratory conditions at 25 ± 1 °C, with 75 ± 5% relative humidity (RH), and a photoperiod of 14:10 h (light/dark) in a climate-controlled chamber.

Technical-grade chlorfenapyr (96%) was provided by Shandong Weifang Rainbow Chemical Incorporated Company (Shandong, China).

2.2. Bioassays

The toxicity of chlorfenapyr against *B. odoriphaga* was determined in newly emerged fourth-instar larvae using a standard stomach and contact joint toxicity method that has been described previously [15]. The active ingredient was dissolved in organic solvent at a ratio of 100 mg/L. Then, serial dilutions with a 0.1% Tween-80 water solution were performed. First, 90 mm-diameter clean filter paper was placed in a 90 mm-diameter Petri dish, after which newly emerged fourth-instar larvae were transferred to the moist filter paper. Next, 1 mL of the test solution was added to the filter paper and larvae. Fresh chive rhizomes (0.5 cm in length) were dipped in the test solution for 30 s with gentle stirring and then air-dried at room temperature, after which four chive rhizomes were transferred to the Petri dish. Five replicates (approximately 25 larvae per replicate) were used to investigate the mortality associated with each concentration, and a control (solvent without chlorfenapyr) was tested at the same time. The larvae were maintained under standard laboratory conditions as described above. Mortality was assessed after 24 h. Larvae were considered dead if their bodies were elongated or they were unable to move when disturbed with the tip of a moist brush.

2.3. Effects of chlorfenapyr on life table parameters of B. odoriphaga

Egg masses laid on fresh Chinese chives within the past 24 h were collected and put into Petri dishes using a moist brush. Approximately 200 eggs were tested for each treatment. Each egg was considered one replicate. The eggs were maintained under standard laboratory conditions as described above, and the hatching conditions and development of the larvae were recorded. Larvae were treated with three concentrations (LC_1, LC_{20} and LC_{50}) of chlorfenapyr using the bioassay method on the fifteenth day (approximately the fourth instar) after the eggs were laid. After 24 h, Surviving larvae were reared with fresh rhizomes of Chinese chive, and the developmental time and survival rate of the larvae were recorded every day. When the larvae transformed into pupae, they were immediately transferred to new Petri dishes, and the developmental time and survival rate continued to be recorded daily. After adults emerged, females and males were paired singly in individual containers containing moist filter paper and fresh Chinese chive stems. The containers were maintained under standard laboratory conditions as described above. The fecundity and longevity of the adults was recorded daily until all adults had died.

2.4. Effects of chlorfenapyr on food consumption and pupal weight

To evaluate the effects on food consumption, newly emerged fourth-instar larvae were exposed to low and median lethal concentrations of chlorfenapyr (as described in Section 2.3), and after 24 h, the survivors were transferred to new Petri dishes. The food consumption of larvae was measured according to a method described previously [24], and five replicates were performed for each treatment. In addition, the effect of chlorfenapyr on pupal weight was measured. After exposure to chlorfenapyr, the larvae were transferred to new Petri dishes and fed with fresh rhizomes of Chinese chives until they developed into pupae. Two-day-old pupae were weighed (weights of both female and male pupae were recorded), and five replicates (ten pupae per replicate) were assessed to evaluate the effects on pupa weight.

2.5. Preparation of samples for biochemical assay

Newly emerged fourth-instar larvae were exposed to the LC_1, LC_{20} and LC_{50} concentrations of chlorfenapyr (as described in Section 2.3). After 24 h, the surviving larvae were selected and weighed. Five larvae were placed in a 1.5 mL centrifuge tube and treated with liquid nitrogen. The samples were stored at −80 °C for preservation. Five insects were tested in each experiment, using 3 replicates for each treatment.

2.6. Measurement of nutrient contents

2.6.1. Total protein content

The total protein content was measured using the method of Bradford [25]. Five larvae (whole body) were homogenized in 100 μL of extraction buffer (20% sucrose, 50 mmol Tris-HCl pH 7.1, 0.5% Triton x-100) on ice, followed by centrifugation at 12,000 rpm for 10 min at 4 °C. Then, 30 μL of the supernatant was mixed with 150 μL of dye (0.01% Coomassie brilliant blue G-250). A standard curve was established with bovine serum albumin (Aladdin). The absorbance was read at 630 nm using a Bio Tek SynergyTM 2 Multi-Mode Reader (Bio Tek Instruments, Inc. Winooski, Vermont, USA).

2.6.2. Lipid content

Lipid content was determined following the procedure of Yuval et al. [26]. Five larvae (whole body) were homogenized in 100 μL of 2% Na_2SO_4 solution. Lipids were extracted in 750 μL of a mixture of methyl alcohol and chloroform (at a ratio of 1:2, respectively) for 4 h. The mixture was subsequently centrifuged at 12,000 rpm for 10 min at 4 °C. Then, 600 μL of the supernatant were removed and dried at 40 °C for 12 h, after which 500 μL of 98% H_2SO_4 was added to the individual tubes, followed by incubation for 10 min at 90 °C in a water bath. Finally, 30 μL aliquots of the samples were mixed with 270 μL of vanillin reagent (60 mg vanillin dissolved in 10 mL distilled water and 40 mL 85% H_3PO_4) (Solarbio). The absorbance was read at 530 nm after 30 min. Lipid content was calculated using the standard curve of cholesterol (Solarbio).