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Toxicity and biochemical study of two insect growth regulators, buprofezin and pyriproxyfen, on cotton leafworm *Spodoptera littoralis*

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ABSTRACT

Lethal and sublethal effects of two insect growth regulators (IGRs) buprofezin and pyriproxyfen were evaluated on larvae of cotton leafworm *Spodoptera littoralis*. Activity of chitinase and polyphenol oxidase (PPO) in surviving larvae after treatment was carried out in order to investigate the biochemical influences of these compounds. The compounds were low toxic against the larvae at 0.05, 0.1, 0.25, 0.5, and 1.0-fold of the field application rate. However, the overall mortalities within 6 days of feeding at 2.0-fold were 46.67% and 100% for buprofezin and pyriproxyfen, respectively. Larval weight gain was considerably decreased as concentration increased. Pyriproxyfen showed high antifeedant activity in a concentration-dependent manner, and larvae stopped to eat from the third day with high dose. Conversely, buprofezin did not significantly show antifeedant except with high concentration (3000 mg (a.i.)/kg diet) that gave 80.68%. The high doses of both compounds showed adverse effects on pupae, and emergence of adults. Buprofezin at the recommended dose (1500 mg (a.i.)/kg diet) caused 93.33% pupation and 53.33% emergence of adults. Otherwise, pyriproxyfen caused 21.33% pupation and zere emergence of adults at the recommended dose (75 mg (a.i.)/kg diet) compared to 100% pupation, and 96.30% emergence of adults in the control. Both compounds varied in their influences on chitinase and PPO activity, and these enzymes could have relation with toxicity of buprofezin and pyriproxyfen against *S. littoralis* larvae.

1. Introduction

The noctuid *Spodoptera littoralis* (Boisduval) is a most important polyphagous pest, widely distributed all over the world [1]. Larvae of this pest can feed on ~90 economically important plant species belonging to 40 families and the rate of development has a strong nutritional component [2]. Food intake by larvae, as well as the quality of food ingested, affects growth rate, development time, final body weight and survival. Commonly, the control of this pest has largely been depending on the use of neurotoxic insecticides including chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids [3,4]. However, the control achieved is not successful because of the insect's high capacity to develop resistance toward the majority of these compounds [5–7].

Selective insecticides with modes of action different from those of broad-spectrum neurotoxic insecticides are highly desirable in integrated pest management (IPM) programmes. Among these insecticides are insect growth regulators (IGRs) that affect the ability of insects to grow and mature normally. IGRs have been developed due to their high activity and selectivity against insects with inherently low toxicity to non-target wildlife. As a result of their mode of action, a subtle effect of these compounds is likely to pose a greater effect to immature stages than to adults of a number of insect species [8–11]. Most compounds that belong to the IGR class are not stomach or neurotoxic poisons, but have a unique mode of action that disrupts the molting process or cuticle formation in insects [12] or interferes with the hormonal balance of insects [9]. They are characteristically slow acting against a narrow range of sensitive stages of the insects' life cycle with harmful effect against target pests [13].

Several studies have been directed to elucidate the biological as well as biochemical effects of IGRs. It was as early as 1974, when Ishaaya and Casida [14] reported that house fly larvae showed an increase of both the cuticle chitinase and phenoloxidase activities up to about 180% and 155%, respectively when treated with one ppm of the compound TH-6040. There are also another reports concerning the biochemical effects of similar compounds (diflubenzuron, Du-19,111 and/or polyoxin D) which showed that such compounds has the inhibition action on the chitin biosynthesis or cuticle production through the blocking action of the terminal polymerization step in chitin synthesis against another species of insects such as *Musca domestica* [15], and *Mamestra Brassica* [16].

Buprofezin and pyriproxyfen are both IGR's and they are highly molt inhibitors for a wide range of insects. Buprofezin, 2-*tert*-butylimino-5-phenyl-3-propan-2-yl-1,3,5-thiadiazinan-4-one, acts spe-

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cifically on immature developmental stages of some homopteran pests by inhibiting the incorporation of *N*-acetyl-[D-H^3] glucosamine into chitin and interfering with cuticle formation resulting in nymphal mortality during ecdysis [17,18]. Pyriproxyfen, 2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine, is a fenoxycarb derivative in which a part of the aliphatic chain has been replaced by pyridyl oxyethylene. It is a potent juvenile hormone mimic affecting the hormonal balance in insects resulting thereby in strong suppression of embryogenesis, metamorphosis, and adult formation [19]. In addition, these compounds interrupt the normal physiological process of molting such as suppressing oviposition of adults and reducing viability of eggs [20–22]. Treated susceptible pests may remain alive on the plant for 3–7 days, but feeding damage during this time is typically very low. Thus, susceptibility monitoring is best examined in the immature stages. Both compounds have given excellent results in controlling the whiteflies which cause a universal problem in cotton production [23]. They were introduced to Arizona cotton in 1996, after resistance to synthetic pyrethroids and other conventional insecticides reached crisis proportions [24]. Furthermore, they are active against a variety of insect species including Diptera, Lepidoptera and Hemiptera [25,26].

The availability of a number of efficient insecticides for *S. littoralis* control would also reduce selection pressure driving resistance against insecticides that are relied upon heavily. For that reason, the objectives of the current study were to investigate the effects of buprofezin and pyriproxyfen on larval development, pupation and the emergence of adults of the cotton leafworm *S. littoralis* (Boisduval). In view of the fact that it is a model lepidopteran and a highly polyphagous insect pest of world economic importance in agriculture and horticulture, as many resistant populations cause a high level of damage in cotton and vegetables. The estimation of efficiency of the two IGRs would provide a greater diversity to integrate them into *S. littoralis* management programs. In addition, these insecticides may manipulate physical and biochemical process of insect by influencing relative enzymes. Therefore, the activities of chitinase and polyphenol oxidase (PPO) were determined in surviving larvae after treatment to elucidate the insecticidal mechanism of buprofezin and pyriproxyfen.

2. Materials and methods

2.1. Chemicals and test insecticides

Formulated buprofezin (Applaud, 25% SC) was obtained from Nihon Nihyaku Co., Japan and pyriproxyfen (Admiral, 10% EC) was obtained from Sumitomo Chemicals (Osaka, Japan). Bovine serum albumin (BSA), Folin–Ciocalteu phenol regent, 3,5-dinitrosalicylic acid and pyrocatechol were purchased from Sigma–Aldrich Chemical Co., USA. All chemicals were used without further purification. The stock solutions of each compound were made in water on the day of experiments and were used immediately. Concentrations of 75, 150, 375, 750, 1500 and 3000 mg (a.i.) buprofezin/kg fresh diet and 3.25, 7.5, 18.75, 37.5, 75 and 150 mg (a.i.) pyriproxyfen/kg fresh diet were prepared. These concentrations represent 0.05, 0.1, 0.25, 0.5, 1.0 and 2.0-fold of the manufacturer recommended label rate of both insecticides, respectively.

2.2. Test pest

A susceptible strain of the cotton leafworm *S. littoralis* Boisduval (Lipidoptera: Noctuidae) was used for toxicity assay. This strain has been colonized for many years in the Department of Economical Entomology, Faculty of Agriculture, Alexandria University, Egypt, without exposure to any pesticides. The colony was reared under laboratory conditions on artificial diet under controlled conditions at 25 ± 2 °C, 70 ± 5% relative humidity and a 16 h light photoperiod [27].

2.3. Toxicity bioassay

2.3.1. Insecticidal and growth-inhibitory assay against S. littoralis

Newly molted second-instar larvae of *S. littoralis* were continuously fed the artificial diet containing different concentrations of buprofezin and pyriproxyfen. Concentrations of 75, 150, 375, 750, 1500 and 3000 mg (a.i.) buprofezin/kg fresh diet and 3.25, 7.5, 18.75, 37.5, 75 and 150 mg (a.i.) pyriproxyfen/kg diet were tested. These concentrations represent 0.05, 0.1, 0.25, 0.5, 1.0 and 2.0-fold of the field application rate, respectively for both compounds. These concentrations were prepared in water and mixed with the diet during its preparation. Untreated diet was provided to control. Larvae (30 per each concentration) were placed in Petri dishes (9 cm in diameter). The tests were conducted in triplicate. After 2, 4 and 6 days of continuous feeding on treated diet, larval mortality was scored; if no movement was observed, larvae were considered as dead. Weight gain of larvae was assayed at days 2, 4 and 6 of feeding. The percentage of antifeedant activity was determined after 6 days of feeding by formula of Abivardi and Benz [28] as follows:

$$\text{Antifeedant}(\%) = \left(\frac{C - T}{C}\right) \times 100$$

where *C* is a weight of diet consumed in untreated (control) and *T* is a weight of diet consumed in treatment. Development of surviving individuals was followed and percent of pupation, pupal weight and the emergence of adults were recorded.

2.4. Total protein assay

The Lowry et al. [29] method was used to determine protein content in surviving larvae obtained after continuous feeding for 2, 4 and 6 days. Definite weight of larvae was homogenized in 3 mL of potassium phosphate buffer (pH 7.0) with a tissue Tearor on ice. The homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was used in protein determination and as the crude enzyme extract. Protein extract (10 μL) was added to 2 mL of alkaline copper reagent [48 mL of 2% (w/v) sodium carbonate in 0.1 N sodium hydroxide + 1 mL of 1% (w/v) sodium–potassium tartrate + 1 mL of 0.5% (w/v) copper sulfate] and immediately mixed. After 10 min, 0.2 mL of Folin–Ciocalteu phenol reagent was added and the samples were thoroughly mixed. The absorbance of the developed blue color was measured at 600 nm using a Unico 1200-Spectrophotometer. The sample protein content was determined by comparing to the standard curve of BSA.

2.5. Chitinase activity assay

Chitinase (EC 3.2.1.14) is specific hydrolyze enzyme which hydrolyse chitin (chitobiose polymer) to *N*-acetyl-D-glucosamine (reduced sugar monomer). The specific activity was determined in the surviving larvae after 2, 4 and 6 days of feeding on diet treated with the tested insecticides. The larvae were homogenized in 0.1 M phosphate buffer (pH 7.0) with a tissue Tearor on ice. The homogenates were then centrifuged at 5000 rpm for 20 min at 0 °C. The supernatants were used as enzyme source for chitinase activity assay. Enzyme activity was measured according to Monreal and Reese [30] method. One ml of colloidal chitin, as a substrate, in 0.05 M citrate phosphate buffer (pH 6.6) was mixed with 1 mL of enzyme extract. Colloidal chitin was prepared by the method that described by Shimahara and Takiguchi [31]. A suspension containing 1% (w/v) of moist colloidal chitin is prepared in