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Insecticidal spectrum of fluralaner to agricultural and sanitary pests

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ABSTRACT

Fluralaner, as an isoxazoline insecticide, has been developed as antiparasitic (namely Bravecto®) with selective toxicity for insect versus mammals. Even though the toxic effect of fluralaner to several insects have been already examined, but also more agricultural and sanitary insects should be tested before it could be used widely. Four insects, including two agricultural insects, one sanitary insect and one stored product insect were tested in the present study. Compared to the broad-spectrum and high activity insecticide of fipronil, fluralaner showed mostly higher activity to tested pests, *Chilo suppressalis, Tribolium castaneum, Spodoptera litura* except *Blattella germanica* with topical application. Through summarizing and analyzing various published data, we concluded that, to date, the toxic activity of fluralaner to 19 insects from 11 orders were already examined, and fluralaner displayed excellent activity. Compared to fipronil, fluralaner mostly shows more excellent activity to tested insect with maximal 1.67×10^4-fold difference so much. In the present study, the toxicity activity of fluralaner was compared to the broad-spectrum phenylpyrazole insecticide fipronil to tested insect pests and the results exhibited that fluralaner would be a novel alternative insecticide with broad insecticidal spectrum and be widely used in more other areas not only as veterinary drug.

Introduction

Fluralaner, was firstly synthesized by Mita et al. (2007) and reported by Ozoe et al. (2010), and was considered as fortuitous with surprising target site discoveries (Casida, 2015). Subsequently, the excellent activities of fluralaner were reported to parasitic and sanitary pests (*Ctenocephalides felis*, *Stomoxys calcitrans*, *Lucilia cuprina*, *Aedes aegypti*, *Rhipicephalus microplus*) (Gassel et al., 2014), and agricultural pests (*Tetranychus urticae*, *Spodoptera frugiperda*, *Helicoverpa zea*, *Empoasca fabae* and *Frankliniella occidentalis*) (Lahm et al., 2013; Zhao et al., 2015). Hence, fluralaner, named Bravecto® (active ingredient: fluralaner) as a new systemically administered parasiticidal and acaricidal product, has been widely used in pets (Walther et al., 2014a) and sold in Europe, USA and Asia (Walther et al., 2014b) with huge market benefit, with little or no toxic to mammals (Ozoe et al., 2010; Zhao et al., 2014). Numerous studies about fluralaner are still conducting as a potent insecticide and acaricide with rapid and persistent efficacy (Lee et al., 2016; Zhao et al., 2014). However, more attention should be required before the usage of fluralaner in agricultural area.

In the present study, the rice stem borer (*Chilo suppressalis*), armyworm (*Spodoptera litura*) and red flour beetle (*Tribolium castaneum*), which are important and representative agricultural pests and always make large economic loss in the agricultural product (Ahmad et al., 2009; Nenaah, 2014; Su et al., 2014; Xu et al., 2016) and the German cockroach (*Blattella germanica*), which is one typical hygeian pest of a potential carrier of various pathogenic organisms, and offensive pests visually, were selected for bioassay (Wei et al., 2001). More importantly, these four insects have developed high resistance to a wide range of insecticides, including organochlorine, organophosphate, pyrethroid, and even some novel insecticides, such as chlorantraniliprole, cyantraniliprole *etc*. (Gao et al., 2013; Song et al., 2016). For example, *C. suppressalis* has developed resistance to flubendiamide

Abbreviations: ANOVA, analysis of variance; CO_2, carbon dioxide; CL, confidence limits; DMRT, Duncan's Multiple Range Test; GABAR, γ-aminobutyric acid (GABA) gated chloride channel receptor; RH, relative humidity; SE, standard error; TLC, thin layer chromatography; TMS, tetramethyl silane

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(Wu et al., 2014) and chlorantraniliprole up to 74-folds in China (Agropages, 2016), and *S. litura* has developed resistance to cyantraniliprole (Song et al., 2016). Therefore, alternative insecticides are necessary to control these pests and to design resistance management stratagem intelligently.

In this study, the toxicity activities of fluralaner to *C. suppressalis*, *T. castaneum*, *S. litura*, and *B. germanica* were examined and compared to other commercial insecticides, and the insecticidal spectrum of fluralaner was classified as well. We expect our study would provide more bioactivity data of fluralaner and promote the use and development of fluralaner in agricultural and hygeian area.

Materials and methods

Insect strain and chemicals

The *C. suppressalis* were reared on potted rice plants under the conditions of 28 ± 1 °C and a 16:8 h (light: dark, L: D) photoperiod as reported in our laboratory (Peng et al., 2017). The *S. litura* were reared under the same condition to *C. suppressalis*.

The German cockroaches *B. germanica* were kindly provided from Professor Shuang-Lin Dong (Nanjing Agricultural University, Nanjing, China) and maintained in an insectarium at a constant temperature of 27 ± 2 °C, a relative humidity (RH) of 60 ± 10%, and a photoperiod of 12:12 h (L: D). The cockroaches were kept in separate labeled plastic box, which was coated on the upper inner rim with petroleum jelly to prevent them from escaping and restrict them to the inner wall of each testing jar and feed with starch, sugar cubes and water ad libitum (Moemenbellah-Fard, 2013).

The red flour beetle *T. castaneum* was maintained in a growth cabinet set at 30 ± 1 °C and 75 ± 5% RH as described by Nenaah (2014) with wheat flour mixture (wheat flour: yeast powder, 9:1).

The insecticides, including fipronil (purity ≥ 97%), dichlorphos (purity ≥ 93%), butene-fipronil (purity ≥ 98%), and beta-cypermethrin (purity ≥ 99%) were obtained from commercial sources except the fluralaner was prepared and identified in our laboratory (Supporting Information).

Bioassay indoor

Bioassay to C. suppressalis and S. litura

Insecticides were dissolved in acetone and serially diluted concentrations were prepared based on the earlier reports and pre-assay (data not shown) (Chen et al., 2000; Li et al., 2007; Qu et al., 2003). Ten 4th-instar larvae (9.00–11.00 mg) for *C. suppressalis* and 3rd-instar larvae for *S. litura* were treated with 1.0 and 0.04 μL, respectively, of the dilution on the middle-abdomen notum by a hand Burkard Arnold microapplicator (Type Lv. 65. needle no. 18 in 1.0 mL syringe) (Burkard Manufacturing, Richmansworth, UK). Each test was triple replications and the acetone treatment was used as control. The tested larvae were reared individually under suitable condition with adequate food. Mortality was assessed at 24 and 48 h for *C. suppressalis* and 48 and 72 h for *S. litura* after micro-application.

Bioassay to B. germanica

Bioassay were conducted only on the first generation adult males, since uniformity in gender would delete sex-biased differences in response to chemicals compared with those of males (Chang et al., 2010). In the formal assay, seven dosages (2.5, 5, 7.5, 10, 12.5, 20, and 40 mg/L) of the test insecticides in 1 μL of acetone were topically applied to CO_2-anesthetized adult males (10 individuals per replication) at the abdominal sternites between 2nd-3rd foot basal inter node with the Burkard microapplicator (Burkard). Control cockroaches were treated with 1 μL acetone alone. After treated, the cockroaches were placed separately in the 300 mL plastic cups and provided with food and water, and the mortalities were determined at 24, 48, 72, 96 and 120 h, respectively. If their bodies or appendages did not move while prodded with fine wooden dowels, males were considered to be dead. Each bioassay was replicated at least three times (Nasirian et al., 2006).

Bioassay to T. castaneum

Insecticidal activity of fluralaner to *T. castaneum* adults were conducted according to published method (Andric et al., 2015; Halliday et al., 1988; Nenaah, 2014) using topical application and the fipronil (200 mg/L) was set as a control group. The beetles (7-day old) were anesthetized for 30 s with CO_2, immobilized and treated with 0.5 μL of each insecticide solution (6.25, 12.5, 25, 50, 100 and 200 mg/L) in acetone for each one by application on the last thoracic segment with a Burkard microapplicator (Burkard). Controls were treated with acetone alone. After treated, 20 beetles as one group in 4 replicates were transferred to clean Petri dishes (9 cm diameter), and 1 g of wheat flour mixture was added to each after 4–6 h. Lethal efficacy was checked at 24, 48, 72, 96 and 120 h after treatment. The experiment was repeated 2–3 times.

Statistical analysis

Correction for mortality in the control treatment using below formula,

Corrected mortality (%) = [survival control (%) − survival treated (%)] × 100 / survival control (%).

Mortality data from the test insects were used to calculate the lethal concentration (LC_{50}) and lethal dose (LD_{50}), which kill 50% of a population and to establish 95% confidence limits (CL) by probit analysis using POLO-PC (LeOra Software, CA, USA), which automatically corrected for control mortality. The means for the different treatments in each bioassay were separated using the least significant difference test at $p = 0.05$ level.

Results and discussion

Insecticidal activity to C. suppressalis

The rice stem borer, as one major and representative pest in rice, was chosen for fluralaner insecticidal test in the present study. A series concentrations (0.625, 1.25, 2.5, 10, 20, 40, and 80 mg/L) were used as formal assay. Fipronil, as an outstanding phenylpyrazole insecticide after methamidophos, bisultap (monosultap) and triazophos, was ever widely used in rice production to control the rice pests including *C. suppressalis* (Fang et al., 2008) since 1997 (Cheng et al., 2010). However, it is banned from 2009 in China although the *C. suppressalis* is still sensitive to it. He et al. (2008) examined field populations of *C. suppressalis* for their toxicological responses to more than 20 insecticides and the LD_{50} value of fipronil is lower than 1.00 ng/insect in Lianyungang (Jiangsu Province), Nanchang (Jiangxi Province), Guiling District (Guangxi Autonomous Region) and Ruian District (Zhejiang Province) of China. Only abamectin and emamectin benzoate have higher activity than fipronil (He et al., 2008). Compared to the fipronil, the fluralaner with LD_{50} value at 3 ng/insect of showed more activity (1.4-fold) than fipronil with LD_{50} value at 4.2 ng/insect (Table 1). Therefore, we could deduce that the fluralaner would has equal or high toxic activity than other 17 insecticides. Chlorantraniliprole and avermectin are the major insecticides for the control of *C. suppressalis* in China. However, in Hengyang city (Hunan province) and Ji'an city (Jiangxi Province) of China, the resistance of *C. suppressalis* to chlorantraniliprole increased quickly, and the efficacy of chlorantraniliprole decreases to ~30% under recommendation usage in 2016 (Agropages, 2016). In future, the fluralaner, functioning on to the target at the GABAR as well, may be a possible alternative insecticide in rice pest control.