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Ecotoxicological Effects of Buprofezin on Fecundity, Growth, Development, and Predation of the Wolf Spider *Pirata piratoides* (Schenkel)

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Abstract The toxicological effects of buprofezin, an insect growth regulator, on the fecundity, development, and pest control potential of the wolf spider *Pirata piratoides* (Schenkel) (Araneae: Lycosidae) were investigated in the laboratory. It was shown that buprofezin had low toxicity to *P. piratoides* and that the median lethal dosage (LD_{50}) 48 h and 10% lethal dosage (LD_{10}) after topical application for female spiders were 653 and 316 mg buprofezin/mg fresh weight of spider, respectively. Buprofezin significantly reduced the percent hatching of spiders' eggs but had only a slight effect on egg production. No negative effects on the development and growth were observed. However, spider predation rates were strongly affected: Insecticide-treated females predated on fewer prey than the controls, and their predation rate did not recover even 5 days after insecticide application. This indicated that their pest control potential might be influenced by buprofezin, and the use of buprofezin in biological control of insects is discussed.

Introduction

Buprofezin is an insect growth regulator (IGR) that disrupts the development of immature forms by interference with chitin synthesis is effective against pest homopterans, such as planthoppers, leafhoppers, and whiteflies on rice. Because this insecticide has generally been considered to have good efficacy against the target pests while being harmless to beneficial insects, it has been used widely in integrated pest management (IPM) programs (James 2004; Gerling and Sinai 1994; Nagate 1986). However, several research projects proved that buprofezin had effects on some beneficial insects or their larvae. Smith (1995) showed that buprofezin caused significant larval mortality and reduced egg production in the scale-feeding coccinellid *Chilocorus circumdatus* Gyllenhal. Other research proved that buprofezin affected egg production and hatching in several coccinellids (Grafton-Cardwell and Gu 2003; James 2004; Magagula and Samways 2000) as well as hemipterans (Smith 1995).

Although there are numerous studies of the effects of buprofezin on the physiology of molting and the feeding behavior of economically injurious insects (Asal et al. 1985; Gu et al. 1993; Heong 1988; Moreno and Nakano 2002; Uchida 1987) and coccinellids, little attention has been paid to the effects of buprofezin on another important group of beneficial arthropods: spiders. Spiders are one of the most abundant beneficial arthropods in agricultural ecosystems and are known to be a potentially important group of natural predators in agricultural ecosystems (Marc et al. 1999). However, they are sensitive to a variety of insecticides, such as deltamethrin, pirimicarb. and imidacloprid (Koichi et al. 2000; Pekar 2002).

In China, buprofezin is widely used in rice for its good efficacy against homopterans. Therefore, the present study was undertaken to evaluate the effects of buprofezin on a wolf spider, *Pirata piratoides* (Schenkel) (Araneae: Lycosidae), which is one of the most abundant spider species in the rice in China (Song 1987). *P. piratoides* is a medium-sized wolf spider; the average range of adult female size is 3.60–5.10 mm. These spiders are wandering hunters that actively purse or ambush their prey. The biology and life history of this species has been studied intensively (Zhao 1992). A behavioral characteristic of the

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wolf spiders is that females carry the egg case until hatching and then carry the newly hatched offspring for about 1 week, at which time they disperse from the mother. During this time, the offspring do not feed.

The aim of this study was to test the effects of buprofezin on physiology and predatory behavior of *P. piratoides*, including the fecundity of the female, growth and development of spiderlings, and their feeding behavior.

Materials and Methods

Test Spiders and Their Maintenance

Female *P. piratoides* with egg sacs were collected in rice fields in Haidian Park (Beijing, China) during May 2005. They were kept individually in glass tubes (40 mm in diameter and 150 mm long) covered with two layers of gauze and with a 20-mm layer of moist sponge at the bottom to maintain high humidity. They were maintained in an illumination incubator at 25°C, 80% relative humidity (RH), and a 14:10-h light:dark photoperiod regime. Wild-type fruit flies *Drosophila melanogaster* Meigen from stock cultures were provided as their prey. After leaving the egg sacs, the spiderlings climb onto the female's abdomen and remain there for about 1 week before dispersal. Upon dispersal, the spiderlings were collected and housed individually in plastic containers under the same environmental conditions described earlier. The offspring of these female were reared following methods outlined by Deng et al. (2006). These offspring provided the subjects used in all subsequent experiments.

Insecticide and Its Application

The buprofezin used in the present experiments was buprofezin 25% wettable powder (61 mg active intergradient/g; Xinghuo, Jingzhou, Hubei, China). In all bioassays, the insecticide was diluted in acetone first for 12 h and then the solution was centrifuged at 1000 rpm (4°C) for 15 min. The supernatant was used as the test material.

In all bioassays, the formulated insecticide was topically applied (Jensen et al. 1997). Briefly, two droplets (each 0.5 μL) of insecticide solution were applied to the dorsal abdomen of spiders using a 5-μL microsyringe, and a control of acetone only was employed. The test spiders were immobilized by CO_2 treatment before application. Spiders were conditioned by starving for 3 days prior to the tests in order to standardize their hunger level. Because the size of individuals varied, test individuals were divided into four weight groups (13–14, 14–15, 15–16, and 16–17 mg for females). Each weight group was treated with a separate buprofezin solution, so that all test individuals received approximately the same dosage. In the present experiments, 50% and 10% lethal dose (LD_{50} and LD_{10}) (48 h) of buprofezin for spiders were chosen as the treatment dosages. Preliminary experiments had established the LD_{10} and LD_{50} values as 316 mg and 653 mg buprofezin/mg fresh weight of female spider, respectively.

Effect on Fecundity of Female Spiders

Eighty adult female spiders from the laboratory were randomly assigned to three treatment groups: the control (treated with acetone), LD_{10}-treated (treated with LD_{10} of buprofezin), and LD_{50}-treated (treated with LD_{50} of buprofezin) groups. To compensate for insecticide-induced mortality, the numbers of spiders assigned to the three groups were uneven: 20 in the control group, 20 in the LD_{10}-treated group, and 40 in the LD_{50}-treated group. After treatment, these spiders were returned to their containers together with a male for 1 or 2 days and fed with sufficient live flies. Over the next month, egg-laying and egg-hatching events were recorded. Only the first egg-laying was recorded, because this species produces the next egg sac only after the previous one has hatched and the spiderlings have dispersed, which required almost 3 weeks. The number of eggs and the emergence of spiderlings were also recorded. The effects of buprofezin on the preoviposition period (time from the last molt to the first egg-laying), number of eggs per clutch, hatching percentage (number of eggs hatched × 100/the total number of eggs in a clutch), and hatching time of eggs (time from be laid to emerging from the sac) for the female spiders were examined.

Effect on Development and Growth of Spiders

Developmental parameters were assessed for spiderlings by measuring differences in abdomen length and cephalothorax width. Therefore, some spiderlings emerging from the eggs of the previous experiment were preserved in ethanol for development measurement. Next, 80 subadult females (*i.e.*, spiders that will become adults after just one more molt), which had just molted the previous day, were assigned to three groups at random: the control (treated with acetone, $n = 20$), LD_{10}-treated (treated with LD_{10} of buprofezin, $n = 20$), and LD_{50}-treated (treated with LD_{50} of buprofezin, $n = 40$) groups. After insecticide application, these spiders were returned to their containers and offered sufficient food. They were checked daily and molting events were recorded. After the last molt, the spiders and their eluvia were preserved in ethanol. As a measure of size, the length of the tibia of the first leg (tibia I) was measured and individual growth rates calculated (Dyar's ratio: length of tibia I of adult divided by length of tibia I of subadult). The presence or absence of successful

molts was another growth parameter assessed for subadults. These measurements were taken over a 70-day period or until the spiderling died. Only growth data on spiderlings that survived until they reached maturity (90 days) were used in statistical analyses. Carapace width measurements were taken with a Unitron dissecting microscope fitted with an ocular micrometer ($N = 224$).

Effect on Predation by Female Spiders

In these tests, ~40 female adults were grouped randomly into three treatment groups: the control (treated with acetone, $n = 8$), LD_{10}-treated (treated with LD_{10} of buprofezin, $n = 10$), and LD_{50}-treated (treated with LD_{50} of buprofezin, $n = 20$) groups. After insecticide application, these spiders were placed separately in containers (80 mm in diameter, 100 mm high, covered with two layers of gauze, and with a 10-mm moist sponge in the bottom) with 12 fruit flies in each. The number of prey killed and consumed by spiders was recorded over the following 5 days, and the remaining prey was replaced by 12 new flies daily. All tests were carried out in an illumination incubator (25°C, 80% RH, 14:10-h light:dark photoperiod regime). The data relating to spiders that died during the tests were ignored. Fruit flies for the tests had just emerged from pupae for 1 day, and a separate test with the same fly densities demonstrated that the death rate of these flies was zero in 24 h; therefore, no adjustments were made to correct for any fly mortality observed in the experiments. The mean predation rate (the number of prey killed) per spider over the 5 days after different treatments was analyzed.

Statistical Analysis

Statistical analyses were performed with SPSS software (Version 13.0 for Windows®; SPSS Inc., Chicago, IL, USA). Nonparametric Kruskal–Wallis tests were used to examine the significance of the differences in the parameters of spiders in the three treatment groups and a comparison was made between the abdomen lengths of offspring from the three treatment groups using one-way analysis of variance (ANOVA). In cases where the tests were significant, multiple comparisons between treatments were carried out using Duncan's multiple-comparison test. A level of $p < 0.05$ was considered to be statistically significant.

Results

Effect of Buprofezin on Fecundity of *P. piratoides*

After insecticide application, no spiders in the control group died, whereas 5 and 19 died in the LD_{10}- and LD_{50}-treated groups, respectively. The effects of buprofezin on the preoviposition period, number of eggs per clutch, hatching percentage, and hatching time of eggs for the spiders are shown in Figure 1. There were no significant differences in preoviposition period among the three treatments (Kruskal–Wallis test, $p > 0.05$). The LD_{10}- and LD_{50}-treated females produced fewer eggs than the control, although these differences were not significant (Kruskal-Wallis test, $p > 0.05$).

Spiderlings molt once before they emerge from the egg sacs and, consequently, the hatching time here was the time spiderlings spent from egg to second instar. The effects of buprofezin on the hatching time of eggs from LD_{10}- and LD_{50}-treated and control females were not significant (Kruskal–Wallis test, $p > 0.05$). However, eggs from LD_{10}- and LD_50-treated females had slightly lower hatching percentages than the controls (Kruskal-Wallis test, $p < 0.05$).

Effect on Development and Growth of Spiders

Treatments affected abdominal length but not width of cephalothorax (Fig. 2). Abdomen length of spiderlings from LD_{10}-treated females were significantly larger than the controls (ANOVA, $p < 0.01$), whereas differences of cephalothorax width were not significantly different compared to the controls (Kruskal–Wallis test, $p > 0.05$).

After insecticide application, 4 of the 20 subadult spiders in the LD_{10}-treated group died before molting, and 17 of 40 died in the LD_{50}-treated group, whereas none died in the control. Moreover, two spiders of the LD_{50}-treated group died during molting. The growth time from subadult to adult of female spiders in insecticide-treated groups was longer than that of the control, although this test was not significant (Kruskal–Wallis test, $p > 0.05$) (Fig. 3). However, the Dyar's ratio of female spiders was affected significantly (Kruskal–Wallis test, $p < 0.05$) and the growth rates in the LD_{50}-treated groups were significantly higher than that of the controls and LD_{10}-treated ones.

Effect on Predation by Female Spiders

Because the efficacy of buprofezin will remain for a long time, even a month, the predation rates of spiders were monitored continuously for 5 days after application. Many spiders were almost motionless just after insecticide application, some died after 24 h, and some partly recovered after several days. However, those spiders that had recovered from application were not as sensitive as the controls to the presence of prey.

Comparing the control (none died, $n = 8$), LD_{10}-treated (1 died, $n = 9$) and LD_{50}-treated (12 died, $n = 8$) groups, the predation rates of the control spiders declined with

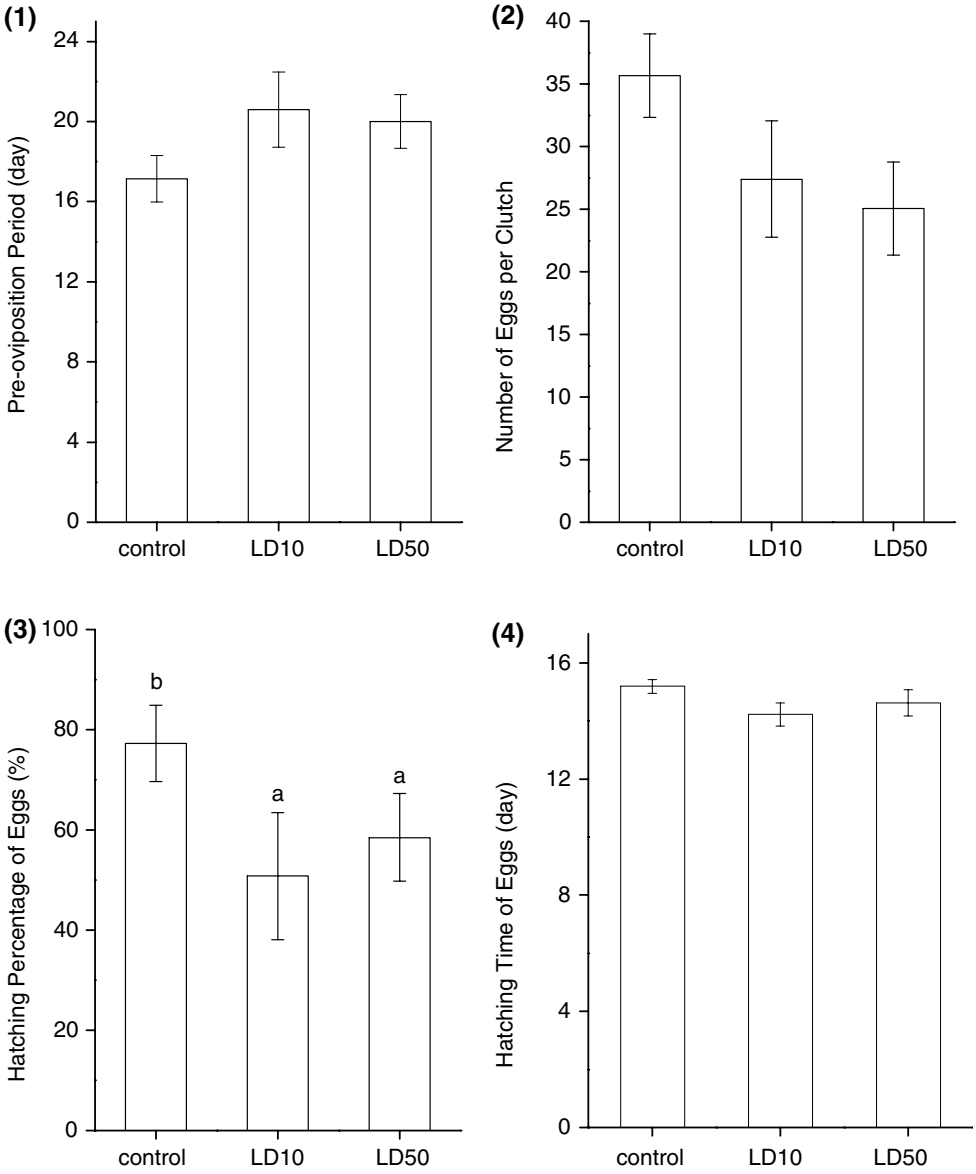

Fig. 1 Comparison of female *P. piratoides* of the preoviposition period **(1)**, number of eggs per clutch **(2)**, hatching percentage **(3)**, and hatching time of eggs **(4)** in LD_{10}- and LD_{50}-treated groups with those in the control. Error bars represent one standard error of the mean. Statistical differences were detected by ANOVA followed by Duncan's multiple-comparison test; comparisons significant at $p < 0.05$ are indicated by different letters

time, wereas the predation rates of those treated with insecticide did not vary with time but were much lower than those of the control (Kruskal–Wallis test, $p < 0.05$) (Fig. 4). The negative effect of buprofezin was greater with increasing application dosage, because the predation rate of the LD_{50}-treated spiders were much lower than those of LD_{10}-treated. As a result, buprofezin had a great effect on the predation by *P. piratoides*.

Discussion

Buprofezin has been shown to suppress insect oviposition and egg fertility. The study of Heong (1988) showed that buprofezin inhibited over 70% egg-laying and over 80% egg-hatching of the brown planthopper. The suppression of insect egg-laying by buprofezin should be attributed to the inhibition of prostaglandin E_2 biosynthesis (Uchida 1987). The result from this laboratory study indicated that buprofezin had effects on the fecundity and development of *P. piratoides*. Not only did insecticide-treated females produce fewer eggs than the controls, but treated females produced a greater proportion of eggs that could not hatch. It should be noted that wolf spiders might eat or abandon their egg sacs if the eggs are not able to hatch. In the test, only two females ate their eggs in the control group, but there were five females in the LD_{10}-treated group and six in the LD_{50}-treated group that did. That is to say, buprofezin application might prevent the development of eggs. However, no negative effects of the insecticide on the growth and development of immature spiders were observed, except that several subadult spiders died during molting.

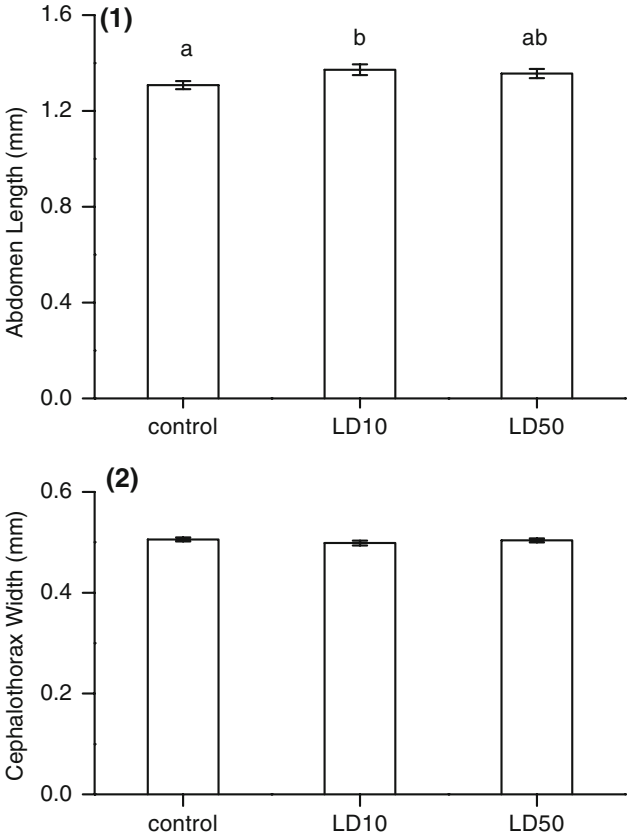

Fig. 2 Comparison of abdomen length **(1)** and cephalothorax width **(2)** of offspring from LD_{10}- and LD_{50}-treated females with those from the control. Error bars represent one standard error of the mean. Statistical differences were detected by ANOVA followed by Duncan's multiple-comparison test; comparisons significant at $p < 0.05$ are indicated by different letters

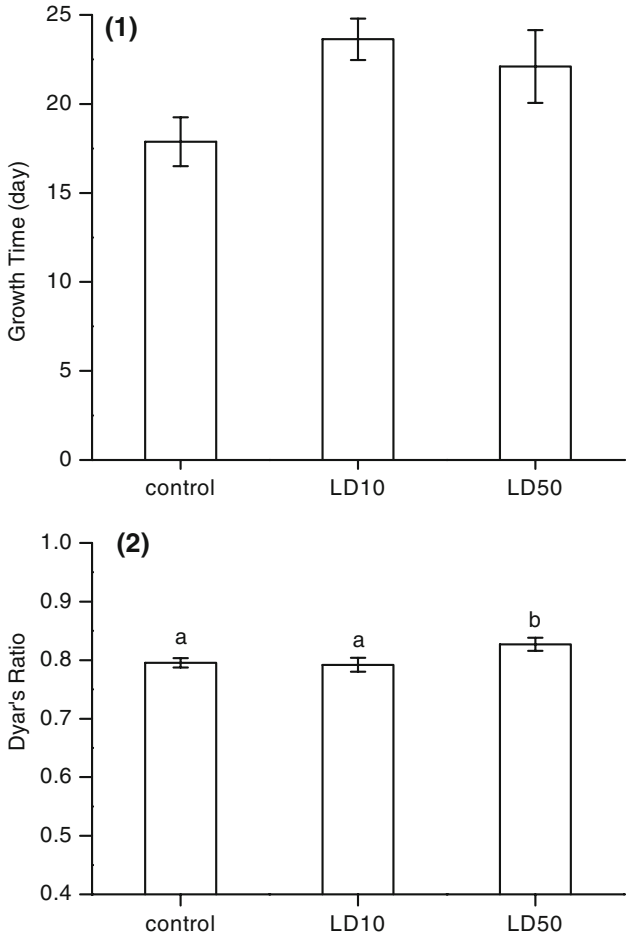

Fig. 3 Comparison of Dray's ratio **(1)** and growth time **(2)** from subadult to adult of spiders in LD_{10}- and LD_{50}-treated groups with those in the control. Error bars represent one standard error of the mean. Statistical differences were detected by ANOVA followed by Duncan's multiple-comparison test; comparisons significant at $p < 0.05$ are indicated by different letters

The differences of abdomen length between insecticide-treated and control spiderlings is also seen. In general, body sizes of spiderlings from LD_{10}- and LD_{50}-treated females were larger than the control. This result might be caused by the decreased egg mass per clutch and the reproductive tradeoff in spiders, whereby relative egg number is inversely related to size of eggs produced (Marshall and Gittleman 1994; Simpson 1995). All in all, the effects of buprofezin on the fecundity and development of *P. piratoides* were much less than those on the pests, but how buprofezin affect the hatching of eggs is still unknown.

A sublethal application of insecticides might induce a stimulatory effect in pests, including physiological and behavioral stimulation, which is called hormoligosis (Luckey 1968). This phenomenon has been proved in some pests such as spider mites (James and Price 2002), but in natural enemies, the effects were negative (Elzen and Elzen 1999). However, in the present study, the growth rates in the LD_{50}-treated subadults were significantly higher than that of the control and LD_{10}-treated group. To our knowledge, this promoting effect has never before been reported in natural enemies It is unknown whether it is hormoligosis. Much more detailed information is required to test this hypothesis.

In addition, the present study showed that buprofezin had adverse influences on the activity of *P. piratoides*, which had not been reported previously. Spiders treated with a high dosage of buprofezin captured fewer prey, even at a high prey density. Two explanations for this are possible. Buprofezin might either inhibit the activity of some enzymes related to the activity of spiders or it might influence the neuroendocrine system of spiders, which might adversely affect predation. The latter is supported by work on the larvae of the brown planthopper (Gu 1993), whereas there is no evidence to support the former explanation. Nevertheless, these possible explanations require further testing and more detailed research.

Although buprofezin had some deleterious impacts on the spiders in this study, such effects on beneficial

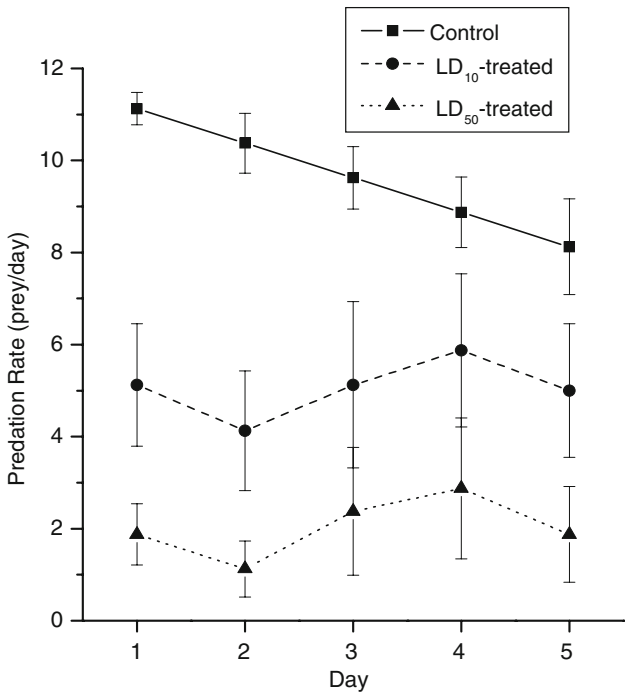

Fig. 4 Predatory rates of female *P. piratoides* for 5 days after insecticide application

arthropod populations are to be expected and are not likely to be catastrophic, as the recommended dosage for buprofezin in the field is 12–15 g active ingredient per are [*i.e.*, 120–150 mg/m^2, which is much lower than the LD_{10} dosage of 316 mg buprofezin/mg fresh weight of spiders used in this study (the mean weight of spiders was 14.38 ± 0.24 mg)]. That means that the exposure dosage of buprofezin to the spiders in the field was probably low enough to be relatively safe to the spiders but had good efficacy against the pests. Consequently, although a few deleterious impacts on spiders can be expected with buprofezin, it is more compatible with biological control than the alternative broad-spectrum insecticides currently applied to rice, such as methamidophos, which was highly toxic to spiders from physiological, reproductive, and behavioral aspects. (Deng et al. 2006, 2007).

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