Full Length Research Paper

Molt disruption and mortality of *Locusta migratoria* var. *manilensis* (Meyen) (Orthoptera: Acrididae) caused by insect growth regulators

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The acute toxicity effects of topical applications of five insect growth regulators (IGRs) on the oriental migratory locust *Locusta migratoria* var. *manilensis* were assessed. Under laboratory conditions, at the highest tested dose rate of 300 ppm, the percent mortality and molt inhibition after two weeks for the five tested compounds were: flufenoxuron (97 and 83%), swallowwort alkaloids (48 and 85%), azadirachtin (100 and 93%), compound RH-5849 (45 and 22%), and pyriproxyfen (48 and 37%) respectively but low doses caused higher molt inhibition for compound RH-5849. These IGRs affected the timing of the subsequent molt, the average timing of the molt (of survivors) was delayed 0-2 days after treatments with flufenoxuron at dose rates between 18.75-300 ppm, RH-5849 at dose rates between 150-300 ppm, but occurred 0-2 days early following treatment with azadirachtin, swallowwort alkaloids or pyriproxyfen at dose rates between 18.75-300 ppm, and RH-5849 at dose rates between 37.5-75 ppm. Initial studies of the insecticidal effects of these insect growth regulators on *L. migratoria manilensis* show them to be very potent locust control agents.

**Key words:** Insect growth regulators, *Locusta migratoria* var. *manilensis* (Meyen), biological control, sustainability.

INTRODUCTION

The oriental migratory locust, *Locusta migratoria manilensis* (Meyen), is an economically important pest that causes significant crop losses in China, Southeast Asia, and the Pacific Region. In northern China, there has been a pattern of increasingly serious locust outbreaks since 1985 (Feng, 2007). The control of the oriental migratory locust in China has been largely based on the use of broad-spectrum chemical pesticides, which can damage human health, agro-ecosystems (example, loss of beneficial insects and increase of insecticide-resistance), and the wider environment (example, effects on non-target species, groundwater, landscapes and communities) (Gregor and Michael, 2007). Biological control has not yet been widely applied for locust control (Dennis, 2008), and work is continuing to find sustainable methods of controlling locusts, including studies on physiology and behavior as mediated by insect growth regulators.

A number of insect growth regulators (IGRs) that have been tested and are used to control a wide variety of insect pests but only a few have been tested against locusts. IGRs are regarded as a third generation of insecticides or biorational pesticides because they differ in their mode of action from other insecticides and have low toxicity to non-target organisms (Zhou et al., 2003). These compounds can potentially affect any insect life stage, including ovicidal effects, inhibition of metamorphosis to the adult stage, or interference with molting of early instar larvae. Because of their desirable characteristics, such as low toxicity, less environmental pollution, high selectivity, and low impact on natural enemies and people, IGRs are used as low-toxicity control agents of various insect pests, and can assist in the development of sustainable agriculture (Wu, 2002; Cedric, 2005; Wang and Wang, 2007).

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Control of locusts requires the availability of safe methods, such as ecological, biological, or integrated controls (Wang, 2007), and the use of IGR pesticides is such an approach because of their low toxicity. However, only a small number of IGRs have been tested against locusts in the field, mainly just a few chitin inhibitors and plant-derived pesticides. Studies have shown that juvenile hormone analogues have a significant effect on the breeding and spawning of the adult oriental migratory locust (Roland and Uwe, 2004); however, there is a general lack of research on the effects of this class of compounds on locusts. Studies of five chitin-inhibiting IGRs in the laboratory showed that the LC$_{50}$ of flufenoxuron and chlorbenzuron to 4$^{th}$ instar nymphs of the oriental migratory locust were 26.5 and 284.4 ppm, respectively, and their LT$_{50}$ values were 4.95 and 5.23 d, respectively (Lei et al., 2002), while diflubenzuron, chlorbenzuron and triflumuron had only very low toxicity to this locust. In another case, 0.3% matrine (at 900 ml/ha) and 1% celangulins (at 300 ml/hm$^2$) killed more than 90% of test locusts (Cheng et al., 2007). As for the widely applied plant-derived pesticide azadirachtin (Dennis, 2008), research on its effects on the Oriental migratory locust has not been done.

This study focused on the effects of different types of IGRs, including the chitin synthesis inhibitors (flufenoxuron), plant-derived pesticides (swallowwort alkaloids and azadirachtin) (Xu, 2002; Cui et al., 2008), molting hormone analogues (RH-5849) and juvenile hormone analogues (pyriproxyfen), on the oriental migratory locust, aiming to find new techniques and methods for the sustainable management of this pest.

MATERIALS AND METHODS

Locusts

*L. migratoria* var. *manilensis* nymphs from the Key Biological Control Laboratory of the China Agricultural University in Beijing, China were used for the tests. A group of 150-180 individuals of both sexes were reared under crowded conditions in cages (15 × 15 × 30 cm$^3$), at 27-30°C and 60-85% RH, and were supplied with fresh maize seedlings for food. The third instar nymphs were selected for the experiment.

Pesticides

The sources of pesticides used in tests were as follows: (1) flufenoxuron: 95% TC, Manufacturer: Zhenjiang Agrosinic Co., Ltd. (2) azadirachtin: 98%. Manufacturers: Yunnan Zhongke Biology Industry, Ltd. and Yunnan Xinlian chemical factory. (3) RH-5849: 92% TC, Manufacturer: Mikania Biological Control Services Ltd. (4) pyriproxyfen: 95% TC, Manufacturer: Hangzhou Yilong Chemical Industry Co., Ltd. (5) swallowwort alkaloids: alkaloids extracted from *Cynanchum komarovi* AL. Iljinski using the following method: At room temperature (25 ± 2°C), 0.2 kg of the plant powder (screened with 40 mesh sieves) was soaked in 2 L industrial alcohol (95.5%, Beijing Chemical Reagent Ltd = B.C.R. Ltd) containing 2 ml hydrochloric acid (37%, B.C.R.Ltd) for 72 h. The solution was then extracted through filter paper (20-30 µm, B.C.R. Ltd) using a vacuum pump (model SHB-III; Zhengzhou Great Wall Industrial and Trading Co.). The extracted solution was dissolved in 2% hydrochloric acid solution, and insoluble particles were removed by filtration and chloroform (RD1.4832 [20/4°C] extraction). The solution was modified to pH 9-11 with sodium hydroxide (98%, B.C.R. Ltd) and the alkaloid solution was obtained through chloroform extraction and vacuum concentration, 3-5 times, with a rotary evaporator (RES2-98, Shanghai Yarong Biochemical Instrument Factory). Berberine chloride (National Institute for the Control of Pharmaceutical and Biological Products of China) was applied to a standard sample; 0.1% BTB (Committee of Chinese Pharmacopeia) was applied for acid dyes and the sample solution (at a pH of 5.4 in a citric acid-sodium citrate buffer) was analyzed with a Three-use Ultraviolet Analysis Instrument (UV-1) (Shanghai Gucun Electro-optical Instrument Factory). The absorbance value of the solution at 419 nm wavelength was recorded and used to determine the alkaloid contents of the sample solution (Wang et al., 2006; Shi et al., unpublished data). All pesticides were diluted with distilled water into different concentrations for use in the experiment.

Toxicity tests

Third instar nymphs of *L. migratoria* var. *manilensis* were selected for the experiment. The wax on the surface of maize leaves was scraped off with knife, and then a 2 × 3 cm rectangle was punched out of the leaf. Onto these maize leaf sections we placed a 1 µL drop of the test concentration of a particular pesticide and then the leaf was air-dried. Distilled water was used to make up the pesticide concentrations that were used in the experiment: 0 (control, distilled water only), 18.75, 37.5, 75, 150 and 300 ppm. A third instar nymph and either a treated or control maize leaf section were placed in muslin-covered plastic container (8 cm diameter and 10 cm height). Each treatment consisted of 20 locusts (only those consuming the whole leaf disc were used in the experiment) and each treatment (by pesticide and concentration) was repeated 5 times. All the insects died or became adults. A toxicity regression equation was calculated for each compound and an LC$_{50}$ obtained using probit analysis.

Statistical analysis

All figures were made using EXCEL 2003 Windows (Microsoft Co., 2003). Statistical analyses were performed using SPSS 11.50 Windows (SPSS Inc., 2001). After arcsine transformation, the molt ratios of the nymphs of oriental migratory locust after treatment with IGRs were analyzed in One Way ANOVA.

RESULTS

Mortality of *Locusta migratoria manilensis* caused by IGRs

Acute lethal toxicity of IGRs against locust

The acute lethal toxicities of flufenoxuron and azadirachtin against the locusts were generally significantly (Figure 1 shows captions of statistical details and P values) higher than those of the swallowwort alkaloids, RH-5849, or pyriproxyfen. With the exception of RH-
5849, mortality generally increased with concentration. There was 100% mortality caused by treatments with high doses of azadirachtin (150 or 300 ppm) and flufenoxuron (300 ppm) (Figure 1).

With 300 ppm azadirachtin, 80% of locusts died within 2 days and 98% in 6 days and mortality was only slightly slower at a dose of 150 ppm. The acute lethal effect of flufenoxuron, at 4 and 8 days after treatment, reached around 80%. The swallowwort alkaloids had a mortality rate of 45% at 14 days post treatment. The lowest mortalities were caused by pyriproxyfen (48% after 14 days) and RH-5849 (45% after 14 days).

**Insect toxicity of flufenoxuron and azadirachtin to locusts**

The LD₅₀ and LC₅₀ of flufenoxuron on 3rd instar nymphs of *L. migratoria var. manilensis* were both lower than the values for azadirachtin, a toxicity index of flufenoxuron was index significantly higher than that of azadirachtin (Table 1). The mortalities caused by the swallowwort alkaloids, RH-5849, pyriproxyfen and in this experiment were below 50%, and therefore their LC₅₀ and LD₅₀ values could not be calculated.

**Influence of IGRs on the molting of locust nymphs**

**Molt disruption of IGRs**

While about 90% of untreated locusts molted, the percent molting was significantly lower when locusts were treated with flufenoxuron, swallowwort alkaloids, azadirachtin (at several concentrations) or RH-5849 (at the lower concentrations). RH-5849 at higher concentrations and pyriproxyfen had no significant effects on molting rates of the nymphs (Figure 2).

**The Influence of IGRs on peak timing of molting by experimental populations**

With untreated locusts the peak time of the subsequent molt was at 3 days but these IGRs affected molting by making it either earlier or later (Figure 3). Flufenoxuron led to a delay in molting that increased with dose. With swallowwort and azadirachtin at 18.75 ppm led to peak
Table 1. Toxicity of the alkaloids flufenoxuron and azadirachtin against *Locusta migratoria manilensis*, giving LC$_{50}$ (ppm) and LD$_{50}$ (mg Kg$^{-1}$) values for 3rd instar nymphs of *Locusta migratoria manilensis*.

<table>
<thead>
<tr>
<th>IGRs</th>
<th>Value</th>
<th>TRE$^a$</th>
<th>RTI</th>
<th>$K^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flufenoxuron</td>
<td>LC$_{50}$ 26.04</td>
<td>Y=-1.33811+0.94521x 136.39</td>
<td>136.39</td>
<td>5.171</td>
<td>0.160</td>
</tr>
<tr>
<td></td>
<td>LD$_{50}$ 0.90</td>
<td>Y=0.04416+0.94521x 136.39</td>
<td>136.39</td>
<td>5.171</td>
<td>0.160</td>
</tr>
<tr>
<td>Azadirachtin</td>
<td>LC$_{50}$ 35.52</td>
<td>Y=-5.05125+3.25792x 100</td>
<td>100</td>
<td>0.783</td>
<td>0.853</td>
</tr>
<tr>
<td></td>
<td>LD$_{50}$ 1.22</td>
<td>Y=-0.28687+3.25789x 100</td>
<td>100</td>
<td>0.783</td>
<td>0.853</td>
</tr>
</tbody>
</table>

$^a$ In regression equations, X denotes the concentration and Y the probability of death. TRE, Toxicity regression equation; RTI, relative toxicity Index; $K^2$, Chi square.

**DISCUSSION**

Insect growth regulators can kill or debilitate treated insects by affecting physiological processes, inhibiting molting, preventing the formation of new integument, or reducing feeding (Bi et al., 2008). The acute lethality and molt-inhibiting effects of five types of IGRs on oriental migratory locust were evaluated. The results demonstra-
Figure 3. Peak timing of molting by experimental populations post-treatment with different concentrations of IGRs. These IGRs affected the timing of the subsequent molt, the average timing of the molt (of survivors) was delayed or forwarded 0-2 days after treatments with these pesticides at dose rates between 18.75-300 ppm respectively.

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References


