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The malformation effect of chlorfluazuron on the reproductive system of land snail *Eobania vermiculata*

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Abstract The effect of chlorfluazuron (IGR) on land snail, *Eobania vermiculata*, was studied under laboratory and field conditions. Snails were treated with different concentrations of chlorfluazuron as contact and bait techniques. LC_{50} value was calculated after 7 days of treatment. The effect of LC_{50} value of the compound was studied on the reproductive system of snails after 7 days of treatment. The effect of the compound was evaluated as a spray under field conditions compared with methomyl (MALR recommended compound). The results revealed that chlorfluazuron was more toxic when used as a contact than as bait. LC_{50} for the contact was 1528.0 ppm and 1992.5 ppm for the bait. On the other side, chlorfluazuron induced malformation on the organs of reproductive system of snails compared with untreated animals. It caused severe swelling in the size of penis, vagina, sperm oviduct and albumen gland, and in the ovotestis the eggs were absent. Under field conditions, the compound achieved good results as it gave 78.7% reduction in snail population. Although this value was less than methomyl (94.4%), chlorfluazuron could be considered satisfied compound to be used in control programs of land snails.

Introduction

Land snails became injurious pests in Egypt. These animals attack different kinds of plants, vegetable, fruit, orchard and ornamental plants. They feed on seeds and damage different parts of plants (Briner and Frank, 1998). The knowledge about the Egyptian terrestrial gastropods is still fragmentary and partial information is available about their biology and control (Reham Ali, 2011). There is no specific compound to be used against pest snails, attacking different agricultural crops in Egypt, except metaldehyde. Thus, this present work aims to study the toxic effect of chlorfluazuron compound (Insect Growth regulator), as a contact poison and as a poison bait against chocolate band land snails (*Eobania vermiculata*), one of the most harmful species to major field crops in Egypt, under laboratory and field conditions.

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Chlorfluazuron is an anti molting agent, inhibits biosynthesis of chitin of an important constituent in insect cuticle, loses cuticle elasticity and firmness and results in abortive molting. chlorfluazuron is benzoyl phenyl urea (BPU) chitin synthesis inhibits larvicidal and ovicidal activity against a number of lepidopteran, coleopteran and dipteran insect pest species at lethal doses, and effects development and reproduction at sublethal treatment (Fang et al., 2007). Also, chlorfluazuron had been used as reproductive inhibitor (Preveen, 2006). Biddinger and Hull (1999) stated that tebufenzoide (IGR) reduced fecundity in mating in the larvae of male and female of tufted apple, *Platynotaidea usalis*. Chlorfluazuron is safe to beneficial insects. Maha Mahmoud (2002) found that diflubenzuron (IGR) caused teratogenic effect on jaws of different ages of land slug *Limax flavus*. The current study is an applied work searching for an alternative compound to be used for the control of land snails attacking different crops in Egypt. It is very important to find new compounds to avoid the development of resistance in snails to the repeated use of only one compound (the recommended). The aim of this work is to study the following:

1. The effect of chlorfluazuron (IGR), as a new compound, against land snail *E. vermiculata*, using contact poison and poison baiting techniques.
2. Pathological changes in the reproductive system (hermaphrodite) and egg production of snails after treatment with chlorfluazuron.
3. The field performance of chlorfluazuron as a spray comparing with methomyl (MALR recommended compound) against the snail species.

Material and methods

Experimental animals

Specimens of chocolate band land snails, *E. vermiculata,* were collected from trees of a citrus nursery belonging to the Ministry of Agriculture, Giza Governorate, and transported to the laboratory. Animals were kept in small glass boxes containing moist soil (8–10 cm high). Each box was provided with fresh green lettuce leaves and was covered with muslin secured with a rubber band, to prevent snails from escaping, and kept under 20 ± 2° c in laboratory.

Tested compounds

Common name: chlorfluazuron

Trade name: Caprice (5% EC) Is an insect growth regulator. LD_{50} for rat is 8500 mg/kg and it is effective at the rate of 10–50 g. a.i./ha. It was obtained from El-Helb for Pesticides and Chemicals Company, Egypt.

Common name: methomyl

Trade name: Newmyl (20% SL) Is a Carbamate compound. LD_{50} for rat is 24 mg/kg. It was obtained from Kafr El-Zayat Company, Egypt. The Egyptian Ministry of Agriculture and Land Reclamation (MALR) recommends this compound for use against land snail infestation in Egyptian fields at a rate of 8–10 kg/feed as bait.

Laboratory experiments

Baiting technique

Chlorfluazuron compound was evaluated as poison bait. Different concentrations of the compound (500, 1000, 1500, 2000, and 2500 ppm) were prepared and tested. Poison bait was prepared by mixing chlorfluazuron with 5% molasses + 93% bran. Five grams of the poison bait was put on a plastic sheet placed on the surface of the soil in each glass box. Animals were exposed to the candidate concentration of the compound. A control test was conducted with plain carriers. Mortality percentages were calculated during the period of 7 days and LC_{50}value (The median lethal concentration) was determined according to Finney (1971).

Contact (thin film) technique

Thin film technique was used according to Asher and Mirian (1981), whereas tested concentrations 500, 1000, 1500, 2000 and 2500 ppm of chlorfluazuron were applied in Petri- dish using water. Two ml of each concentration of the compound was spread on the inner surface of each Petri-dish by moving the dish gently in circles. Water was evaporated in a few minutes, under room conditions, leaving a thin layer film of the tested compound. Animals were exposed to the candidate concentrations of the tested compound for 7 days. A parallel control test was conducted using plain water. The killed animals were daily counted and removed. Mortality percentages were calculated and LC_{50} value was determined.

Anatomical studies

Snails were treated with LC_{50} value of chlorfluazuron compound. After 7 days the treated snails were anesthetized in 1% solution of chloral hydrate for 12 h (Shoieb, 1997). Reproductive systems of the treated snail were carefully removed using binocular stereoscope, then photographed and compared with those of untreated ones. Ovotestis was separated from each treated and untreated snail at the time of reproduction and photographed.

Field application

The two tested compounds, chlorfluazuron and methomyl (the MALR recommended compound) were evaluated as a spray application against land snail, *E. vermiculata,* at Giza Governorate i.e., citrus nursery trees. Three plots (each of 4 m^2) were chosen. One plot was used for chlorfluazuron, the second for methomyl and the third was left without treatment as control. Each plot was far from the other by at least 2 m. Live snails were counted daily pre and post treatment during the next 7 days. The efficiency of each compound was calculated based on the reduction of snail population after 7 days of treatment according to the formula of Henderson and Tilton (1952).

Results and discussion

Laboratory studies

Toxicity tests

Data in Table 1 show the efficacy of chlorfluazuron, when used as a contact and bait techniques, against land snail,