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Toxicity of Hexaflumuron as an insect growth regulator (IGR) against *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae)

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Abstract
The exploit of insect growth regulators (IGR) for insect pest management are good option because they do not accumulate in the environment and are less toxic to man and domestic animals. At the present study, a chitin synthesis inhibitor from insect growth regulator; Hexaflumuron was tested for its toxicity and side effects on the 4th instar larvae of *Helicoverpa armigera* Hubner. Estimated LC_{50} and LC_{90} values of leaf dip bioassay of Hexaflumuron on these larvae after 120 hours of treatment were 8.47, and 82.26 mg /L, respectively. Hexaflumuron at doses of 1, 3.2, 9.8, 31.6, and 100 mg/L were caused significant mortality on prepupae and pupae as compared to untreated control. Also abnormalities in adults were observed with treated pupae. We conclude that the sub-lethal and lethal effects of Hexaflumuron might exhibit significant effects on the population of *H. armigera* and can be used to its management.

Keywords: IGR, Hexaflumuron, *Helicoverpa armigera,* prepupae, pupae.

1. Introduction
Helicoverpa armigera Hubner (Lepidoptera: Noctuidae) is a pest with broad distribution of surfaces in worldwide that is causing damage on many agricultural crops and early instars are foliar feeders and later instars damnify to seeds, fruits, and bolls [28, 7]. This pest would damage in crops like cotton, tomato, pigeon pea, chickpea, groundnut, sorghum, pearl millet, and other crops of commercial value [23].

The utilization of synthetic chemicals such as endosulfan, profenofos and thiodicarb has been introduced as main method for control of *H. armigera* in Iran [18]. The various methods have been used and lots of money is expensed every year to control of *H. armigera*. In such cases insect growth regulators are better option that do not persist or accumulate in the environment and are degraded to simple molecules that don't have a problem of environmental contamination and are less toxic to man and domestic animals [25]. Hexaflumuron [N-(((3,5-dichloro-4-(1,1,2,2-tetrafluroethoxy) phenyl)- amino) carbonyl) 2,6 diflurobenzamide] is a chitin synthesis inhibitor that have ability of controlling various insect pests of agriculture [1, 14]. Insecticides based with organophosphates (OPs), carbamates and pyrethroids have caused increasing of resistance, resurgence and outbreaks in *H. armigera* populations by deleterious effects on natural enemies are reason outbreaks of pests [8, 2] while the hexaflumuron is seemed to have a lower toxicity to *Habrobracon hebetor* (Say) as a commercial parasitoid [22].

Due to adverse effects of the use of conventional insecticides and also existence of a high level of resistance in this pest to all groups of pesticides, other options were requirement. Therefore insect growth regulators (IGRs) via inhibiting the synthesis of chitin in insects are most suitable [4, 12]. This study is therefore, aimed to test the efficacy of hexaflumuron against caterpillar of *H. armigera* so that their population can be managed.

2. Materials and methods
2.1. Insect rearing
Larvae of *Helicoverpa armigera* Hubner was collected from the Moghan cotton farms in Iran and reared on modified Shorey and Hale's [24] pinto bean-based artificial diet in the laboratory at 27±2 °C under a 16 h light:8 h dark (L:D 16:8) photoperiod and 50±5% relative humidity between April to October 2013.

2.2. Bioassays

In this study, the toxicities of the hexaflumuron (Consult 10 EC) were assessed against 4th instars of larvae, prepupae and pupae of *H. armigera* on the treated tomato leaves. The concentration of various treatments was 1, 3.2, 9.8, 31.6, and 100 mg /L. The tomato leaf were dipped into different concentrations of the insecticide for 10 seconds and let dried for 15 minutes. The 4th instars of *H. armigera* were then transferred on the leaves. Twenty larvae of *H. armigera* were put in each treatment. The mortality of larvae was recorded in 48, 72, 96 and 120 hours after treatments. All processes have done for control group with distilled water instead hexaflumuron. Experiments have repeated three times.

Prepupae were treated with 30 µl of the hexaflumuron via topical application, So that it is attempted to cover completely whole the body of prepupae. Pupae were dipped into different concentrations of the insecticide for 2 seconds. Twenty prepupae and pupae instars of *H. armigera* were put in each treatment. Three replicates were used in all experiments.

2.3. Data analysis

The experiment data were subjected to one-way analysis of variance (ANOVA) after checking for normality. Means were compared by Duncan's test and significant differences were recorded at $\rho = 0.05$ with SPSS software.

3. Results and discussion

3.1. Toxicity Symptoms

Chitin synthesis inhibitors caused disruption of chitin synthesis in the insect pests. In some treated larvae, white ring inside of head capsule filled with brown fluid (probably hemolymph) was seen and also the larvae body was slimy and black in color (Fig 1. A and B). Some of the prepupae had partial molting and finally dead and the prepupal-pupal intermediates and partial smudge on body was seemed (Fig 1. C and D). Short wings and condensation of wings was observed in some of adults (Fig 1. E and F). Hughes *et al.* [9] and Karimzadeh *et al.* [13] also were reported some of these symptoms and introduced that hexaflumuron probably decreases chitin synthesis in endocuticle of various instars. The creation of larval-pupal intermediates and defective pupae have been reported in *Spodoptera mauritia* (Bois du val) [10] and *H. armigera* when treated with diflubenzuron [15] and also *Tribolium castaneum* (Herbst) and *T. Confusum* Jacquelin du Val when treated with cyromazine and pirimiphos-methyl [11].

Fig 1: The White ring filled with brown fluid of 4th instar (A) and it has been seem black and slimy (B), partial molting of prepupae (C), prepupal-pupal intermediates and partial smudge of it (D), the wing short (E) and condensation of wing (F) in adults of *Helicoverpa armigera* after treatment with hexaflumuron

3.2. LC values

The results of assessing the toxicity of hexaflumuron on the 4th instars of *H. armigera* in various hours after treatment are shown in Tables 1. The data obtained suggests that hexaflumuron is a highly toxic insecticide against *H. armigera*. As shown in table 1, mortality after 96 and 120 hours considerably was higher than 48 and 72 hours which are depict slow effect of IGRs.

Table 1: LC_{50} values of hexaflumuron on 4th instars of *Helicoverpa armigera* 48, 72, 96 and 120 hours after using the leaf dip method

Time	LC_{10} (mg /L) (95% CL)	LC_{50} (mg /L) (95% CL)	LC_{90} (mg /L) (95% CL)	slope ± SE	χ^2
48	5.69 (1.99-10.56)	345.4 (133.23-2517.11)	20970 (2774.62-192000)	0.72 ± 0.14	0.26^{ns}
72	1.59 (0.61-2.89)	42.25 (27.23-77.35)	1123 (417.33-5969.98)	0.90 ± 0.12	0.41^{ns}
96	0.90 (0.41-1.54)	12.77 (9.19-18.02)	180.8 (100.69-430.08)	1.11 ± 0.12	1.78^{ns}
120	0.87 (0.44-1.39)	8.47 (6.28-11.36)	82.26 (52.02-156.94)	1.29 ± 0.13	3.25^{ns}

Hexaflumuron at doses of 100, 31.6, 9.8, 3.2 and 1 mg /L caused mortality of the 4th instar larvae. Mortality in insecticide treatments was significantly higher than in the untreated control ($f = 97.857$, $df = 5$). It was increased with increase in hexaflumuron concentration (Fig. 2). Figure 2 presents the relationship between the probit of percentage mortalities and the logarithm of the concentrations of the tested chitin synthesis inhibitor. There is a report that hexaflumuron has a high contact toxicity to cotton bollworm, with 0.7434 mg /L of LD_{50} in topical application test, and high stomach toxicity with 2.0592 mg/L of LC_{50} in leaf dipping bioassay on cotton [6]. Karimzadeh *et al.* [13] reported that hexaflumuron and lufenuron were more effective than the other insecticides on *Leptinotarsa decemlineata* (Say). Also the spinosad and hexaflumuron are more useful than the other insecticides due to their higher toxicity to *H. armigera* and lower toxicity to *H. hebetor* (Say) [22].

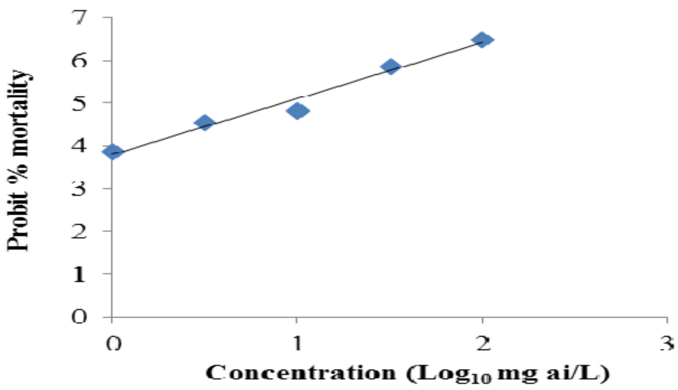

Fig 2: Concentration-response relationship of chitin synthesis inhibitor on the 4th instars of *Helicoverpa armigera* after 120 hours from treatment

3.3. Mortality of prepupae and pupae

Hexaflumuron at doses of 100, 31.6, 9.8, 3.2 and 1 mg /L caused mortality of prepupae instar. Mortality was found to increase in a dose dependent manner and was significantly higher in insecticide treatments than in the untreated control ($f = 26.044$, $df = 5$). Post-exposure effects were observed in pupae if prepupae were exposed to hexaflumuron. After change to pupae, mortality in insecticide treatments was significantly higher than in the untreated control ($f = 10.522$, $df = 5$) (Fig. 3) and at last the adult's emergence was decreased. The duration of nymphal development was not prolonged and treated survivor change to adults. In treatment of *H. armigera* with Spinosad, has been reduced the pupation ratio, pupal survival and the adult emergence ratio [27]. It is showed that hexaflumuron had a significant reduction on the percentage of pupation and adult emergence on the *Plutella xylostella* (L.) [17] and *Ephestia kuehniella* Zeller [16]. Vasuki and Rajavel [26] also suggested that hexaflumuron significantly decreased the adult emergence rate of *Anopheles stephensi* Liston.

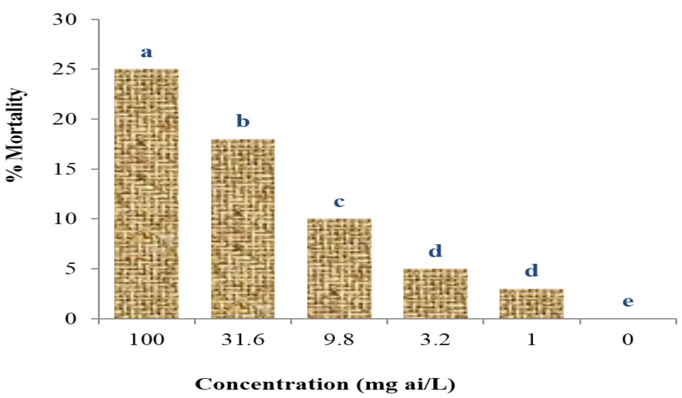

Fig 3: Mortality of prepupae observed at different concentration of hexaflumuron. The mortality depended on concentrations ($F=26.044$, $df= 5$, $P=0.0001$ for prepupae and $F=10.522$, $df= 5$, $P=0.0001$ for pupae). Mean mortality with the same letter on columns are not significantly different ($P=0.05$, Duncan's test)

3.4. Abnormality of adults

Post-exposure effects were observed in adult if pupae were exposed to hexaflumuron. Hexaflumuron at doses of 100, 31.6, 9.8, 3.2 and 1 mg /L caused abnormality of adults (Fig 4). Abnormality in insecticide treatments was significantly higher than in the untreated control and was increased with increase of hexaflumuron concentrations ($f = 29.581$, $df = 5$). Also in all concentrations adult emergence was 100 percent. Diflubenzuron (10-1000 ppm) caused 24.8% adult abnormalities on the 2nd instar larvae of *H. armigera* [15]. In study of Bakr *et al.* [3] all nymphs of *Schistocerca gregaria* (Forskal) with 75 and 100 mg/L of hexaflumuron had not conversion into adults and it has been reported that adult emergence was inhibited completely in *Spodoptera litura* (F.) with diflubenzuron at 50 ppm and in *Culex quinquefasciatus* Say with 23 diphenylureas chitin inhibitors at 1 ppm [5, 20].

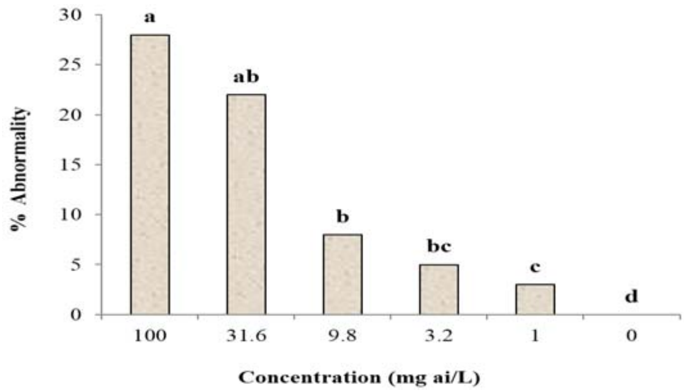

Fig 4: Abnormality of adults observed at different concentration of hexaflumuron. The activity depended on concentrations ($F=29.581$, $df= 5$, $P=0.0001$). Mean abnormality with the same letter on columns are not significantly different ($P=0.05$, Duncan's test)

In summary, the present results suggest that hexaflumuron has an effective role on all studied stages of the *H. armigera*. Also sub-lethal and lethal doses of hexaflumuron had effects on *H. armigera*, such as mortality and abnormality in larvae, prepupae and pupae and abnormality in adults. It is reported that survival of parasitoids and rates of parasitism were higher in cotton fields sprayed with IGRs compared with those sprayed with conventional insecticides [21]. Inhibition of chitin synthesis can be effective by having selective properties, efficacy on immature stages of pest and have ability to be used in Integrated Pest Management (IPM) programs [19]. If our results can be supported in the field situation, the hexaflumuron may be considered as alternative chemical with a high potential for controlling of *H. armigera*.

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