

Effects of Anti-Juvenile Hormone, Precocene-I, on Egg Development of Sunn Pest, *Eurygaster integriceps*, and Its Progenies

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ABSTRACT

The Sunn pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae), is a key pest of wheat in Iran. The nymphs and adults reduce wheat yield quality and quantity by feeding on leaves, stems, and grains. Pesticide application is the main method of Sunn pest control. Precocene, a juvenile hormone inhibitor, exerts cytotoxic effects on corpora allata of insects' species. . In this research, the effect of precocene-I (7-methoxy-2, 2-dimethyl chormene) on Sunn pest egg development and subsequent progenies was evaluated in the laboratory. Concentration range on eggs bioassay was 1, 5, 10, 20, and 50 $\mu\text{g mL}^{-1}$. The results of bioassay tests on 2- and 5-day old eggs showed that LC_{50} values were 15.4 and 15 $\mu\text{g mL}^{-1}$, respectively. Therefore, old eggs were more sensitive than young eggs. The effects of LC_{30} and LC_{50} of precocene-I on the egg and nymphal mortality were significantly stronger than the control, and the highest mortality rates in the first and third instars were 14.28 and 13.26%, respectively. Also, precocene-I did not affect embryonic period, but increased nymphal period (39.3 days), compared to the control (34.8 days). Morphological changes on nymphs that emerged from treated eggs included large wings and hemelytron. The fifth instars showed deformed scutellum. Also, typically, some old cuticules were not completely separated.

Keywords: Juvenile hormone inhibitor, Nymphal mortality, Pesticide, 7-methoxy-2, 2-dimethyl chormene.

INTRODUCTION

The Sunn pest, *Eurygaster integriceps* Puton (Hem: Scutelleridae) is one of the most destructive pest of wheat and barley fields in Iran and vast areas of Asia that causes direct and indirect damage to the wheat crop (Radjabi, 2000; Kazzazi *et al.*., 2005).

Increased use of organophosphorus pesticides, such as fenitrothion, and their adverse effects on the environment and human health has necessitated implementation of safety methods (Hoffmann and Lorenz, 1997). The emergence and identification of chemical compounds with natural origin, affecting the

juvenile hormone of insects, enhanced efforts to produce synthetic bioactive analogues.

The natural compounds which interfere with insect growth are called 'Insect Growth Regulators'. They intervene biosynthesis of juvenile hormone via stimulating or inhibiting its synthesis which has been used as a solution for insects control (Edwards and Menn, 1981). So far, some growth regulators such as Juvenile Hormone (JH) analogues have been used against Sunn pest in several studies. For instance, effect of the pyriproxyfen on different stages of eggs and nymphs of Sunn pest was tested (Zarnegar and Nouri, 2006;

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Mojaver and Bandani, 2010; Zibae et al., 2011). Increasing concentration of Fenoxycarb increased egg production in females and created wrinkles on egg shells (Pour et al., 2014). Precocene-I, which is extracted from *Ageratum houstonianum* Mill., restrain cytochrome P-450 in *Corpora Allata* (CA) of insects through competition with oxidizing enzymes, in the final stages of juvenile hormone biosynthesis. Therefore, inactive and unsustainable epoxide of Precocene is produced. The involvement of cytochrome P-450 in Precocene metabolism leads to decrease in JH biosynthesis. In addition, Precocene epoxide alkylates cellular components of CA and finally eliminates cells of this endocrine organ (Pratt, 1983; Polivanova, 1985). Generally, Precocenes have toxic effects on CA and results in transformation of premature larvae and sterility of adult insects by stopping the production of JH. Also, it prevents the ectoderm formation and the high mortality rate of eggs (Bower et al., 1976; Dorn, 1982). There are several studies on impact of Precocene on insects (Dorn, 1982; Bitsch and Bitsch, 1984; Aboulafia-Baginsky et al., 1984; Stall, 1986; Bradley and Bowers, 1992; Triseleva, 2007; Farazmand and Chaika, 2008). For instance, the effects of Precocene-II were examined on the first instar larvae of *E. integriceps* (Polivanova, 1985). It is shown that Precocene-II causes morphological changes in subsequent developmental stages and its effects depend on concentration and time of larvae exposure. Also, Precocene-II has an effect on CA in male and female and represents similar effects on both genders of *E. integriceps* (Triseleva, 1990). Influence of Precocene-I on growth, reproduction and hemolymph protein was investigated and its impacts on the number of eggs and their hatching were proved (Amiri et al., 2010). The effects of JH inhibitor compounds on fifth

instar of nymphs in Sunn pest, was studied and showed that treatment of nymphs with Precocene-I and -II led to abnormality of the adults (Kafi et al., 2016). So far, little research has been conducted on the role of Precocene-I on developmental stages of Sunn pest. Therefore, this research aimed to investigate the effect of Precocene-I on the egg development of Sunn pest and its progenies.

MATERIALS AND METHODS

The insects were collected from wheat fields near Tehran, Iran, and were maintained in the laboratory ($25\pm1^{\circ}\text{C}$, RH: $65\pm5\%$, 16 hours light: 8 hours dark). To obtain two-and five-day old eggs, adults were moved from the nurturing cage made for *E. integriceps* to medium-size plastic Petri dishes (60 mm in diameter). Anti-juvenile hormone compound used in the experiment was Precocene-I, 99% (7-methoxy-2, 2-dimethyl chormene) (Sigma-Aldrich Co., Germany).

After preliminary tests, five concentrations of Precocene-I, including 1, 5, 10, 20 and $50\text{ }\mu\text{g mL}^{-1}$, were chosen for bioassay. Eggs were dipped in to the Precocene-I solutions for 10 seconds. The control eggs were dipped in acetone. After application, all eggs were left to dry on filter paper. The treated eggs were transferred into Petri dishes (5 cm in diameter) ($25\pm1^{\circ}\text{C}$, RH: $65\pm5\%$, 16 hours light: 8 hours dark). Each concentration had three replications. In each replication, 24 eggs were treated. In all experiments, acetone was used as a solvent.

Effects of Sub-Lethal Concentrations on the Nymph

The effect of LC_{30} and LC_{50} of Precocene-I on egg mortality rate, nymphal period, mortality rate of nymphs, survival rate (number of insects survived for 10 days after emergence as an adult) and abnormality

percentage of the appeared insects were estimated.

Young 24-hour eggs were immersed into Precocene-I concentrations of 8.5 and 14.4 $\mu\text{g mL}^{-1}$. They were transferred to culture containers after being dried in the air. Then, they were kept up to 10 days after becoming adult. All experiments had six replications.

Statistical Analysis

Statistical analysis was performed using SAS (ver. 9). Stat Direct Software (2012) was used to calculate LC_{30} and LC_{50} . Means were compared using Tukey test ($\alpha=0.05$)

RESULTS

The results of the Precocene-I bioassay on the 2- and 5-day old eggs showed that the required dose to kill 50% of 2- and 5-day old eggs were 15.4 and 15 $\mu\text{g mL}^{-1}$, respectively (Table 1). According to the results, old eggs were more sensitive to Precocene-I than young eggs. So, the rate of mortality in older eggs was higher than young eggs in all experiments.

Effect on Eggs

Precocene impact on the mortality rate of Sunn pest eggs is presented in (Table 2). The highest percentage of egg mortality in treatment of LC_{50} was $50\% \pm 4.40$ while this value was $3.06\% \pm 1.44$ in the control ($F_{2, 18} = 58.51$, $P = 0.0001$). The mortality percentage of eggs with LC_{30} was 30.61 ± 4.03 , which was significantly different than the control and LC_{50} treatments.

Effect on Nymphs

Based on the results on total mortality percentage of nymph, the highest mortality rate, which was $82.65 \pm 3.06\%$, was observed in LC_{50} treatments (Table 2). Total nymphal mortality rate was different from the control ($F_{2, 18} = 10.88$; $P = 0.0008$). According to the results, the most Precocene-I impact on mortality rate of nymphal instars was in the first and third instars and the lowest mortality rate was observed in the fifth instar (Figure 1).

Effect on Survival Rate of Adults

The highest survival rate of adult Sunn

Table 1. Results of Probit Analysis and Precocene toxicity calculation in different ages of Sunn pest, *Eurygaster integriceps*.

Egg treatment	Lethal Concentration ($\mu\text{g mL}^{-1}$) (95% Confidence interval)			Slope \pm SE	Chi-Square (df)	P-value
	LC_{30}	LC_{50}	LC_{90}			
2-Day old egg	9.423 (6.904- 11.760)	15.395 (12.414- 18.688)	51.099 (38.795- 77.800)	2.46 \pm 0.22	6.153 (3)	0.1044
5-Day old egg	10.00 (8.034- 11.815)	14.95 (12.711- 17.099)	39.940 (31.550- 56.575)	3.01 \pm 0.36	1.204 (3)	0.752

Table 2. Effect of sub-lethal concentrations of Precocene-I, applied on eggs of Sunn pest.^a

Treatment	Egg mortality (%) (Means \pm SE)	Nymphal mortality (%) (Means \pm SE)	Survival (%) (Means \pm SE)
Control	3.06 \pm 1.44a	60.20 \pm 4.08 a	34.69 \pm 3.63 a
LC_{30}	30.61 \pm 4.03 b	73.47 \pm 3.00 b	20.41 \pm 2.42 b
LC_{50}	50.0 \pm 4.40 c	82.65 \pm 3.06 b	17.34 \pm 3.06 b

^a Means within each column, followed by the same letter, are not different based on Tukey test ($P < 0.05$).

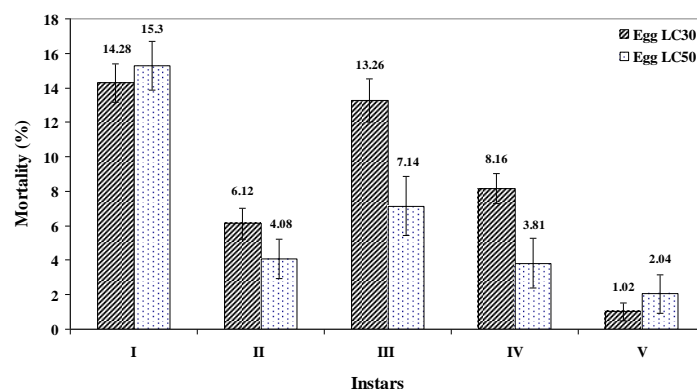


Figure 1. Effect of Precocene I on eggs of *Eurygaster integriceps* and subsequent nymphal mortality.

pest was observed in control ($34.69 \pm 3.63\%$) and the lowest survival rate of the adult was $17.34 \pm 3.06\%$, which was observed in individuals treated with LC_{50} . In general, results showed that adults' survival rate was dose-dependent. There were differences between the control and the treatments ($F_{2,18} = 8.17$, $P = 0.003$).

When eggs were directly treated with Precocene-I, in addition to losses on eggs, indirect effects of the compounds were observed in subsequent instars and also the exuviae was remained on the adults body.

Adult abnormality included deformed

scutellum or accumulation of transparent fluid. The upper part of wings was filled with transparent liquid and their bodies were deformed. Development of different body parts of the abnormal adults was disproportionate, including small and slim or enlarged and deformed stomachs (Figure 2).

Effect on the Length of Embryonic and Nymphal Stage

Precocene-I did not have any significant effect on embryonic stage of *E. integriceps* but could increase the length of nymphal stage

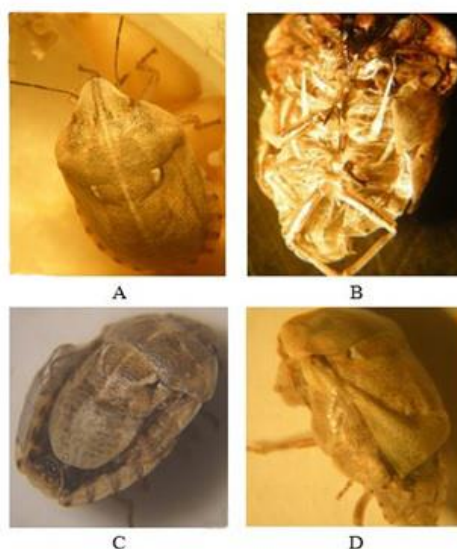


Figure 2- Induced morphological changes in adult Sunn pest, *Eurygaster integriceps*, obtained from eggs treated with Precocene. (A) Control; (B) Morphological changes in ventral abdomen; (C) Morphological changes in hemelytron, and (D) Morphological changes in hemelytron and scutellum, (original figure).

Table 3. Effect of sub-lethal concentrations of Precocene-I, applied on eggs of Sunn pest, *Eurygaster integriceps*, to investigate its effect on the length of embryonic and nymphal stage.^a

Mean (±SE) length of embryonic period (Day)	Mean (±SE) length of nymphal stage (day)					Mean (±SE) fetal period to adult (Day)
	First instar	Second instar	Third instar	Fourth instar	Fifth instar	
0.5±0.21a	6.92±0.17a	6.24± 0.31a	5.57±0.21ab	4.97±0.25a	6.11±0.12a	34.84±0.54a
5.14±0.14a	6.83±0.16a	6.81±0.21a	5.14±0.20a	6.28±0.16b	6.88±0.26a	37.10±0.48ab
4.85±0.14a	7.1±0.35a	5.97± 0.29a	6.24±0.21b	6.85±0.23b	8.26±0.42b	39.29±1.14b

^a Means within each column, followed by the same letter, are not different based on Tukey test ($P < 0.05$)

(39.29 days) (Table 3).

While Precocene had not significant effect on the first and second nymphal stages, the length of 3rd, 4th and 5th stages were increased (3rd: $F_{2, 18} = 5.74$; $P = 0.011$; 4th: $F_{2, 18} = 19.59$; $P = 0.0001$; 5th: $F_{2, 18} = 13.32$; $P = 0.003$).

DISCUSSION

In this study, it was shown that use of Precocene-I on *E. integriceps* eggs increased egg and nymphal stages mortality and also reduced insect survival rate (Table 2). Based on the results of bioassay tests, effect of Precocene-I on mortality of older eggs (5-days-old) was more than young ones. In the other words, older eggs showed more sensitivity to Precocene-I (Pener *et al.*, 1986). In the identification of juvenile hormone extracted from *Locusta migratoria migratoriodes* eggs, using gas chromatography, Pener *et al.* (1986) mentioned that the amount of the hormone was the highest in 8 days after laying eggs. Also, treatment of old eggs with Precocene-II caused reduction in egg hatching. Therefore, because of the formation of CA in old eggs, Precocene treatment led to their destruction and caused disorder in JH metabolism (Pener *et al.*, 1981). Hatching rate also declined, that

is consistent with our results. As expected, the mortality rate in this experiment during embryonic and nymphal stages was directly associated with increase in concentration. The highest mortality rate during the embryonic period was 92% in treated eggs with a dose of $50 \mu\text{g mL}^{-1}$. These results were consistent with results of other research on insects with incomplete metamorphosis (Unnithan and Nair, 1979; Feyereisen *et al.*, 1981; Pener *et al.*, 1981; Darvas *et al.*, 1985; Pradeep and Nair, 1989; Kafi *et al.*, 2016). According to Bownes (1989), any change in the JH may directly or indirectly lead to change in duplication of the genes encoding the protein yolk. For example, it was represented in the study of Robinson (1992) on honeybee that high levels of JH suppressed vitellogenin synthesis. In the present study, the eggs that were laid without any yolk were dried up after a few days after a few days, may be due to changes in the JH as a result of Precocenes effect. The mortality rate of nymphs in sub lethal concentrations was 82.86% and indicated that Precocene-I had powerful anti-hormonal effects on survived eggs and nymphs as well. Egg treatment of Sunn pest with Precocene-I led to the creation of abnormality in adults and the maximum deformation was observed in sub-lethal



concentrations. In a similar study, application of Precocene -I and -II on the fifth nymphal instar of Sunn pest indicated abnormality of adult insects (Kafi *et al.*, 2016). Transitional effects of this compound caused serious abnormalities on created structures of adult insect by disrupting the insect hormone and changing the physiology of hormones, in which case these disorders eventually led to insect mortality.

Based on the results obtained in this study, treatment of Sunn pest eggs with various concentrations of Precocene I increased nymphal period. These results are similar to experimental observations of Kafi *et al.* (2016) in which the effect of Precocene-I and-II on the fourth instar of Sunn pest resulted in delay moulting and increased nymphal period. In another study, the effect of Precocene-II on the fourth instar of *L. migratoria* (L.) led to delayed moulting. In a study conducted by Pener *et al.* (1981), treatment of nymphs of *Nilaparvata lugens* (Stal) with different concentrations of Precocene-II indicated the increased nymphal period, which was due to direct and indirect effects of Precocene on prothoracic glands producing moulting hormone (Pradeep and Nair, 1989). Delay in larvae to pupae development was observed in *Spodoptera littoralis* (Boisduval) due to treatment with Precocene-I and II (Srivastava and Kumar, 1997). In a study on the effects of Precocene on growth and reproduction of *Rhodnius prolixus* Stal, Garcia *et al.* (1987) concluded that Precocene prevented moulting and reproduction (egg laying). Application of ecdysteroid and JH-III led to restoring the disorder occurred in moulting and reproduction, respectively.

Due to high mortality rate of nymphs produced from eggs exposed to Precocene-I and also due to high abnormality percentage of adults, Precocene-I can be used in Sunn pest management programs as an environmentally-friendly compound with low adverse effect on non-target organisms. Therefore, conducting further tests to

determine its impact on different biological stages of the pest and the possibility of combining it with other conventional control methods in the field is recommended.

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تأثیر پریکاسن I، مهار کننده هورمون جوانی، روی رشد و نمو تخم سن گندم، *Eurygaster integriceps* و نتاج حاصل از آن

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چکیده

سن گندم (*Eurygaster integriceps* Puton (Hemiptera:Scutelleridae) مهمترین آفت گندم در ایران است. تغذیه از برگ، دانه و ساقه توسط پوره ها و حشرات کامل منجر به ایجاد خسارت کمی و کیفی زیادی به محصول گندم می شود. استفاده از آفت کش ها مهمترین راه کنترل این آفت می باشد، پریکاسن I، مهار کننده هورمون جوانی، روی غدد آلایات حشرات اثر سمیت سلولی دارد. در این تحقیق اختلالات رشد و نمو ایجاد شده توسط پریکاسن I (۷-متوکسی-۲و۲-دی متیل-۳-کورمن) بر روی تخم و نتاج سن گندم بررسی شد. دامنه غلظت های پریکاسن I برای زیست سنجی بر روی تخم های ۲ و ۵ روزه شامل ۱، ۵، ۱۰، ۲۰ و ۵۰ میکروگرم بر میلی لیتر بود. میزان LC₅₀ برای تخم های مسن و جوان به ترتیب شامل ۱۵ و ۱۵.۴ میکروگرم بر میلی لیتر برآورد گردید و نشان داد تخم های مسن حساس تر می باشند. تاثیر LC₃₀ بر روی تخم و تلفات پوره های آنها نسبت به شاهد دارای تفاوت معنی دار می باشد و بیشترین تلفات روی پوره سن اول و سن سوم، به ترتیب، به میزان ۱۴.۲۸ و ۱۳.۲۶ درصد مشاهده شد. همچنین، پریکاسن I روی طول دوره جنینی تاثیر نداشت، اما طول دوره پورگی را به میزان ۳۹.۳ روز در مقایسه با شاهد (۳۴.۸ روز) افزایش داد. تغییرات مرفولوژیکی در نمونه هایی که از تخم تیمار شده به وجود آمده بودند، شامل تغییر شکل دربال ها، بالپوش ها، ایجاد سپرچه های بسیار بزرگ و بدشکل در سن پنجم پورگی و در مواردی حفظ کوتیکول قدیم بود.