

Full Length Research Paper

# Effect of an anti-juvenile hormone agent (Precocene I) on Sunn pest, *Eurygaster integriceps* (Hemiptera: Scutelleridae) development and reproduction

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Sunn pest (*Eurygaster integriceps* Put.) (Hemiptera: Scutelleridae) is a serious pest of cereals causing severe quantitative and qualitative damage by feeding on leaves, stems and grains. Pesticide application is the main method of Sunn pest control, thus a search for developing new control methods is needed to diminish reliance on insecticides for insect control. So in the current study, the effect of an anti-juvenile hormone agent (precocene I) on the growth, reproductive biology and adult hemolymph proteins of *E. integriceps* females and males were investigated. The results indicated that precocene I had no acute toxicity to adults and second instars of *E. integriceps*. The treatment of second instars did not lead to death even in high doses, nor did it induce abnormality. The treatment of the third instar nymphs caused disorder in the physiology and increased mortality. The mortality of third instar was dose dependent ( $R^2 = 0.9774$ ). Hemolymph protein concentrations in control males and females were high ( $2497.95 \pm 0.04$  and  $2088 \pm 0.04$ , respectively), but they decreased with the starting of oviposition. Hemolymph protein concentrations in treated females were lower than the control, at first day after treatment. With passage of time, hemolymph protein concentrations remained constant and decreased near oviposition. Total protein concentration in males and females was nearly equal, and there were no significant differences among them ( $P > 0.01$ ). Precocene I also affected the number of eggs laid by females and the percentage of hatched eggs. It can be said that the effect of precocene I was stage- and age-specific, that is, its effects were varied with stage of the insect and its age in that stage. When used in early growth stage, its effect was less. However, when used in the late developmental stage its effect was more apparent and increased mortality as well as abnormalities.

**Key words:** *Eurygaster integriceps* Put, precocene I, hemolymph protein concentration, immature development, reproduction.

## INTRODUCTION

Sunn pest (*Eurygaster integriceps* Put.) (Hemiptera: Scutelleridae), is a serious pest of cereals globally from near and middle east to east and south Europe and north Africa (Radjabi, 2000). The insect causes severe quantitative and qualitative (destruction of gluten protein) damage to crops (sometime up to 100%) by feeding on leaves, stems and grains.

Pesticide spraying is the main method of the Sunn pest

control in area where infestation is high. In addition to the high cost of chemical control, insecticides pose a risk to nature's balance, human health, water quality, wildlife, and the environment as a whole. Thus, the search for developing new control method is needed to diminish reliance on insecticides for insect control. Another approach to pest control is the use of insect growth regulators (IGR's) and anti-juvenile hormone as insecticides. Many of these compounds have potential for the control of various pests (Staal, 1986).

Juvenile hormone (JH) is known to play an important role in the growth, development, reproduction, diapause behaviour and caste differentiation of insects

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(Wigglesworth, 1970). Adult diapause in female insects is characterized by suppression of ovarian development and the cessation of secretion of JH by the corpora allata (CA). Corpus allatum has been shown to be the key factor in the suppression of the ovarian development in many insects (Denlinger et al., 2005). There are evidence that JH plays a major role in regulating diapause, for example, *E. integriceps* (Burov et al., 1972), the green bug *Plautia stali* Scott (Kotaki and Yagi, 1989), the pear psylla *Cacopsylla pyricola* Foerster (Krysan, 1990), and the Colorado potato beetle *Leptinotarsa decemlineata* Say (Koopmanschap et al., 1989).

In many insect groups, anti-juvenile hormone agents inhibit the biosynthesis of JH of the CA. The insufficiencies of these substances exert some abnormalities in certain biological phenomena, which are controlled by the JH (Goodman and Granger, 2005).

Plants defend themselves by producing toxicants that are effective against herbivores. Of these, precocenes, the natural products produced by *Ageratum houstonianum* Blumink have been shown to be cytotoxic to the insect CA, thus preventing the production of JH (Bowers et al., 1976; Schooneveld, 1979; Pratt et al., 1980). These compounds are more effective against heteropteran insects as well as some other insect species such as grasshoppers and cockroaches (Pratt and Bowers, 1979). It has been reported that some larvae of holometabolous insects are less susceptible to the action of precocenes (Ohta et al., 1977; Burt et al., 1979). However, holometabolous insects such as *Spodoptera mauritia* Boisduval, *Tenebrio molitor* Linnaeus and *Spodoptera littoralis* Boisduval have been reported to be sensitive to precocenes (Mathai and Nair, 1983, 1984; Kozhanova and Nemeč, 1991; Khafagi and Hegazi, 2001).

In Hemiptera, removal of the CA suppresses ovarian development in nondiapausing females (Hodková, 1977; Kotaki and Yagi, 1989; Morita and Numata, 1997) and implantation of an active CA or application of JH or its analogue induces ovarian development in diapause females (Hodková, 1977; Kotaki and Yagi, 1989). From these results, adult diapause has also been considered to be induced by a lack of JH in heteropterans. Diapause induction was noticed in precocene-treated coleopterans (Bowers, 1976).

In Hemiptera, precocene sensitive species have been found in the Lygaeidae, Pyrrhocoridae, Reduviidae, Coreidae and the Cimicidae (Belles and Baldellou, 1983; Bowers et al., 1976; Bowers 1982). However, it has been reported that the phytophagous bug *E. integriceps* was an insensitive target for precocene II (Polivanova, 1981; Polivanova et al., 1983).

Thus, the aim of the current study was to investigate the influence of precocene I on the growth and reproductive biology of *E. integriceps* females and males. Also, the effect of precocene I on adult hemolymph protein concentrations as well as on low and high molecular proteins were studied.

## MATERIALS AND METHODS

### Insects

A stock colony of *E. integriceps* was maintained in the laboratory under 16 L: 8 D photoperiod at  $26 \pm 1^\circ\text{C}$  and 55% RH on soaked wheat seeds (developed in insect physiology laboratory for Sunn pest rearing). Water was provided in circle dishes plugged with cotton wool. Freshly ecdysed second and third instars (<24-h-old) selected for experiments were transferred from stock colonies to Petri dishes containing 10 animals. Adults (<24-h-old) were collected from stock colonies, placed in plastic boxes in groups of five females and five males.

### Precocene treatment

Fresh solutions of precocene I (Obtained from Sigma-Aldrich Chemical Company, Germany) in acetone was prepared. Acetone was used as solvent because the topical application method requires a volatile solvent evaporating rapidly to leave substantially pure insecticide in the epicuticular wax. Adults were treated with concentrations of 0.1, 0.45, 0.8, 1.5 and 2  $\mu\text{g}/\mu\text{l}$ , third instars with concentrations of 0.046, 0.139, 0.417, 1.25, 2.5, 5 and 10 ppm and second instars with the concentrations of 1.25, 2.5, 7.5, 5, 10, 20, 40, 80, 160, 320, 640 and 1000 ppm (these doses had been determined by performing dose determination experiments, previously). Precocene I concentrations were applied topically to the ventral abdominal segments of adult females and males or the thoracic surfaces of the nymphs. Adults and nymphs were treated individually with 1 and 0.5  $\mu\text{l}$  of the precocene concentrations using a microapplicator, respectively. Controls were treated only with acetone alone. Each treatment (dose) consisted of three replicates and in each replicates 10 insects were used.

Treated adults were monitored over 25 days and their mortality, number of laid eggs and percentage of hatched eggs were recorded. Also, hemolymph protein concentration over 11 days period and changes in low and high molecular proteins were determined.

For treated nymphs, percentages of mortality, abnormality and percentage of adult emergence was recorded.

### Determination of protein concentration

Protein concentration was measured according to the method of Bradford (1976), using bovine serum albumin (Bio-Rad, Munchen, Germany) as a standard.

For protein determination of one, three, seven and eleven days after treatment, hemolymph from the control and treated adults (females and males) was collected in a chilled calibrated micro capillary pipette through amputated forelegs and diluted (1:1) with anticoagulant buffer (41 mM citric acid, 1.7 mM EDTA, 98 mM NaOH and 186 mM NaCl; pH 4.5). The samples were centrifuged at 10,000 g for 10 min at  $4^\circ\text{C}$  to remove hemocytes and other tissue fragments. The resulting supernatants were stored at  $-20^\circ\text{C}$  for further analyses.

### Electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was conducted on 10% slab gels according to Laemmli (1970). Samples were diluted (1:1) in sample buffer (0.5 M Tris-HCl, pH 6.8; 10% SDS; Glycerol, 2-mercaptoethanol), boiled for 5 min and loaded into the gel with bromophenol blue as tracking dye. Gels were run in tris-glycine buffer (pH 8.3). Following electrophoresis, gels were stained in 0.1% coomassie brilliant blue R-250 in 40% methanol and 10% acetic acid at room temperature. Gels

**Table 1.** Effect of different concentrations of precocene I on third instars of *E. integriceps*.

Concentration (ppm)	Abnormality (%)				Mortality (%)			Adult emerge (%)	Stadium (Day)
	Third instar	Fourth instar	Fifth instar	Adult	Third instar	Fourth instar	Fifth instar		
0 (Control)	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	16.67 ± 2.94	33.33 ± 2.94	43.33 ± 2.22	2.22 ± 2.22	28 ± 1.04
0.046	0 ± 0.00	0 ± 0.00	25 ± 4.79	50 ± 16.67	30 ± 1.92	23.33 ± 2.94	40 ± 3.33	4.44 ± 1.11	28 ± 1.16
0.139	0 ± 0.00	0 ± 0.00	0 ± 0.00	25 ± 8.33	36.67 ± 1.11	10 ± 1.92	40 ± 0.00	5.55 ± 1.11	29 ± 1.54
0.417	0 ± 0.00	14.3 ± 16.67	0 ± 0.00	40 ± 6.67	43.33 ± 2.93	23.33 ± 2.94	16.67 ± 1.11	4.44 ± 1.11	28 ± 1.58
1.25	0 ± 0.00	0 ± 0.00	0 ± 0.00	25 ± 8.33	63.16 ± 1.07	7.89 ± 0.65	18.42 ± 0.65	2.63 ± 1.07	29 ± 1.65
2.5	0 ± 0.00	50 ± 4.77	0 ± 0.00	100 ± 0.00	68.42 ± 0.76	5.26 ± 0.76	15.79 ± 0.76	2.63 ± 1.07	28 ± 0.96
5	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	86.84 ± 0.65	2.63 ± 0.66	2.63 ± 0.66	1.97 ± 0.66	28 ± 1.08
10	-	-	-	-	100 ± 0.00	-	-	-	-

Abnormality in different doses of precocene was not dose dependent. The mortality of third instar was dose dependent ( $R^2 = 0.9774$ ). The mortality of fourth instar in high doses (1.25, 2.5, 5 and 10 ppm) was dose dependent ( $R^2 = 1$ ). Stadium and percentage of adult emergence was not significant among control and treatments.

were then destained in 40% methanol and 10% acetic acid until bands appeared.

#### Statistical analysis

Data were compared by regression analysis as well as by one-way and two-way analysis of variance (ANOVA), followed by Duncan's multiple range test where significant differences were found at  $P \leq 0.05$ . Software Statgraphics Plus 5.1 was used for all statistical analysis.

## RESULTS

### Mortality

Anti-JH Precocene I had no acute toxicity to adults and second instars of *E. integriceps*. The treatment of second instars did not lead to death even at high doses (1000 ppm) nor did it induce abnormality. The treatment of third instars caused disorder in the physiology and increased mortality. The mortality of third instar was dose dependent ( $R^2 = 0.9774$ ). The mortality of fourth instars in high doses (1.25, 2.5, 5 and 10 ppm) was dose dependent ( $R^2 = 1$ ) although third instars were

directly treated and fourth instars were not directly treated; but in low doses (0.046, 0.139 and 0.417 ppm), the effect was not in a dose dependant manner (Table 1). The mortality of fifth instars also did not show dose dependant manner since they were not directly treated with precocene. There were no significant differences among stadium developmental time and percentage of adult emergence in control and treatments (Table 1).

### Abnormality

Precocene I did not induce abnormality in treated second instars through whole developmental stages. In treated third instars, surviving nymphs developed through third stadium as normal. However, disorder in physiology led to appearance of deformed insects in fourth and fifth instars and adults. Abnormality in different doses of precocene was not dose dependent. Deformed nymphs had a small and narrow abdomen or the abdomen was abnormally big. The whole body was disordered and the cuticle was bigger in

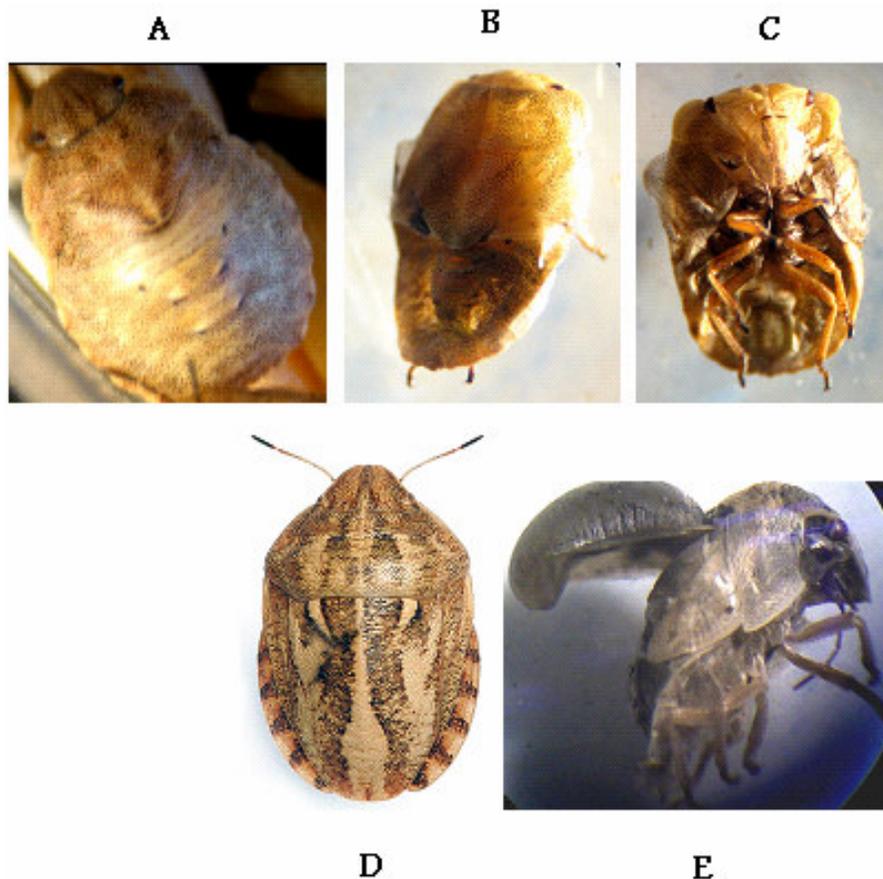
comparison to the body. Deformed nymphs would die in less than 24 h. In adult of F1 the deformation was seen as their scutellum was small and full of transparent liquid. Sometimes, upper wings were full of transparent liquid. These nymphs were immediately lost after formation. Deformed adults would die mostly in the first day of appearance (Table 1 and Figure 1).

### Precocious metamorphosis

Topical application of the precocene on second and third instars did not induce precocious metamorphosis in *E. integriceps*.

### Effect of precocene on total protein in hemolymph

In control males and females, hemolymph protein concentrations, at first were high ( $2497.95 \pm 0.04$  and  $2088 \pm 0.04 \mu\text{g/ml}$ , respectively), but they decreased with the starting of oviposition ( $1776.12 \pm 0.13$  and  $1460.91 \pm 0.08 \mu\text{g/ml}$ , respectively).



**Figure 1.** Abnormalities resulting from precocene I treatment, in forth instars and adult *E. integriceps* treated in third instar. (A) Normal fourth instar, (B) and (C) abnormal fourth instar, (D) normal adult, (E) abnormal adult.

Hemolymph protein concentrations in treated females were lower than the control, at first. With passage of time, hemolymph protein concentrations remained constant and decreased near oviposition. In treated males hemolymph protein concentrations, was at first lower than the control, then increased and again decreased with passage of time. Total protein concentration was not significantly different between males and females. Different doses of precocene did not cause significant differences in hemolymph protein concentrations by itself, but interaction of day and precocene and also effect of day by itself caused significant differences in concentration of protein ( $P < 0.01$ ) (Figures 2 and 3).

#### **Effect of precocene on high and low molecular weight proteins in hemolymph**

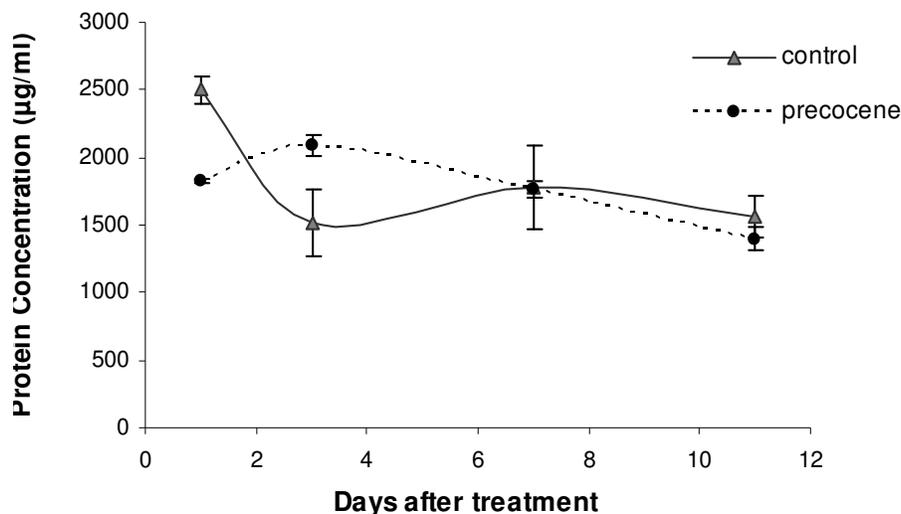
In males and females hemolymph, with passage of time (7 and 11 days post treatment), the concentration of high molecular proteins increased in both control and precocene treated insects, but the concentration of low molecular proteins decreased. The amount of increment

in high molecular proteins in precocene treatment was more than the control. The concentration of these proteins in precocene treatment was more than control. Low molecular proteins in females hemolymph were more than males (Figures 4 and 5).

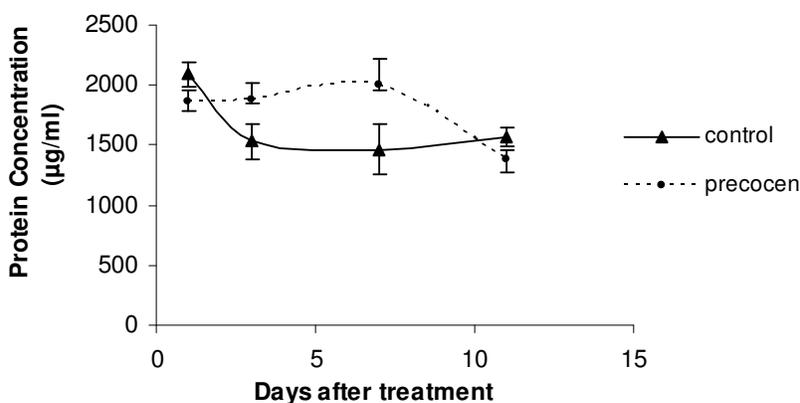
#### **Egg number**

Daily averages of each female's egg over 25 days, in 0.1, 0.45 and 0.8  $\mu\text{g}/\mu\text{l}$  treated insects were 3.87, 3.87 and 3.89, respectively. So, the treated insect with doses of 0.1, 0.45 and 0.8  $\mu\text{g}/\mu\text{l}$  laid eggs at 2.31, 2.31 and 2.9%, respectively, more than the control. However, with the insects treated with doses of 1.5 and 2  $\mu\text{g}/\mu\text{l}$ , they laid eggs 4.43 and 12.82% less than the control.

The number of each female's egg during the whole period in different doses did not show significant differences. The number of eggs in different days showed significant differences ( $P < 0.01$ ) and the interaction of day and precocene treatment caused significant differences on the number of eggs ( $P < 0.05$ ), but precocene treatment did not have any effect on the total number of eggs, by



**Figure 2.** Changes in the hemolymph protein concentrations of *E. integriceps* males over 11 days in control and precocene I (2 µg/µl) treatment.



**Figure 3.** Changes in the hemolymph protein concentrations of *E. integriceps* females over 11 days in control and precocene I (2 µg/µl) treatment.

itself (Figures 6 and 7).

### Hatching

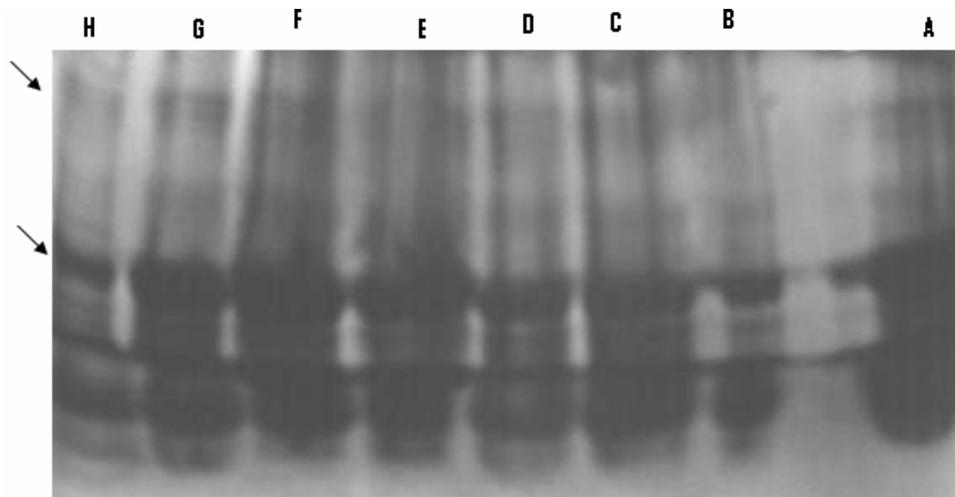
By increasing the doses, percentage of eggs hatch also increased ( $R^2 = 82/64$ ). Egg's hatch in concentrations of 0.10, 0.45, 0.8, 1.5 and 2 µg/µl was 74.3, 70.92, 72.27, 83.12 and 79.3%, respectively. Therefore, with insects treated with concentrations of 0.10, 0.45, 0.8, 1.5 and 2 µg/µl, their egg hatched 18.8, 12.8, 14.95, 32.21, 25.13%, respectively, more than the control (62.87) (Figure 8).

### DISCUSSION

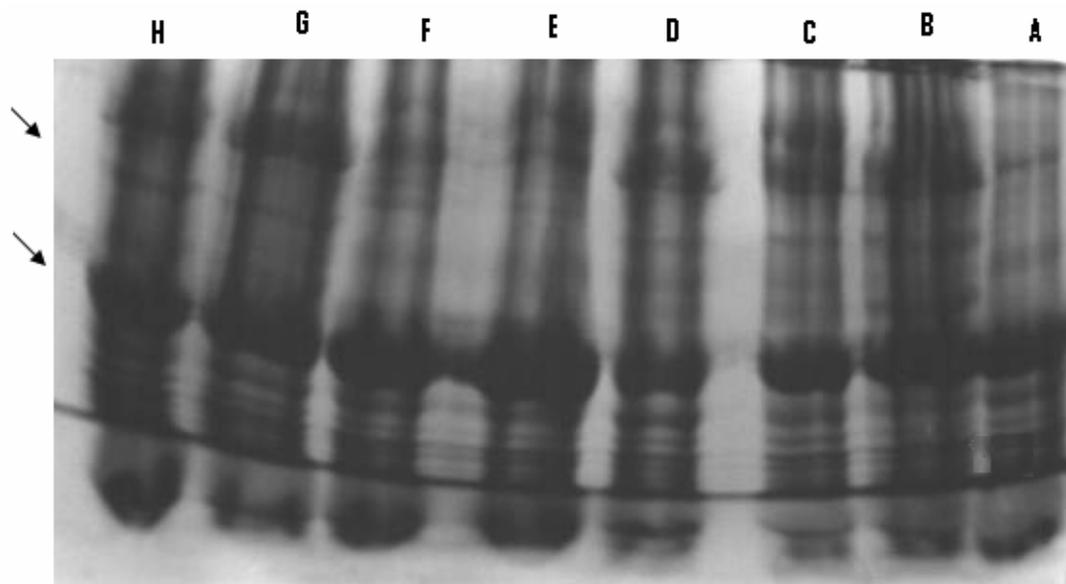
Reproductive diapause was not induced perfectly by the single application of the precocene I in *E. integriceps*.

However, the egg production reduced in early days after treatment. A termination of diapause with precocene II was reported by Sieber and Benz (1980) in *Laspeyresia pomonella* Linnaeus, which is known to have diapause when JH is high. Precocenes caused diapause induction in *Conotrachelus nenuphar* Herbst (Bowers, 1982) and *Leptinotarsa decemlineata* Say (Bowers et al., 1976).

Hemolymph protein concentration in treated females was initially lower than the control, probably because of chemical destruction of the corpora allata, the endocrine glands that synthesize and release JH. With application of precocene I, vitellogenins titer (the main hemolymph proteins of female insect) decreased. With passage of time, hemolymph proteins concentration remained constant and decreased near oviposition (probably because of vitellogenins absorbed to ovaries). From the physiological point of view, diapausing insects differ distinctly from their non-diapausing counterparts in many aspects (Danks,



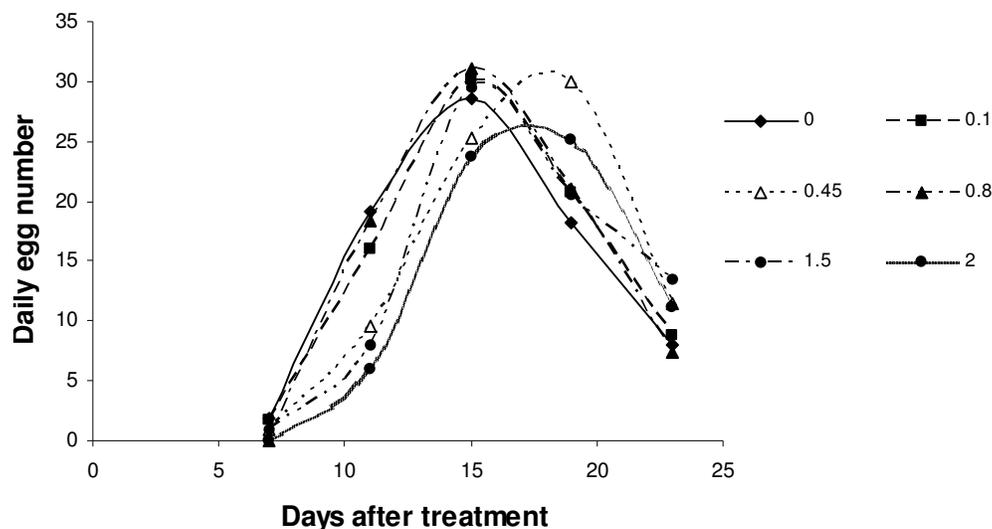
**Figure 4.** SDS-PAGE pattern of hemolymph proteins at day 1, 3, 7 and 11 after topical application with precocene I ( $2 \mu\text{g}/\mu\text{l}$ ) on adult males of *E. integriceps*. (A) Control, day 1; (B) control, day 3; (C) control, day 7; (D) control, day 11; (E) precocene, day 1; (F) precocene, day 3; (G) precocene, day 7; (H) precocene, day 11.



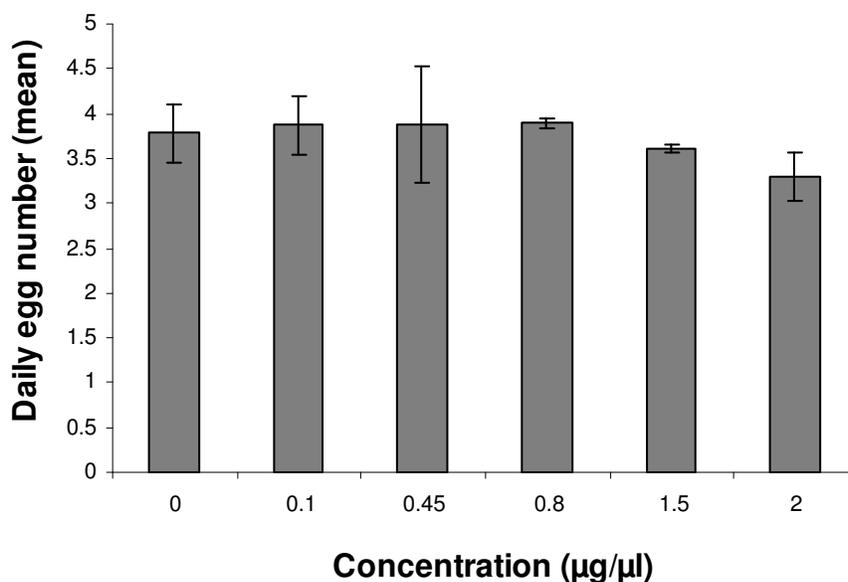
**Figure 5.** SDS-PAGE pattern of hemolymph proteins at day 1, 3, 7 and 11 after topical application with precocene I ( $2 \mu\text{g}/\mu\text{l}$ ) on adult females of *E. integriceps*. (A) Control, day 1; (B) control, day 3; (C) control, day 7; (D) control, day 11; (E) precocene, day 1; (F) precocene, day 3; (G) precocene, day 7; (H) precocene, day 11.

1987; Chippendale, 1988). One of these differences is the synthesis and accumulation of "diapause-associated proteins" (Peferoen et al., 1982; Chippendale, 1988). Some of the accumulating proteins have been named "diapause-associated proteins" (Chippendale, 1988). Most of these were detected in larvae or pupae and only a few reports have been concerned with diapause-associated proteins in adult insects (Wyatt et al., 1990; Miura et al., 1991; Chinzei et al., 1992).

Different doses of precocene I did not cause significant difference in hemolymph protein concentration, nor did it affect the number of eggs, but interaction of day and precocene and effect of day by itself caused significant differences. Chemical allatectomy by precocene I in *Dysdercus koenigii* Fabricius (Hemiptera: Pyrrhocoridae) at emergence resulted in negligible amounts of vitellogenins in fat body and hemolymph. However, at 70 h post-emergence of adult, chemical allatectomy did not



**Figure 6.** Temporal mean number of eggs laid by female *E. integriceps* treated with different concentrations of precocene I ( $\mu\text{g}/\mu\text{l}$ ).



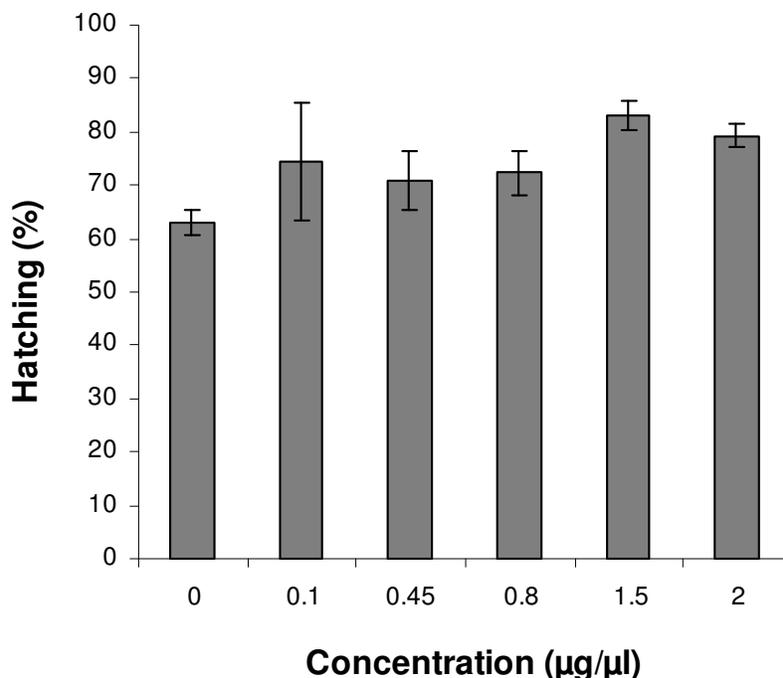
**Figure 7.** Daily mean number of eggs laid by female *E. integriceps* treated with different concentrations of precocene I over 25 days period.

changed hemolymph vitellogenin levels (Venugopal and Kumar, 2000).

In various adult insects, treatment with an appropriate dose of precocene prevents ovarian development and can lead to sterility (Bowers and Martinez-Pardo, 1977; Pener et al., 1978; Woodard and Rankin, 1980). In *Drosophila melanogaster* Hardcover (Diptera: Drosophilidae), exposure to 0.14  $\mu\text{mol}$  of precocene decreased oviposition by about half (Ringo et al., 2005). In *Panstrongylus megistus* Burmeister (Hemiptera: Reduviidae) males treated with precocene II and

ethoxyprecocene II, the average number of eggs produced per couple *P. megistus* which copulated did not differ statistically from that of the control groups. Precocene II and ethoxyprecocene II applied to males had no effect on egg hatching of *P. megistus* (Cavalcante and Regis, 1992).

The number of each female's egg during whole period in different doses did not show significant differences. It is probable that the effect of precocene disappears very soon, and to keep its effect, it should be applied repeatedly. Repeated applications on *S. littoralis* Boisduval



**Figure 8.** Hatching percent of eggs laid by female *E. integriceps* treated with different concentrations of precocene I.

larvae of either JH, Precocene I or Precocene II were more effective in disrupting parasitoid development than application of single doses (Khafagi and Hejazi, 2004). Egg production decreased dose-dependently but transiently in *Acheta domesticus* Linnaeus and *Nemobius fasciatus* De Geer both species after treatment with either precocene I or II (Bradley and Haynes, 1991).

Precocene I was ineffective during the second instar when the CA were presumed to have a low level of activity, however, in using precocene to effect chemical allatectomy of *E. integriceps* nymphs, it became apparent that precocene I is effective in the third instar and induces mortality and abnormality. Apparently, CA is not active during whole developmental stages. It is known that the ability of juvenile hormone to regulate developmental switches is restricted to a specific part of the molting cycle known as the juvenile hormone-sensitive period or critical period (Willis, 1974; Nijhout, 1982). The periods sensitive to precocene II in *Nilaparvata lugens* Stal and the effects observed clearly differ according to the developmental stages (the second, third and fourth stadia) being treated (Bertuso et al., 2002). The time-response curves for the induction of juvenile characters and for the degeneration of the CA in *Locusta migratoria* Linnaeus suggest that the CA may be active during the early 5th instar (Miall and Mordue, 1980). The treatment of second instars did not lead to death even in high doses, nor did it induce abnormality. The time of application is important and only application of precocene early in the intermolting period caused its effects in the hemipteran *Rhodnius prolixus* Stal nymphs (Garcia et al.,

1987). The critical periods for juvenile hormone sensitivity were examined in *Diploptera punctata* Eschscholtz female larvae. Second-instar larvae allatectomized at the beginning of the stadium underwent one or more additional ecdyses after the operation, but most of them retained their larval form; all of them died without completing metamorphosis. Thus, at this stage, juvenile hormone may not be morphogenetically functional. In contrast, allatectomy of 3rd instars during the first 8 days of the stadium resulted in a high incidence of precocious metamorphosis at the next ecdysis (Kikukawa and Tobe, 1986).

When last instars of *S. mauritia* Boisduval (Lepidoptera: Noctuidae) treated with a single dose of 80 µg or 160 µg of precocene II at various ages, they showed different percentage of mortality, e.g. early treated larvae had high mortality than late treated larvae (Santha and Nair, 1986).

Disorder in physiology led to appearance of deformed insects in fourth and fifth instars and adult. Precocene II, applied topically in the early 4th instar has potent anti-allatotrophic activity in *L. migratoria* (Pener, 1978). In the early 5th instar of *L. migratoria* Linnaeus, precocene II mimics JH-activity, but is still active in causing degeneration of the CA (Miall and Mordue, 1980).

Topical application of the precocene I did not induce precocious metamorphosis in the *E. integriceps*. However, precocene II induced precocious metamorphosis in *Rhodnius prolixus* Stal and *Triatoma dimidiata* Latreille (Hemiptera) when applied by either contact exposure or fumigation (Tarrant et al., 1982). Anti-juvenile hormone agents induce precocious metamorphosis in a restricted

number of insect species (Darvas et al., 1990). In *Nilaparvata lugens* Stal periods sensitive to precocene II for precocious metamorphosis induction appeared from early second stadium to 18h after molting to the third stadium (Bertuso et al., 2002). Precocene II did not induce precocious metamorphosis in *Schistocerca gregaria* Forskal (Islam, 1995). Induction of precocious metamorphosis in the bug *Oxycarenus lavaterae* Fabricius by application of precocenes on third instars is reported (Belles and Baldellou, 1983).

In conclusion it can be said that the effect of precocene I was stage- and age-specific, i.e. its effects were varied with stage of the insect and its age in that stage. When used in early growth stage, its effect was less. However, when used in the late developmental stage, its effect was more apparent and increased mortality as well as abnormalities.

## ACKNOWLEDGMENTS

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