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Insecticidal and Anti-juvenile Hormone Activities of Precocene II against the Grasshopper *Euprepocnemis plorans plorans* (Charp.) (Orthoptera: Acrididae).

K. Ghoneim
Faculty of Science, Al-Azhar University, Cairo, Egypt

A. Basiouny
Faculty of Science, Al-Azhar University, Cairo, Egypt

ABSTRACT

The grasshopper *Euprepocnemis plorans plorans* caused a considerable damage to crops of the Nile Delta, Egypt. The present study was conducted aiming to assess the insecticidal and anti-hormonal effects of Precocene II on this grasshopper. The newly moulted 2nd or 4th (penultimate) instar nymphs were exposed to a series of doses: 60, 40, 20 and 10 µg/cm². Exposure of 2nd instar nymphs to the higher two doses resulted in complete mortality of nymphs within 24 h. At the lower two doses, PII exhibited a considerably extended low toxicity on the subsequently moulted instars and emerged adults. LD_{50} was calculated as 0.388 µg/cm². After exposure of the 4th instar nymphs to PII, no complete mortality was observed, but various mortality percentages among the treated nymphs, 5th instar nymphs and adults. LD_{50} was calculated as 17.022 µg/cm². PII exerted a slight inhibitory action on the nymphal growth of both 4th and 5th instars, after treatment of 2nd instar nymphs, regardless the dose level, but the growth rate was remarkably regressed after treatment of 4th instar nymphs with 40 and 20 µg/cm². Exposure of 2nd instar nymphs to PII led to 3.33% precociously moulted nymphs into 4th instar, skipping off the 3rd instar (only at the lowest dose). After exposure of 4th instar nymphs to PII, some treated nymphs precociously metamorphosed into adultoids, omitting the 5th instar, only at the higher tow doses. Another noticeable feature of the deranged development was 'permanent nymphs' which induced in 2nd instar nymphs (3.85%) after exposure only to 20 µg/cm². Also, similar permanent nymphs were induced during the 4th instar. No permanent nymphs had been induced after exposure of 4th instar nymphs to PII.

***Keywords**: adult, development, growth, metamorphosis, mortality, nymph, permanent, toxicity*

I. INTRODUCTION

Grasshoppers have become a serious pest in **Egypt** especially in the newly reclaimed area [1]. The most economic species of grasshoppers that caused a serious damage is the grasshopper *Euprepocnemis plorans plorans* (Charp.)(Orthoptera: Acrididae). This species caused 95% damage to crops of the Nile Delta, Egypt [2]. The control strategies against this grasshopper still depend on the conventional insecticides. Although these chemicals are often effective, but not always appropriate.

As a result of improper and excessive uses, these insecticides usually exhibit several adverse impacts on the human health and beneficial animals as well as cause serious toxicological problems to the environmental systems because these chemicals have a long half-life, which causes their retention in the environment for long periods [3-6]. Furthermore, these chemicals have a tendency to accumulate in different trophic levels of the food net [7, 8]. In addition, the repeated use of many conventional insecticides has caused resistant insect strains to emerge [9, 10]. Therefore, eco-friendly insecticides have received global attention in recent years as alternative for the conventional insecticides. These

alternative compounds are characterized by a short period of half-life in the environment, lower toxicity to non-target organisms than conventional insecticides and they are effective at low concentrations [11, 12]. Also, they are biodegradable into harmless compounds, which allows for avoiding the problems of environmental pollution [6, 13, 14].

It should be mentioned that the use of juvenile hormone (JH) or JH-based compounds for pest control was early suggested by some authors [15-17] as "third generation insecticides". Screening new targets involved in JH-biosynthesis within the corpora allata (CA), JH-producing organs in insects, has been a subject of study in the past four decades [18]. So, compounds that interact with JH, stimulate JH-biosynthesis, inhibit JH-biosynthesis or interfere with its catabolism can be utilized as new insecticides against insect pests [19]. All these compounds can be collectively called as 'insect growth regulators' (IGRs). IGRs belong to a group of compounds which are not directly toxic, but act selectively on normal growth, development, metamorphosis and/or reproduction in insects *via* disrupting the hormonally regulated physiological processes [20, 21]. Because of their desirable characteristics, such as low toxicity, less environmental pollution, high selectivity, and low impact on natural enemies and human health, IGRs are used to control various insect pests [22-24]. Compactin, fluoromevalonate, imidazoles, and precocene are insect growth regulators (IGRs) that are anti-juvenile hormone agents that affect either the mevalonate pathway in JH biosynthesis, or the corpora allata (CA) directly, the organ that produces JH [25].

Ageratochromes (precocenes), plant compounds causing precocious metamorphosis in insects, had been isolated by Bowers et al. [26] and described their action as "chemical allatectomy" [27]. An extensive review on the effects of precocenes on different pests belonging to various insect orders was provided by Staal [25]. However, Precocene-I (7 methoxy-2,2-dimethylchromene, PI) and Precocene-II (6,7-dimethoxy-2,2-dimethylchromene, PII) have been used as insect regulators by inducing symptoms of JH-deficiency in insects [28-30]. Consequently, this inhibition can disrupt the embryonic development, induce premature metamorphosis, and disturb the insect behavior, beside their effects as antifeedants and repellents [31-37]. PI and PII have been shown to halt the reproductive potential in adults of several insects by prevention of the normal vitellogenesis of the oocytes, leading to sterility [35, 38, 39]. As reported by some authors [25, 40, 41], precocenes either inhibit JH biosynthesis or are inhibitors of the enzyme action. Therefore, precocenes and other anti-JH agents are widely used as effective tool in experimental endocrinology of arthropod animals and have been considered prototypes of "fourth-generation pesticides" [42-46]. It has been demonstrated that the design of JH mimics or anti-JH agents is an effective strategy for insecticide discovery [18]. Compounds with anti-JH activity are considered as new representatives of insect growth regulators (IGRs) lacking some disadvantages of juvenoid-type chemicals [17, 47]. These chemicals are potentially efficacious for control of the major insect pests where most of the damage is caused by larval stage [48]. The grasshopper *E. plorans plorans* received a little attention of research in Egypt [2, 49-54]. Therefore, the present study was conducted aiming to assess the insecticidal and anti-hormonal activities of PII against this grasshopper.

II. MATERIALS AND METHODS

1. Experimental insect

A culture of the grasshopper *Euprepocnemis plorans plorans* (Charp.) (Orthoptera: Acrididae) was originated by a sample of nymphs and adults from the susceptible culture maintained for several generations along some years in Locust and Grasshopper Department, Plant Protection Research Institute, Doqqi, Giza, Egypt. It was reared under laboratory controlled conditions (32±2°C, 65±5% R.H. and 12h dark: 12h light) at Department of Zoology and Entomology, Faculty of science, Al-Azhar University, Cairo. Both adults and nymphs were raised in glass fronted cages (30 x 30 x 30 cm in diameter). The top of each cage had a small wire-gauze opening door. The bottom of cages was covered with a layer of sterilized sand (10 cm in depth). All cages were held at the laboratory controlled conditions. Insects of all developmental stages were fed on the maize leaves (*Zea mays*), and a daily routine feeding and cleaning manipulations were continuously carried out.

2. Precocene II treatment

Precocene II (6,7-dimethoxy-2,2-dimethylchromene, PII) was kindly provided by Dr. Heba Hassan, Prof. at Plant Protection Research Institute, Agricultural Research Center, Doqqi, Giza, Egypt. PII was diluted

in acetone to prepare a series of doses: 60, 40, 20 and 10 µg/cm².

A contact technique, originally described by Unnithan et al. [55] for PII against *Schistocerca gregaria*, was used. By this technique, the precocene fumigant could presumably reach the corpora allata of the insect rapidly *via* the tracheal system. Bottom of a Petri dish (15x2 cm) was coated with each dose. After acetone evaporation, groups of 15 newly moulted nymphs of 2nd or 4th (penultimate) instar of *E. plorans plorans* were confined in each dish for 24h (exposure period). Groups of 15 newly moulted nymphs of 2nd or 4th instar nymphs were confined in acetone-treated Petri dishes and used as controls. All treated and control nymphs were kept under the controlled conditions. After the exposure period, treated and control nymphs were transferred into clean Petri dishes and provided with suitable pieces of maize leaves, as fresh food, every day.

3. Criteria of study

3.1. Toxicity: Initial mortality (%) was recorded within 24 h post-treatment whereas the extended toxic effect of PII was determined according to the recorded mortalities of all developmental stages. Only female nymphs and adults were used in the present study. LD_{50} values were calculated by Microsoft office Excel, 2007, according to Finney [56].

3.2. Growth rate: After exposure of 2nd instar nymphs to doses of PII, the successfully moulted nymphs of 4th and 5th instars were daily weighed (in mg). Also, the treated 4th instar nymphs and the successfully moulted 5th instar nymphs were daily weighed. Growth rates (GR) of 4th and 5th instar nymphs were calculated according to Waldbauer [57] as follows: GR = G/TA. Where G: fresh weight gain (mg) of nymphs along the instar. T: the instar period (in days). A: mean fresh body weight of nymphs during the feeding period.

3.3. Development and metamorphosis: After exposure of 2nd and 4th instar nymphs to PII, all features of retarded development and impaired metamorphosis were recorded (in %).

4. Statistical analysis of data

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction [58] for the test significance of difference between means.

III. RESULTS

1. Toxic effect of PII on *E. plorans plorans*

After exposure of the newly moulted 2nd instar female nymphs to different doses of PII, the toxic effect was detected by mortality (%) of nymphs. Data of mortality had been assorted in Table (1). According to these data, all treated 2nd instar nymphs completely died at 24 h post-treatment (initial mortality) by the higher two doses (60 and 40 µg/cm²). The lower two doses (20 and 10 µg/cm²) of PII caused only 26.92 and 20.00% mortality, respectively, at 24 h post-treatment. At 11 days post-treatment, the same lower doses caused 28.30 and 23.20% mortality, respectively, compared to 10.67% mortality of control nymphs. Thus, toxicity of PII increased by the time within the same instar. The insecticidal activity of PII extended to the subsequently moulted nymphal instars and adults. Mortality of 3rd instar nymphs were 20.10 and 18.12%, at 20 and 10 µg/cm², respectively, *vs*. 6.0% of control mortality. At the lower two doses, mortality of 4th instar nymphs were 16.99 and 14.98%, respectively. As obviously seen, the toxic effect of PII appeared in a dose-dependent course against 2nd, 3rd, and 4th nymphal instars. PII, only with its lowest dose, affected the survival of 5th (last) instar nymphs, since 7.98% mortality was recorded. With regard to the successfully emerged adult females, PII exhibited a weak mortal potency after treatment of 2nd instar nymphs (3.85%), only with 20 µg/cm². Depending on the determined total mortality, PII toxicity was recorded in a dose-dependent manner. LD_{50} was calculated as 0.388 µg/cm² (Table 1).

After exposure of the newly moulted 4th instar nymphs to different doses of PII, data of the insecticidal activity were summarized in Table (2). Depending on these data, it was obviously observed that all doses caused various mortalities of treated 4th instar nymphs and the successfully moulted 5th instar nymphs. At 24 h post-exposure, the nymphal mortalities were recorded in 22.22, 40.74, 10.20 and 25.00%, at 60, 40, 20 and 10 µg/cm², respectively. Six days later, the toxic potency of PII decreased, since mortalities were 12.12, 30.85, 07.69 and 15.00%, at 60, 40, 20 and 10 µg/cm², respectively, vs. 5.00% mortality of control nymphs. Regardless the dose level, toxic potency of PII decreased by the moulting, since mortalities of 5th instar nymphs were 10.10, 21.00, 05.18 and 05.00%, at 60, 40, 20 and 10 µg/cm², respectively. In addition, PII displayed its

insecticidal activity in no certain trend. In respect of the successfully emerged adult females, only the lower two doses caused 7.69 and 10.00% mortality, respectively. Depending on data of the total mortality, no certain trend could be detected (44.44, 92.59, 30.76 and 55.00%, at 60, 40, 20 and 10 µg/cm², respectively, compared to 05.00% mortality of control adults). LD_{50} was calculated as 17.022 µg/cm² (Table 2). Depending on these data, the 2nd instar nymphs were more sensitive to PII toxicity than 4th instar nymphs.

2. Effect of PII on growth of *E. plorans plorans*

Table (3) contains data of nymphal growth during the latter two instars (4th and 5th) as affected by exposure of 2nd and 4th instar female nymphs to different doses of PII. In the light of these data, PII exerted a slight inhibitory action on growth of 4th instar nymphs (0.026±0.003 and 0.025±0.005, at 20 and 10 µg/cm², respectively, *vs*. 0.030±0.004 of control nymphs). Also, a slight inhibitory action was exerted on growth of 5th instar nymphs (0.026±0.002 and 0.027±0.004, at 20 and 10 µg/cm², respectively, *vs*. 0.029±0.005 of control nymphs).

After exposure of the penultimate instar nymphs to PII, data of nymphal growth were also arranged in the same table. As exiguously shown, PII exerted a weak suppressing action on growth of 4th instar nymphs after exposure to the highest and lowest doses (0.038±0.009 and 0.039±0.007, at 60 and 10 µg/cm², respectively, *vs*. 0.043±0.015 of control congeners). Similarly, the growth rate of 5th instar nymphs was insignificantly suppressed at these doses (0.037±0.004 and 0.038±0.005, at 60 and 10 µg/cm², respectively, *vs*. 0.039±0.001 of control congeners). On the basis of data arranged in the aforementioned table, PII exerted potent suppressing action on the nymphal growth only at the middle two doses. Concerning the 4th instar nymphs, growth rates were considerably regressed (0.032±0.001 and 0.030±0.005, at 40 and 20 µg/cm², respectively, *vs*. 0.043±0.015 of control nymphs). Similarly, the growth rate of 5th instar nymphs was remarkably regressed (0.028±0.005 and 0.029±0.001, at 40 and 20 µg/cm², respectively, *vs*. 0.039±0.001 of control nymphs).

3. Effects of PII on development and metamorphosis of *E. plorans plorans*

Depending on the data distributed in Table (4), two major features of retarded development and deranged metamorphosis could be recorded (in %) as permanent nymphs and precocious metamorphosis. After exposure of 2nd instar female nymphs to PII, the development was suspended in a feature of permanent nymphs which survived two-fold period of their control congeners and failed to moult into the next instar ending in death. Permanent nymphs were induced in 3.85% in the 2nd instar (only at dose 20 µg/cm²) and 3.33% in the 4th instar (only at dose 10 µg/cm²). As clearly seen in the same table, 3.33% of treated 2nd instar nymphs precociously moulted into 4th instar, skipping off the 3rd instar. These precociously developed nymphs survived more than 20 days and eventually perished.

After exposure of 4th instar female nymphs to PII, no permanent nymphs were observed. On the other hand, the metamorphosis program was impaired; since precocious adultoids (omitting the 5th instar) had been produced only at the higher two doses (11.11 and 3.70%, at 60 and 40 µg/cm², respectively). These precocious adultoids appeared normally in colour and behaviour but without wings. They survived more than one month and eventually perished with no ability to mate.

Table 1: Mortality (%) of *E. plorans* after exposure of 2nd instar female nymphs to PII

Dose (µg/cm²)	Nymphal instars					Adult females	Total mortality	LD_{50} (µg/cm²)
	2nd		3rd	4th	5th			
	24 h post-treatment	11 days post-treatment						
60	100.00	--	--	--	--	--	100.00	**0.388**
40	100.00	--	--	--	--	--	100.00	
20	26.92	28.30	20.10	16.99	0.00	3.85	96.16	
10	20.00	23.20	18.12	14.98	7.98	0.00	84.28	
Controls	00.00	10.67	06.00	0.00	0.00	0.00	16.67	

Table 2: Mortality (%) of *E. plorans* after exposure of 4th instar female nymphs to PII.

Dose (µg/cm²)	Nymphal instars			Adult females	Total mortality	LD_{50} (µg/cm²)
	4th		5th			
	24 h post-treatment	6 days post-treatment				
60	22.22	12.12	10.10	00.00	44.44	17.022
40	40.74	30.85	21.00	00.00	92.59	
20	10.20	07.69	05.18	07.69	30.76	
10	25.00	15.00	05.00	10.00	55.00	
Controls	00.00	05.00	00.00	00.00	5.00	

Table 3: Effects of PII on the growth rate (mean±SD) of the 4th and 5th instar female nymphs of *E. plorans*.

Dose (µg/cm²)	After exposure of 2nd instar nymphs		After exposure of 4th instar nymphs	
	4th instar	5th instar	4th instar	5th instar
60	---	---	0.038±0.009 a	0.037±0.004 a
40	---	---	0.032±0.001 b	0.028±0.005 c
20	0.026±0.003 a	0.026±0.002 a	0.030±0.005 b	0.029±0.001 c
10	0.025±0.005 a	0.027±0.004 a	0.039±0.007 a	0.038±0.005 a
Controls	0.030±0.004	0.029±0.005	0.043±0.015	0.039±0.001

Mean±SD followed with the letter (a): not significantly different ($p > 0.05$), (b): significantly different ($p < 0.05$), (c): highly significantly different ($p < 0.01$).

Table 4: Effect of P II on development and metamorphosis of *E. plorans*

Dose (µg/cm²)	Exposure of 2nd instar female nymphs			Exposure of the 4th instar female nymphs (% precocious adultoids) (3)
	% Permanent nymphs (1)		% Precocious development to 4th instar (2)	
	2nd instar	4th instar		
60	---	---	---	11.11
40	---	---	---	03.70
20	3.85	0.00	0.00	0.00
10	0.00	3.33	3.33	0.00
Control	0.00	0.00	0.00	0.00

(1): Permanent nymphs survived two-fold period of control nymphs and eventually died. (2): Precocious development into the 4th instar (skipping off the 3rd instar). (3): Precocious adultoids (skipping the 5th instar) had no wings and eventually died without mating.

IV. DISCUSSION

1. Affected survival of *E. plorans plorans* by PII

The currently available literature has been enriched with many reported works on the toxic effects of several anti-juvenile hormone (anti-JH) compounds against different insect species. For examples, both PI and PII exhibited larvicidal activities against several mosquito species, such as the mosquito species *Aedes aegypti*, *Anopheles sacharovi* and *An. stephensi* [59, 60]. The precocenes exhibited larvicidal effects, in a dose-dependent course, on the Colorado potato beetle *Leptinotarsa decemlineata* [61]. A toxicological effect of PII was reported by Abdullah [62] against larvae of red palm weevil *Rynchophorus ferrugineus*. Also, PII exhibited larvicidal and pupicidal effects on the grey flesh fly *Parasarcophaga dux*, in a dose-dependent course [63]; larvicidal effect on the lepidopterous pest *Pericallia ricini* [31]; and larvicidal effect on the Asian tiger mosquito *Aedes albopictus* [64]. Apart from precocenes, other anti-JH compounds displayed different degrees of toxicity against some insects, such as synthesized EMD (ethyl (E)-3-methyl-2-dodecenoate) [65] and some synthesized analogues of FMev (tetrahydro-4-fluoromethyl-4-hydroxy-2H-pyran-2-one) [66] against the mulberry silkworm *Bombyx mori*.

Results of the present study on *E. plorans plorans* were in agreement with those reported results of toxicity of some anti-JH compounds, since exposure of 2nd instar nymphs to the higher two doses (60 and 40 µg/cm²) of PII resulted in complete mortality of nymphs within 24h (initial mortality). At the lower two doses (20 and 10 µg/cm²), PII exhibited a considerably extended toxicity on the subsequently moulted instars but weak toxicity on adult females. After exposure of the 4th instar nymphs to PII, various mortality percentages among the treated nymphs and 5th instar nymphs had been recorded. Only at the lower two doses, PII affected the adult survival. For explication of the nymphicidal effect of PII, it may be attributed to the prevention of moulting nymphs to swallow volumes of air for splitting the old cuticle and expand the new one during ecdysis [67]. Also, these nymphal deaths may be due to the prevented feeding and continuous starvation of the present insect [68]. The adult mortalities, after exposure of 2nd or 4th instar nymphs to PII, can be explained by the retention and distribution of this compound in the insect body as a result of rapid transport from the gut of treated nymphs into other tissues, by the direct and rapid transport *via* the haemolymph to other tissues, and/or by lower detoxification capacity of adults against the tested compound [69].

As reported in the currently available literature, LC_{50} (or LD_{50}) value depends on several factors, such as susceptibility of the insect and its treated stage or instar, lethal potency of the tested compound and its concentration levels, method and time of treatment, as well as the experimental conditions. For examples, LD_{50} of PII against the red cotton bug *Dysdercus koenigii* has been found to be 85.46 and 82.37 mgl^{-1} for 4th and 5th instar nymphs, respectively [45]. After treatment of 4th instar larvae of the Asian tiger mosquito, *Aedes albopictus* with PI and PII, LC_{50} values were estimated in 41.63 µg/ml and 43.55 µg/ml, respectively [64]. LC_{50} of PII against the booklice *Liposcelis bostrychophila* was calculated in 30.4$\mu g/cm^2$ but LC_{50} of PI was found as 64.0$\mu g/cm^2$ [37]. LC_{50} of PI against the cat flea *Ctenocephalides felis* was estimated in 10.97 ppm [70]. LC_{50} values of Pitavastatin against the tobacco hornworm *Manduca sexta* and the viviparous cockroach *Diploptera punctata* were estimated in 5.23, and 395.2 µM, respectively [14]. In the present study on *E. plorans plorans*, LD_{50} values were 0.388 and 17.022 $\mu g/cm^2$, after exposure of 2nd instar nymphs and 4th instar nymphs, respectively. Depending on these data, the 2nd instar nymphs were more sensitive to PII toxicity than 4th instar nymphs.

2. Growth inhibition of *E. plorans plorans* by PII

In the present study on *E. plorans plorans*, PII exerted a slight inhibitory action on the nymphal growth of both 4th and 5th instars, after treatment of 2nd instar nymphs, regardless the dose level, but the growth rate was remarkably regressed after treatment of 4th instar nymphs with 40 and 20 $\mu g/cm^2$. These results were, to some extent, in accordance with some of the reported results of inhibited growth of various insects by different anti-JH compounds. Several chromene derivatives inhibited the growth of the last instar larvae of the mealworm beetle *Tenebrio molitor* [71].

PI and PII exhibited growth-inhibiting activities against *A. aegypti*, *An. sacharovi* and *An. stephensi* [59, 60]. Larvae of *M. sexta* were fed on HMG-CoA reductase inhibitors, Fluvastatin, Lovastatin or Pitavastatin-treated food, starting with 1st instar. The treated larvae grew in significantly slow growth rate [14].

To understand the growth inhibition of *E. plorans plorans*, in the current study, PII might block the release of morphogenic peptides, causing alteration in the ecdysteroid and juvenoid titers [72]. Also, PII may affect the tissues and cells undergoing mitosis [73]. In addition, PII might exert an inhibitory action on the haemolymph and fat body protein contents, as suggested by Lange et al. [74] for locusts after treatment with precocenes.

3. Disrupted development and metamorphosis of *E. plorans plorans* by PII

3.1. Precocious metamorphosis

In the current investigation, exposure of 2nd instar nymphs of *E. plorans plorans* to PII led to 3.33% precociously moulted nymphs into 4th instar, skipping off the 3rd instar (only at the lowest dose). These precocious 4th instar nymphs survived for more than 20 days and eventually perished. After exposure of 4th instar nymphs to PII, some treated nymphs precociously metamorphosed into adultoids, omitting the 5th instar, only at doses of 60 and 40 µg/cm². These precocious adultoids appeared normally in body colour and behaviour but without wings. They survived more than one month and eventually perished without mating.

These results were, to a great extent, in corroboration with those reported results of precocious metamorphosis in several insects of various orders by different anti-JH compounds. Within Orthoptera, exposure of 4th instar nymphs of the desert locust *Schistocerca gregaria* to PII (15 µg/cm²) induced precocious adultoids [75, 76]. Different doses of PI and PII (20-100 µg/insect) induced different degrees of precocious metamorphosis in the Mediterranean splendid grasshopper *Heteracris littoralis* [77]. Among Hemiptera, PII induced precocious metamorphosis in the kissing bugs *Rhodnius prolixus* and *Triatoma dimidiata* when applied by either contact exposure or fumigation [78]. Ayoade et al. [79] observed precocious metamorphosis in the brown plant hopper *Nilaparvata lugens* after exposure to PII. In Coleoptera, topical application of PI and PII onto the 2nd larval instar of *L. decemlineata* induced the precocious adultoids [61]. In addition, precocious metamorphosis had been induced by precocenes in several insects of Diptera, such as the flesh fly *Neobellieria bullata* [80] and the house fly *Musca domestica* [36] as well as Lepidoptera, such as the tobacco cutworm *Spodoptera litura* [81] and *P. ricini* [31]. Moreover, other anti-JH compounds induced such feature of impaired metamorphosis in various insects, such as Fluoromevalonate (FMev) against the fall webworm *Hyphantria cunea* [82] and the lawn armyworm *Spodoptera mauritia* [83]; ETB [65], KK-42 [84,85], KK-22 [86,87] and 3-pyridine derivatives [88] against *B. mori*. Treatment of *N. bullata* larvae with KK-110 and J-2710 resulted in precocious pupation [80].

For interpretation of the induction of precocious 4th instar nymphs, after exposure of 2nd instar nymphs of *E. plorans plorans* to PII, or precocious adultoids, after exposure of 4th instar nymphs to the same compound, in the present study, it is well known that the cells of corpora allata, CA, JH-producing organs in insects, are selectively destroyed by precocenes [26, 89-91]. Thus, precocious metamorphosis in the present grasshopper indicated the prohibition of JH production by PII. On the molecular basis of JH action, Wilson [92] reported that the effects of JH may be due to interference with the expression or action of certain genes, particularly the *broad* complex (*br*-C) transcription factor gene, that direct changes during metamorphosis. In hemimetabolous insects (like the present grasshopper), Erezyilmaz et al. [93] studied the role of *br* for inducing the precocious adult molt in *O. fasciatus* after application of PII to 3rd instar nymphs, and suggested that a loss of *br* mRNA was caused at the precocious adult molt. However, a deep discussion on the action mechanisms of anti-JH compounds in insects was clearly shown in the available literature [34, 38, 39, 91, 94- 99].

3.2. Suspended development

In insects, a state of suspended development attracts a great attention of some entomologists. This state is usually expressed in 'permanent nymphs or larvae'. Depending on the available literature, the induction of permanent nymphs or larvae was recorded in some insect species as a response to some insect growth regulators (IGRs) or botanicals. Among IGRs, some authors [75,100-102] observed permanent (over-aged) nymphs of *S. gregaria* (Orthoptera) after treatment

with certain IGRs. Permanent larvae of the European corn borer *Ostrinia nubilalis* (Lepidoptera) were induced depending upon the dose of Fenoxycarb (juvenile hormone analogue) and the timing of application onto the 5th instar larvae [103]. Permanent larvae of the grey flesh fly *Parasarcophaga argyrostoma* (Diptera) were induced after topical application of last instar larvae with 100 µg/larva of chlorfluazuron (Chitin synthesis inhibitor) [104]. In addition, some botanicals, plant extracts or isolated plant products, had been reported to induce permanent nymphs in various insects, such as the milkweed bug *Oncopeltus fasciatus* (Hemiptera) after injection of the newly moulted last instar nymphs with azadirachtin [105]; *O. fasciatus* and the cotton stainer bug *Dysdercus peruvianus* (Hemiptera) after topical application of *Manilkara subsericea* (Sapotaceae) extracts onto 4th instar nymphs [106]; *S. litura* (Lepidoptera) after treatment of larvae with acetone leaf extract of *Withania somnifera* (Solanaceae) [107]; and the confused flour beetle *Tribolium confusum* (Coleoptera) after treatment of 5th instar and 6th instar larvae with 1µg/µl of Andrographolide (a terpenoid isolated from the leaves of *Andrographis paniculata*, Acanthaceae) [108]. Apart from IGRs and botanicals, El-Gammal et al. [109] observed permanent nymphs in *S. gregaria* after exposure of gamma irradiation (dose of 20 gray) against the 3rd instar nymphs.

In the present work, another noticeable feature of the deranged development was 'permanent nymphs' which induced in 2nd instar nymphs (3.85%) after exposure only to 20 µg/cm² of PII. Also, similar permanent nymphs were induced during the 4th instar. No permanent nymphs had been induced after exposure of 4th instar nymphs to PII. All permanent nymphs survived two-fold period of control congeners, rejecting the food, and eventually perished as nymphs. As far as our literature survey could ascertain, no information was available on the induction of permanent nymphs in insects by precocenes or other anti-JH compounds. Therefore, the present study provides the first report of this feature of suspended development in *E. plorans plorans* by Precocene in the world. To explicate the induction of permanent nymphs of *E. plorans plorans*, in the current investigation, PII exerted an inhibitory action on the prothoracic gland (ecdysone-producing gland) and hence the ecdysone could not be synthesized and/or released. It is well known that the absence of ecdysone leads to failure of ecdysis. An appreciable suggestion of Gaur and Kumar [107] is the compound may disrupt the ecdysteroid metabolism or may alternatively act directly to inhibit the release of ecdysis-triggering hormone.

The current investigation obviously revealed that PII exhibited multiple activities against *E. plorans plorans*: anti-JH activity and anti-ecdysteroid activity. These data have validated the reported anti-ecdysteroid activity of other anti-JH compounds in some insects. The imidazole compound KK-42 was found to delay/inhibit the ecdysteroid production in *O. nubilalis* and *S. gregaria* [110, 111]. Another imidazole SDIII had been reported to exert strong anti-JH and anti-ecdysteroid actions on *B. mori* [112]. Results obtained by Yoshida et al. [88] revealed that the 3-pyridine derivatives temporarily act as anti-ecdysteroids against *B. mori*.

CONCLUSION

Precocene II exhibited an insecticidal activity against nymphs and adults of the grasshopper *E. plorans plorans*, inhibited the nymphal growth, induced precocious moult to last nymphal instar and precocious sterile adultoids. In addition to this anti-JH activity, PII exhibited anti-ecdysteroid activity as appeared in permanent nymphs. In spite of these findings, it may be recommended to use PII for pest control but after study its activity and persistence under the field condition in the foreseeable future.

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