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Evaluation of the toxic and physiological activities of Precocene I (Anti-juvenile hormone agent) against *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae)

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Abstract

The cotton leafworm, *Spodoptera littoralis* is a destructive pest of cotton and various crops all over the year in Egypt as well as in some countries. Objective of the present study was to assess the effects of Precocene I (PI) on survival, growth, development and metamorphosis of this pest. Five doses: 150, 120, 90, 30 and 15 µg/larva had been topically applied (once) onto 1-day old penultimate instar larvae or 1-day old last instar larvae. Various mortality percentages were recorded among the larvae, pupae and adults. LD_{50} values were calculated in 70.48 and 234.96 µg/larva, after treatment of 5th and 6th larval instars, respectively. PI exerted a diverse action on the maximal body weight (max. wt) of treated larvae, depending on the dose. The larval growth was considerably inhibited. The larval duration was conspicuously shortened while the pupal duration was slightly or significantly prolonged, depending on the dose. Regardless the larval instar under treatment, the developmental rate of pupae was generally regressed. PI failed to induce the precocious pupation, regardless the applied dose. PI exerted a strong inhibitory action on the pupation, regardless the larval instar under treatment. The adult emergence was partially blocked. Some morphologically abnormal pupae were produced after treatment of 6th instar larvae with the higher two doses.

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Introduction

In Egypt, the cotton leafworm, *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) has been considered as destructive phytophagous pest of cotton and several vegetable and field crops all over the year (Hosny *et al.*, 1986; Shonouda and Osman, 2000; El-Khawas and Abd El-Gawad, 2002; Adham *et al.*, 2009). The insect attacks almost 112 plant species belonging to 44 families in tropical and temperate regions of the old world (Magd El-din and El-Gengaihi, 2000).

For controlling this pest, different types of conventional insecticides have been used over the past 40 years (Casida and Quistad, 1998). The intensive and indiscriminate use of insecticides against this pest results in the development of resistance to many registered pesticides making their control even more difficult (Ishaaya *et al.*, 1995; Smagghe *et al.*, 1999; Miles and Lysandrou, 2002; Aydin and Gurkan, 2006; Davies *et al.*, 2007, Mosallanejad and Smagghe, 2009).

As a result of improper and excessive uses, also, these insecticides usually exhibit several adverse impacts on the human health and beneficial animals as well as cause serious toxicological problems to the environment because these chemicals have a long half-life and retention in the environmental systems for long time (Van Der Gaag, 2000; Costa *et al.*, 2008; Relyea, 2009; Tiryaki and Temur, 2010). In addition, these chemicals have a tendency to accumulate in different trophic levels of the food net (Damalas and Eleftherohorinos, 2011; Chowański *et al.*, 2014).

Therefore, eco-friendly chemicals have received a great attention in the world during the recent years. These alternative compounds have lower toxicity to non-target organisms than conventional insecticides and they are effective at low concentrations (Attathom, 2002; Gade and Goldsworthy, 2003). Also, they are biodegradable into harmless compounds, and hence the problems of environmental pollution can be avoided (Tiryaki and Temur, 2010; Walkowiak *et al.*, 2015; Li *et al.*, 2017). Owing to the dangerous attacks of S. littoralis, it is a subject to intensive research for searching new measures to control it and for improving the effects of known control methods (Hussain, 2012).

At present, the use of insect growth regulators (IGRs) is considered as possible alternative agents of conventional insecticides for controlling this pest (Raslan, 2002). IGRs are regarded as a 'third generation of insecticides'because they differ in their mode of action from other insecticides and have low toxicity to non-target organisms (Zhou *et al.*, 2003). Because of their desirable characteristics, such as less environmental pollution, high selectivity, and low impact on natural enemies and people, IGRs are used to control various insects (Wu, 2002; Cedric, 2005; Wang and Wang, 2007).

Juvenile hormone (JH) is necessary for the developmental stages in insects (Staal, 1986). Also, JHs play important roles in other physiological processes, such as behaviour, polymorphism, migration, metabolism, reproduction, diapause, and innate immunity (Riddiford, 1994; Gilbert *et al.*, 2000; Mitsuoka *et al.*, 2001; Tatar *et al.*, 2001a,b; Truman and Riddiford, 2007; Riddiford, 2008; Flatt *et al.*, 2008; Denlinger *et al.*, 2012; Amsalem *et al.*, 2014a).

The anti-juvenile hormone agents that affect either the mevalonate pathway in JH biosynthesis can be considered insect growth regulators (IGRs) affecting the corpora allata (CA) directly (Staal, 1986). Precocenes are originally plant-derived chromenes (2H-1-benzopyran) with insecticidal activities (Bowers, 1976; Proksch *et al.*, 1983; Isman *et al.*, 1986).

In the context of exploring the growth regulatory activities of compounds related to precocenes, rational synthetic methods for 2, 2-dimethyl chromenes were developed for the preparation of precocene analogues or precocenoids (Banerji and Goomer, 1984; Banerji and Kalena, 1989; Szczepanik *et al.*, 2005; Banerjee et al., 2008).

Some entomologists paid a great attention to precocene analogues due to their twin advantage; avoiding the former surgical allatectomy and as an effective agent in devising 'fourth generation insecticides' in future (Muraleedharan *et al.*, 1986; Sariaslani *et al.*, 1987; Moya *et al.*, 1997; Szczcpanik *et al.*, 2005; Singh and Kumar, 2011).

The hemimetabolous insects, such as grasshoppers, aphids and cockroaches, are more susceptible to precocenes than some larvae of holometabolous insects (Burt *et al.*, 1979; Chênevert *et al.*, 1980; Kiss *et al.*, 1988). However, some authors (Mathai and Nair, 1984; Kozhanova and Nemec, 1991; Khafagi and Hegazi, 2001) reported that a number of holometabolous insects, such as *Spodoptera mauritia*, *Tenebrio molitor* and *Spodoptera littoralis* are sensitive to Precocenes.

It has been demonstrated that the design of anti-JH agents is an effective tool for insecticide discovery (Bede *et al.*, 2001). In addition, compounds with anti-JH activity are considered as new representatives of IGRs lacking some disadvantages of juvenoid-type chemicals (Bowers, 1982; Staal, 1982).

These compounds are potentially effective for controlling the major insect pests, since the larval stage is the dangerous among the insect developmental stages (El-Ibrashy, 1982). Objective of the present study was to assess the effects of Precocene I on the survival, growth, development metamorphosis and morphogenesis of *S. littoralis*.

Materials and methods

Experimental insect

A pupal sample of the Egyptian cotton leafworm, *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) was kindly obtained from the culture of susceptible strain maintained for several generations in Plant Protection Research Institute, Agricultural Research Center, Doqqi, Giza, Egypt. In Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, Cairo, a culture was raised under laboratory controlled conditions (27±2°C, 65±5% R.H., photoperiod 14h L and 10h D). Rearing procedure was carried out according to Ghoneim (1985) and improved by Bakr *et al.* (2010). Larvae were provided daily with fresh castor bean leaves *Ricinus communis*. The emerged adults were provided with cotton pieces soaked in 10% honey solution as a food source. Moths were allowed to lay eggs on *Oleander* branches. The egg patches were collected daily, and transferred into Petri dishes for another generation.

Precocene I administration

Precocene I (PI)(7-methoxy-2,2-dimethyl chromene) was kindly provided by Dr. Heba Hassan, Prof. at Plant Protection Research Institute, Agricultural Research Center, Doqqi, Giza, Egypt. Molecular formula: $C_{12}H_{14}O_2$ PI was diluted in acetone to prepare five doses: 150, 120, 90, 30 and 15 µg/larva. Each dose was topically applied (once) onto the thoracic sterna of 1-day old 5th (penultimate) and 1-day old (last) instar larvae by Hamilton microapplicator (NHN 737). Groups of 20 healthy larvae were used as replicates for each dose.

The newly moulted larvae did not be used, in the present study, because the first 24h, in each larval instar, is the critical period of corpora allata of *S. littoralis* (El-Ibrashy, 1971) after which the juvenile hormone is released in the haemolymph. Control larvae had been topically applied only with 1µl acetone. All treated and control larvae were kept individually under the previously mentioned laboratory controlled conditions.

All larvae were provided with fresh castor bean leaves every day, during the feeding period. Starting from the day after treatment all treated and control insects were observed daily to record all criteria of study.

Criteria of study

Mortality in larvae, pupae and adults were determined in %. LD_{50} values were calculated by Microsoft office Exel, 2007, according to Finny (1971). Coefficient of growth:Coefficient of growth was calculated according to El-Ibrashy and Aref (1985) as follows: maximal body weight (mg)/ duration (in days) for each larva. Developmental duration: Dempster's equation (1957) was used for calculating the developmental duration (in mean days±SD) and Richard's equation (1957) was used for calculating the developmental rate.Pupation rate:Pupation rate was expressed in % of the developed pupae. Adult emergence was determined in %. Precocious metamorphosis was determined in % of precocious pupation. Impaired morphogenesis was determined in % of deformed larvae, pupae and adults.

Statistical analysis of data

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) for the test significance of difference between means.

Results

Lethal action of PI against S. littoralis

After topical application of PI (once) onto the 1-day old 5th (penultimate) instar larvae, insecticidal action of PI was expressed in mortality among larvae, pupae and adults. According to data arranged in Table (1), PI exerted a toxic effect on the treated 5th instar larvae, in no certain trend.

The successfully moulted 6th instar larvae suffered the toxic effect only at higher two doses (14.3 and 11.1% mortality, at 150 and 120 µg/larva, respectively, *vs.* 0% mortality of control congeners). Also, PI exhibited toxicity against pupae only at the higher two doses.

In a similar trend, the emerged adult females had been subjected to a toxic effect of PI only after larval treatment with the higher two doses. LD_{50} value was calculated in 70.48 µg/larva.

Table 1. Toxicity (%) of PI against *S. littoralis* topically treated as penultimate (5th) or last (6th) instar larvae.

Dose (µg/larva)	Treatment of 1-day old 5th instar larvae						Treatment of 1-day old 6th instar larvae				
	Larval mortality		Pupal mortality	Adult mortality	Total mortality	LD_{50} (µg/ larva)	Larval mortality	Pupal mortality	Adult mortality	Total mortality	LD_{50} (µg/larva)
	5th instar	6th instar									
150	53.3	14.3	16.67	20.00	73.33	70.48	20.00	8.33	9.09	33.33	234.96
120	40.0	11.1	12.50	28.57	60.00		13.33	53.85	16.67	66.67	
90	33.3	0.0	0.00	0.00	33.33		0.00	6.67	0.00	6.67	
30	60.0	0.0	0.00	0.00	60.00		0.00	0.00	0.00	0.00	
15	6. 7	0.0	0.00	0.00	6.67		0.00	0.00	0.00	0.00	
Control	0.0	0.0	0.00	0.00	0.00		0.00	0.00	0.00	0.00	

The same table contains data of PI toxicity after topical application onto 1-day old 6th (last) instar larvae. Depending on these data, PI exhibited an insecticidal activity against larvae only at the higher two dose levels. Pupal mortality was observed after topical application of the higher three doses. With regard to the successfully emerged adult females, these moths were subjected to the toxic effect of PI only at the higher two doses. LD_{50} value was calculated in 234.96 µg/larva. As obviously seen, sensitivity of *S. littoralis* to PI toxicity was higher when the larvae were treated as 1-day old 5th instar.

Effect of PI on growth of S. littoralis

After topical application of PI doses onto the 1-day old 5th instar larvae, data of the maximal body weight (max. wt), duration and coefficient of growth (CG) of the treated larvae and the successfully moulted 6th instar larvae were assorted in Table (2). In the light of these data, max.wt of treated 5th instar larvae significantly decreased at the higher two doses. In contrast, max. wt remarkably increased as a response to the action of PI, at the lower three dose levels. Apart from this diverse effect of PI on max.wt, it exerted a strong inhibitory action on larval growth, since the CG was considerably regressed, especially at the higher three dose levels. With no exception, PI enhanced the successfully moulted 6th instar larvae to attain increasing max. wt , in a dose-dependent course. In a similar trend, CG was pronouncedly induced in a dose-dependent manner (details table 2).

Table 2. Growth of *S. littoralis* after topical application of PI onto 1-day old penultimate instar larvae.

Dose (µg/larva)	Penultimate instar larvae			Last instar larvae			Precocious pupation (%)
	Maximal body weight (mean mg±SD)	Duration (mean days ± SD)	Coefficient of growth (mean± SD)	Maximal body weight (mean mg±SD)	Duration (mean days ± SD)	Coefficient of growth (mean± SD)	
150	102.8±17.5 b	1.0±0.0 b	102.8±17.5 c	622.5±32.7 b	4.0±0.7b	160.4±29.4 b	0
120	103.7±5.8 b	1.0±0.1 b	103.7±7.8 b	610.8±45.0c	4.1±0.6 b	148.9±11.11 c	0
90	138.3±8.7 b	1.4±0.1 a	98.8±6.8 c	605.2±55.8c	4.2±0.7a	148.0±39.3 b	0
30	151.20±17.6 b	1.5±0.2 a	108.0±21.4 a	572.8±67.0a	4.5±0.5a	127.9±13.4 a	0
15	162.0±10.8 c	1.5±0.3 a	108.0±19.5 a	560.7±77.9 a	4.5±0.5a	124.6±15.0 a	0
Control	119.0±38.9	1.9±0.3	110.4±19.8	513.4±1238	4.5±0.5	108.5±29.8	0

Mean±SD followed with the same letter a: insignificantly different (P >0.05), b: significantly different (P<0.05), c: highly significantly different (P<0.01).

After topical application of PI onto the 1-day old last instar larvae, data of max. wt and CG were summarized in Table (3). On the basis of these data, a diverse action of PI on max.wt was recorded, since it significantly increased at the higher two doses but drastically reduced at the lower three doses. Also, a diverse action of PI was exerted on the CG, since the compound promoted larvae to gain more growth at the higher two dose levels but slightly suppressed the CG at the lower three doses.

Table 3. Growth of *S. littoralis* after topical application of PI onto 1-day old last instar larvae.

Dose (µg/larva)	Maximal body weight (mean mg±SD)	Duration (mean days±SD)	Coefficient of growth (mean±SD)
150	572.7±151.5 b	5.63±0.8 a	111.7±49.9 b
120	682.0±149.1 b	6.40±1.5 a	114.8±57.7 b
90	468.1±18.8 b	5.71±0.8 a	085.7±35.8 a
30	472.2±78.1 b	5.33±0.9 b	092.0±25.3 a
15	493.8±47.4 a	5.18±0.7 b	089.3±13.5 a
Control	558.8±104.0	6.50±0.7	105.3±27.9

a, b: see footnote of Table (2).

Effects of PI on development, metamorphosis and morphogenesis of S. littoralis

Affected duration and developmental rate

After topical application of PI onto 5th instar larvae, data of affected larval duration were assorted in Table (2). In view of these data, duration of the treated larvae was conspicuously shortened, almost in a dose-dependent course. Also, duration of the successfully moulted 6th instar larvae was considerably shortened; especially at the higher three doses.

As obviously shown in Table (3), topical application of PI onto 6th instar larvae resulted in an abbreviation of larval duration, especially at the lower two doses. Data of distributed in Table (4) revealed a slight prolongation of pupal duration, after treatment of 5th instar larvae.

Also, a slight prolongation of pupal duration was easily observed in Table (5) after treatment of 6th instar larvae. Moreover, the pupal duration was remarkably prolonged at the lower two doses of PI.

As easily seen in tables 4 and 5, the developmental rate of pupae was generally regressed, in no certain trend, after treatment of 5th or 6th instar larvae with PI.

Impaired metamorphosis and morphogenesis

As obviously observed in Table (2), PI failed to induce the precocious pupation in *S. littoralis* after treatment of the penultimate instar larvae. With regard to the pupation rate, PI unexceptionally exerted a strong inhibitory action on pupation, regardless the larval instar under treatment (see tables 4 and 5).

Table 4. Development and metamorphosis of *S. littoralis* after topical application of PI onto 1-day old penultimate instar larvae.

Dose (µg/larva)	Pupation rate	Pupal Duration (mean days±SD)	Pupal Developmental Rate	Abnormal pupae (%)	Morphogenic efficiency (%)	Adult emergence (%)
150	45.0	7.65±2.9 a	13.00	0	0	50.0
120	48.5	7.65±3.1 a	13.00	0	0	58.3
90	62.5	7.85±2.8 a	12.80	0	0	66.6
30	63.5	7.50±3.3 a	13.33	0	0	100
15	69.5	7.50±3.1 a	13.33	0	0	100
Control	100	7.30±1.9	14.70	0	---	100

a: See footnote of Table (2).

After topical application of penultimate instar larvae, the inhibitory action of PI on pupation increased parallel to the dose level (Table 4). After treatment of last instar larvae, PI exhibited an inhibitory effect on the pupation, in a reverse trend with the dose level, i.e., the pupation rate was regressed consecutively with the decreasing dose level (Table 5).

In respect of the adult emergence, PI exerted a blocking action on this crucial metamorphosis event, after treatment of 5th instar larvae only with the higher three doses (Table 4). A similar arresting action of PI on adult emergence was recorded after treatment of 6th instar larvae only with the higher two doses (Table 5).

Table 5. Development and metamorphosis of *S. littoralis* after topical application of PI onto 1-day old last instar larvae.

Dose (µg/larva)	Pupation rate	Pupal Duration (mean days±SD)	Pupal Developmental Rate	Abnormal pupae (%)	Morphogenic efficiency (%)	Adult emergence (%)
150	86.9	7.53±1.1 a	13.28	50.0	60.0	85.7
120	86.7	7.50±3.5 a	13.33	06.7	07.1	95.5
90	83.8	7.39±1.4 a	13.53	00.0	00.0	100
30	83.3	8.00±0.8 c	11.95	00.0	00.0	100
15	80.5	8.44±0.7 b	11.85	00.0	00.0	100
Control	100	7.35±1.1	13.61	00.0	---	100

a, b, c: See footnote of Table (2).

After treatment of 5th instar larvae, PI failed to exhibit morphogenic efficiency on the present insect, since no malformed pupae or adults had been observed.

On the other hand, PI exhibited morphogenic efficiency on pupae, after treatment of 6th instar larvae only with the higher two doses, since morphologically abnormal pupae had been observed (50.0 and 6.7% abnormal pupae, at 150 and 120 µg/larva, respectively, *vs.* 0% abnormality in control pupae). As clearly shown in Fig. (1), some features of the impaired pupation program appeared in dwarf-sized pupae which failed to metamorphose into adults.

Discussion

Affected survival of S. littoralis by PI

Toxicity of several anti-juvenile hormone (anti-JH) compounds had been reported against different insect species. For examples, bothPI and PII exhibited larvicidal activities against several mosquito species, such as*Aedes aegypti,Anopheles sacharovi* and *An. stephensi* (Saxena et al., 1994; Yasyukevich and Zvantsov, 1999). Precocenes exhibited larvicidal effects, in a dose-dependent course, on the Colorado potato beetle *Leptinotarsa decemlineata* (Farazmand and Chaika, 2008). A toxicological effect of PII was reported by Abdullah (2009) against larvae of the red palm weevil *Rynchophorus ferrugineus*.

Also, PII exhibited larvicidal and pupicidal effects on the grey flesh fly *Parasarcophaga dux* (Nassar *et al.*, 1999); larvicidal effect on the lepidopterous pest *Pericallia ricini* (Khan and Kumar, 2000); and larvicidal effect on the Asian tiger mosquito *Aedes albopictus* (Liu and Liu, 2014). After exposure of the newly moulted 2nd or 4th (penultimate) instar nymphs of the grasshopper *Euprepocnemis plorans* to some doses of PII, various mortality percentages were recorded among the treated nymphs of different instars and the emerged adults (Ghoneim and Basiouny, 2017). 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)(Kuwano *et al.*, 1988) and some synthesized analogues of FMev (tetrahydro-4-fluoromethyl-4-hydroxy-2H-pyran-2-one)(Shuto *et al.*, 1988) against the mulberry silkworm *Bombyx mori.*

Fig.1. Pupal abnormalities of *S. littoralis* after treatment of 6th instar larvae with the higher two doses of PI (150 and 120 µg/larva). Normal pupa (at left) and dwarf pupa (at right). These dwarf-sized pupae failed to metamorphose into adults.

Results of the present study on the Egyptian cotton leafworm *Spodoptera littoralis* were in agreement with those previously reported results, since topical application (once) of PIonto 1-day old 5th (penultimate) or 6th (last) instar larvae affected the survival of the insect, as recorded in various mortality percentages among larvae, pupae and adult females. However, the larval deaths may be attributed to the prevention of moulting larvae to swallow air volumes for splitting the old cuticle and expand the new one during ecdysis (Linton *et al.*, 1997). Also, these larval deaths may be due to the prevented feeding and continuous starvation of the insect (Ghoneim *et al.*, 2000). The pupal deaths can be directly related to the hormonal activity of the tested anti-JH compound or may be to other causes, such as bleeding, desiccation and suffocation, due to imperfect exuvation, failure of vital homeostatic mechanisms, *etc.* (Smagghe and Degheele, 1994). The adult mortalities can be explained by the retention and distribution of PI in the insect body as a result of direct and rapid transport *via* the haemolymph to other tissues, and/or by lower detoxification capacity of adults against the tested compound (Osman *et al.*, 1984).

The reported LD_{50} (or LC_{50}) values of anti-JH compounds are variable in different insects. For examples, LD_{50} of PII againstthe red cotton stainer *Dysdercus koenigii* has been found to be 85.46 and 82.37 mgl^{-1} for 4th and 5th instar nymphs, respectively (Banerjee *et al.*, 2008). After treatment of 4th instar larvae of *A. albopictus*withPI and PII, LC_{50} values were estimated in 41.63 µg/ml and 43.55 µg/ml, respectively (Liu and Liu, 2014). LC_{50} of PII against the booklice *Liposcelis bostrychophila* was calculated in 30.4 µg/cm² but LC_{50} of PI was found as 64.0µg/cm² (Lu *et al.*, 2014). LC_{50} of PI againstthe cat flea *Ctenocephalides felis* was estimated in 10.97 ppm (Rust and Hemsarth, 2017). LC_{50} values of the anti-JH agent Pitavastatin against the tobacco hornworm *Manduca sexta* andthe viviparous cockroach *Diploptera punctata* were estimated in 5.23, and 395.2 µM, respectively (Li *et al.*, 2017). LD_{50} values of PII against *E. plorans* were 0.388 and 17.022 µg/cm² after topical treatment of newly moulted 2nd and 4th (penultimate) instar nymphs, respectively (Ghoneim and Basiouny, 2017). In the current study, LD_{50} values were estimated in 70.48 and 234.96 µg/larva, after treatment of 5th and 6th instar larvae, respectively. Thus, the 5th instar larvae were more sensitive to PI than 6th instar larvae.

As reported in the currently available literature, LD_{50} (orLC_{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.

Growth inhibition in S. littoralis by PI

After treatment of 5th or 6th instar larvae of *S. littoralis* with PI doses, in the current investigation, the compound exerted a diverse action on the maximal body weight (max. wt) of treated larvae, depending on the dose. The coefficient of growth was considerably regressed, irrespective of the instar of treated larvae. 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. Growth of the last instar larvae of the mealworm beetle *Tenebrio molitor* was remarkably inhibited after treatment with several chromene derivatives (Roberto *et al.*, 1998). PI and PII exhibited growth-inhibiting activities against the mosquito species *A. aegypti, An. sacharovi* and *An. stephensi* (Saxena *et al.*, 1994; Yasyukevich and Zvantsov, 1999). After feeding of *M. sexta* larvae on a treated diet with HMG-CoA reductase inhibitors, Fluvastatin, Lovastatin or Pitavastatin, the growth rate of treated larvae was significantly slow (Li *et al.*, 2017).After exposure of the newly moulted 2nd or 4th (penultimate) instar nymphs of *E plorans* to different doses of PII, the nymphal growth of both 4th and 5th instars had been slightly inhibited after treatment of 2nd instar nymphs, but remarkably regressedafter treatment of 4th instar nymphs (Ghoneim and Basiouny, 2017). To understand the inhibition in growth of S. littoralis, in the current study, PI might affect the tissues and cells undergoing mitosis (Nasiruddin and Mordue, 1994). Also, PI might exhibit an inhibitory effect on the protein contents in haemolymph and fat body (Lange *et al.*, 1983).

Developmental disturbance in S. littoralis by PI

The developmental rate of an insect stage is usually reversely related to the developmental duration, i.e. shorter duration indicates faster rate and vice versa. The larval duration in several insect species (holometabolous or hemimetabolous) had been prolonged as a response to the action of different anti-JH compounds. For examples, Bowers and Aldrich (1980) recorded a prolongation of 5th nymphal instar in the milkweed bug *Oncopeltus fasciatus* after treatment with PI. Treatment of the 4th instar nymphs of the desert locust *Schistocerca gregaria* with PII resulted in prolongation of the duration of both 4th and 5th nymphal instars (Eid *et al.*, 1982). Treatment of 6th instar larvae ofthe lawn armyworm *Spodoptera mauritia* with PII resulted in prolongation of duration in last larval instar (Mathai and Nair, 1983, 1984). The nymphal period of the grasshopper *Aiolopus thalassinus* was prolonged after topical application of PIII onto 5th instar nymphs (Osman, 1988). Treatment of the tobacco cutworm *Spodoptera litura* larvae with PI, PII or ethoxyprecocene (a synthetic analog of P II) resulted in prolongation of larval period (Srivastava and Kumar, 1997, 1999). After treatment of 4th instar nymphs of *D. koenigii* with PII, duration of the successfully moulted 5th instar nymphs was prolonged(Banerjee *et al.*, 2008). Apart from precocenes, prolongation of the larval period in the fall webworm *Hyphantria cunea* was recorded after treatment with FMev (Farag and Varjas, 1983). Similar results of prolonged larval duration were reported in *B. mori* by KK-22 (phenylimidazoles) (Kuwano and Eto, 1983; Asano *et al.*, 1984). After treatment of 4th instar larvae of *B. mori* with the synthesized 3- (2-methy l-l-pheny l-l-propenyl) pyridine, the larval period was prolonged (Yoshida *et al.*, 2000). Results of the present investigation disagreed with those previously reported results, since topical application of PI onto 5th instar larvae of *S. littoralis* resulted in conspicuously shortened larval duration, almost in a dose-dependent course. Also, the larval duration was remarkably shortened after treatment of 6th instar larvae with PI, especially at the lower two doses.

On the other hand, our results were in accordance with those reported results of shortened larval duration after treatment with some anti-JH compounds, such as *P. dux* after treatment of the 3rd instar larvae with PII (Nassar *et al*., 1999); the flesh fly *Sarcophaga ruficornis* after treatment of the last instar larvae with PI, PII or PIII (Srivastava and Kumar, 1996); the house fly *Musca domestica* after treatment of the larvae withPII (Gaur and Kumar, 2009); *B. mori* after treatment of the 3rd and 4th instars with the imidazole compound SSP-11 (Kiuchi *et al*., 1985). Moreover, topical application of PI onto 5th or 6th instars larvae of *S. littoralis*, in the present study, resulted in a slight or significant prolongation of the developed pupae**,** depending on the dose level. In general, the developmental rate of these pupae was slightly regressed. As far as our literature survey could ascertain, no information was available for the effects of anti-JH compounds on the pupal duration or developmental rate.

To explicate the shortened larval duration of *S. littoralis*, in the currentinvestigation, it may be due to the response of these treated larvae for avoiding the adverse action of PI, as a xenobiotic agent. On the other hand, the prolongation of pupal period in *S. littoralis*, in the present study, indicated a retarding action of PI on the development as expressed in regression of the developmental rate. This prolongation in the pupal duration may be attributed to the indirect interference of this compound with the neuroendocrine organs responsible for the synthesis and release of tropic hormones, like prothoracicotropic hormone (Subrahmanyam *et al*., 1989). Also, the recordedprolongation of pupal stage may be attributed to a disturbing action of PI on the persistence of JH in the haemolymph where it is only in the absence of JH that ecdysone could be activated and lead to the formation of the next stage (Bowers, 1982; Kuwano *et al*., 1988). In addition, PI may exhibit a delaying effect on the pupal transformation into adults (Linton *et al*., 1997). In particular, the final step of chitin biosynthesis pathway was inhibited by PI and the precursor was not converted into chitin leading to a prolongation of developmental duration (Djeghader *et al*., 2014).

Impaired metamorphosis and morphogenesis of S. littoralis by PI

Precocious metamorphosis

In the current study, PI failed to induce the precocious pupation or other features of precocious metamorphosis of *S. littoralis*. This result disagreed with many reported results of precocious pupation or metamorphosis in several insect species as a response to different anti-JH compounds, such as the precocious pupation in *H. cunea* (Farag and Varjas, 1983) and *S. mauritia* (Balamani and Nair, 1989) after treatment with the anti-JH compound Fluoromevalonate(FMev), the flesh fly *Neobellieria bullata* after treatment with PII (Darvas *et. al*., 1990), *P. ricini* after treatment with PII (Khan and Kumar, 2000) and *B. mori* after treatment of 4th instar larvae with a synthesized 3-pyridine derivative (Yoshida *et al*., 2000).Exposure of 2nd instar nymphs of the grasshopper *Euprepocnemis plorans* to PII led to precociously moulting into 4th instar, skipping off 3rd instar (only at the lowest dose). Also, exposure of 4th instar nymphs to PII, some treated nymphs precociously metamorphosed into adultoids, omitting the 5th instar (Ghoneim and Basiouny, 2017).

However, the failure of PI to induce precocious pupation or other features of precocious metamorphosis, in the present study on *S. littoralis*, was in agreement with those reported results of failure of some anti-JH compounds to induce precocious forms, such as PI againstthe sunn pest *Eurygaster integriceps* (Tarrant *et al*., 1982), PII against *S. mauritia* (Mathai and Nair, 1983), the synthesized anti-JH compound EMD or its analogues against *B. mori* (Kuwano *et al*., 1988), the synthesized compound (S)-(+)-FMev against *B. mori* (Shuto *et al*., 1988) and PII against *S. gregaria* (Islam, 1995).

The failure of PI to induce precocious pupation of *S. littoralis*, in the current study, can be understood because it did not exhibit an anti-JHactivity indicating the inability to affect the larval JH levels (Edwards *et al*., 1983). Some authors (Burt *et al*., 1978; Haunerland and Bowers, 1985; Staal, 1986) reported that the larvae of holometabolous insects-with few exceptions- are less susceptible to the action of Precocenes than hemimetabolous insects. In contrast to our finding, some holometabolous insects, such as *S. mauritia* and *S. littoralis* have been reported to be sensitive to the anti-JH activity of Precocenes (Mathai and Nair, 1984; Khafagi and Hegazi, 2001).

*Pupation process*In the current investigation, PI exerted a strong inhibitory action on the pupation rate in *S. littoralis*, irrespective of the larval instar under treatment, 5th or 6th instar. No information is available in the current literature for the inhibitory effects of anti-JH compounds on the pupation rate in insects. However, the present result was consistent with those reported inhibitory effects of some juvenile hormone analogues (JHAs) or other insect growth regulators (IGRs) on the pupation rate in various insects, such as the diamondback moth *Plutella xylostella* by Hexaflumuron (Mahmoudvand *et al.*, 2012); *S. littoralis* by Novaluron (Ghoneim *et al.*, 2015); the lesser mulberry pyralid *Glyphodes pyloalis* by Lufenuron (Aliabadi *et al.*, 2016); the pink boll worm *Pectinophora gossypiella* (Ghoneim *et al.*, 2017a) and the olive leaf moth *Palpita unionalis* (Ghoneim *et al.*, 2017b) by Novaluron.

Deranged morphogenesis

Precoceneshave been reported to be anti-juvenile hormone, accelerating the development of insects and inducing dwarfness associated with low survival rates (Ghosh *et al.*, 2012). In thepresent studyon *S. littoralis*, no malformed pupae or adults had been observed after treatment of 5th instar larvae with PI. On the other hand, some morphologically abnormal pupae were produced after treatment of 6th instar larvae with the higher two doses. These dwarf-sized pupae failed to metamorphose into adults.This result was, to a great extent, in agreement with those reported results of adverse action of some anti-JH compounds on the morphogenesis of a number of insects. For examples, Treatment of 3rd instar larvae of *B. mori* with some terpenoid imidazole compounds led to the formation of miniature pupae after molting to 4th instar larvae(Kuwano *et al.*, 1983). Topical application of PIII onto eggs or 5th instar nymphs of *A. thalassinus* resulted in some prothetelic morphogenic disturbances (Osman, 1988).

Production ofabnormal puparia was recorded in *S. ruficornis* after administration of PI, PII or PIII to the last instar larvae (Srivastava and Kumar, 1996). Treatment of *S. litura* larvae with PI, PII or ethoxyprecocene (a synthetic analog of P II) resulted in the production of abnormally formed pupae (Srivastava and Kumar, 1999). Puparial malformations were observed in *P. dux* after topical application of PII onto the 3rd instar larvae (Nassar et al., 1999). Treatment of *M. domestica* maggots with PIIled to the formation of abnormal puparia (Gaur and Kumar, 2009). Larval treatment of *E. integriceps* with PI led to the production of some morphological abnormalities (Amiri *et. al.*, 2010).

For interpretation of the pupation rate regression and impairment of the pupation program in *S. littoralis*, as caused by PI in the present study, PI might exert a prohibiting 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. Whatever the mode of action, PI suppressed the chitin synthesis and prevented the normal deposition of new cuticle during apolysis leading to the production of pupal deformities (Retnakaran *et al.*, 1985). In addition,PI might block the release of morphogenic peptides, causing alteration in both ecdysteroid and juvenoid titers (Barnby and Klocke, 1990). A suggestion of Gaur and Kumar (2010) may be appreciated. PI might disrupt the ecdysteroid metabolism or might alternatively act directly to inhibit the release of ecdysis-triggering hormone.

Inhibited adult emergence

Scarce studies have examined the effects of anti-JH compounds on the adult emergence in insects. Inhibition of adult emergence in *S. ruficornis* was recorded after larval treatment with PII (Khan and Kumar, 2005). KK-42 (a terpenoid imidazole)was reported to inhibit the adult emergence of *B. mori* when applied to the newly formed pupae (Kadono-Okuda *et al.*, 1987).

In the present study on *S. littoralis*, the adult emergence was slightly or drastically blocked, especially at the higher three or two doses of PI, depending on the larval instar under treatment, 5th or 6th instar.

For interpretation of this result, it important to point out that the adult emergence in insects is a crucial physiological process and regulated by the eclosion hormone. The disturbance of this hormone appears to partially or completely arrest the adults to emerge. The present result of blocked adult emergence can be interpreted by the disruptive effect of PI on the normal metabolism of insect hormones during the development of the immature stages leading to failure of adult emergence (Trigo *et al.*, 1988). In particular, PI might disturb the adult eclosion hormone release and/or inhibition of the neurosecretion (Al-Sharook *et al.*, 1991; Joseph rajkumar *et al.*, 1999). On the molecular basis, anti-JH compounds, like PI, might cause misexpression of certain genes, particularly the *brood* complex (*br*-C) transcription factor gene, leading to symptoms of deranged metamorphosis, like blocking of adult emergence (Wilson, 2004; Nandi and Chakravarty, 2011).

In general, it is still unclear whether the effects of precocenes on insects are restricted to the corpora allata (JH-producing organs) (Garcera *et al.*, 1991; Burks *et al.*, 1992), or are more general due to their toxicity (Ergen, 2001), or indirect as consequence of the decreased JH titers (Amsalem *et al.*, 2014b), since many studies revealed multiple targets for the effects of Precocenes in insects (Chen *et al.*, 2005 a,b; Amiri *et al*, 2010).

Conclusion

According to the obtained results of the present study, it can be concluded that PI exhibited various degrees of toxicity against all developmental stages of *S. littoralis*, as well as some disruptive effects on growth and development but failed to induce anti-JH activity against this pest. Therefore, PI may be recommended to use in the integrated pest management program after further investigation on its activity under the field conditions.

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