

# Effects of Anti-Juvenile Hormone (Precocene II) Treatment on Phase Changes and Pheromone Production in the Desert Locust, *Schistocerca gregaria* (Forsk.) (Orthoptera: Acrididae)

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**Abstract:** The roles of anti-juvenile hormone (precocene II) on phase changes and pheromone production were examined in laboratory-bred gregarious desert locust, *Schistocerca gregaria*. Precocene II was applied to last instar nymphs and newly emerged adults in gregarious phase. Generally, the 5<sup>th</sup> instar nymphs exhibited a higher sensitivity to topical application of precocene II than the adults. In contrast, the adults exhibited a higher sensitivity to injection of precocene II in comparison to 5<sup>th</sup> instar nymphs. Topical application or injection of precocene II inhibited pheromone production (as measured by the amount of phenylacetone nitrile released). Both topical application to 5<sup>th</sup> instar nymphs and injection to adults affected the external body coloration, sexual behavior and mating of gregarious adult males, *S. gregaria* in comparison to control insects.

**Keywords:** Anti-juvenile hormone, locust, phase polyphenism, pheromone, precocene, *Schistocerca gregaria*.

## 1. INTRODUCTION

Locust phase polyphenism is a remarkable form of phenotypic plasticity in which the expression of numerous physiological, morphological and behavioral traits occurs in response to changes in local population density. This polyphenism is continuous and all the intermediate stages are found between the two extreme phases, depending on the direction of the transformation. The gregarious desert locust, *S. gregaria*, is the most destructive international pest, with its distribution extending from India in the east to Africa in the west [1-5]. Therefore, the endogenous factors that regulate the transition from a solitary to a gregarious state and then maintain the gregarious phase are of considerable interest.

The involvement of endocrine factors in the regulation of locust phase transformation has been extensively reviewed [6-11]. It has long been known that the corpora allata (CA) and their product, the juvenile hormone (JH), undeniable affect some phase characteristics and may involve in locust phase change. For example, implantation of extra CA or administration of JH or JH analogs, to crowded hoppers induces green color which is considered as a solitary characteristic. This effect was demonstrated in *Locusta migratoria* [12, 13], *S. gregaria* [14, 15], and in other grasshoppers [16]. On the other hand, Joly and Joly [17] and Joly [18, 19] reported that implantation of extra CA into crowded *Locusta* hoppers results in a decrease of the E/F (length of elytron/length of hind femur) ratio. They even obtained "hyper-solitary" values. Also, allatectomy of the gregarious adult

male resulted in complete absence of the yellow color, but re-implantation of CA or administration of JH restored yellowing [20, 21].

Previous work on the desert locust, *S. gregaria*, and the African migratory locust, *L. migratoria*, had implicated a number of pheromonal and interspecific chemical signals in their phase dynamics [22, 23]. The existence of aggregation pheromone in the air surrounding gregarious *S. gregaria* has been shown in behavioral experiments [24, 25]. Obeng-Ofori *et al.* [24] revealed the presence of two aggregation pheromone complexes analyzing volatiles from the faeces as well as from the air surrounding second- to fifth-instar nymphs and adults. Thereafter, Torto *et al.* [25] identified six electrophysiologically active components in the volatiles of sexually mature male *S. gregaria*: anisole, veratrole, benzaldehyde, guaiacol, phenylacetone nitrile and phenol. Phenylacetone nitrile was the dominant component, consisting of 80 % of emission of the older and mature males. In spite of this important result concerning the identification of the aggregation pheromone systems in the desert locust, little is known about the interaction between pheromones and hormones in phase dynamics of the desert locust, *S. gregaria*.

So far, seven juvenile hormones [MF (Methyl farnesoate), JH 0, JH I, 4-methyl-JH I, JH II, JH III and JHB3] have been identified in insects [26, 27]. They differ in the number of methyl groups. Results by Baehr *et al.* [28] showed that the hemolymph of *L. migratoria* contained JH I, II and III. Also, Fuzeau-Braech *et al.* [29] reported JH I and JH II in *L. migratoria*. However, when more reliable physicochemical analyses were employed, both *Schistocerca* and *Locusta* had exclusively JH III, and no JH I or JH II can be detected [30-32], or in other orthopteroid insects [33, 34]. Most insect species contain only JH III. JH 0, JH I, and JH II are generally confined to Lepidoptera. The form JHB3 first isolated

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from *Drosophila* and thereafter, has been found in other dipterans and ticks. Methyl farnesoate (MF), which is JH III lacking the epoxide group, has been isolated from some insects and crustaceans, where it may serve as the active JH. On the other hand, Bowers *et al.* [35] reported that certain chromene derivatives from the plant *Ageratum houstonianum* induce symptoms of JH-deficiency in the large milkweed bug, *Oncopeltus fasciatus* as well as in acridids and other Hemipteran species. These authors termed the active substances precocene I (PI) and precocene II (PII). In many insect species precocene (I or II) can act as a JH antagonists [35-37]. Thus, in the present study P II has been used as anti-juvenile hormone.

The effects of shifting *S. gregaria* adult members of a solitary-reared colony to crowd-rearing conditions and *vice-versa* were monitored by GC analyses of the pheromone emission from the males [38, 39]. Crowding of solitarious insects resulted in the production of the pheromone within days. Interestingly, even pairing of two solitarious insects led to detectable production of the pheromone [38]. Aggregation-maturation titer, therefore, could provide a more sensitive measure in monitoring phase changes in desert locust [38]. On the other hand, in locusts, pheromone communication may be regulated by the neuroendocrine system [9, 11]. Thus, hormones may regulate the biosynthesis and /or release of the pheromone. Nevertheless, no attempt has been made to investigate the effects of exogenous anti-hormones on the pheromone production of the desert locust. Therefore, the objective of the experiments described in the present paper was to examine in more details the effects of exogenous anti-JH (P II) on the phase changes (external coloration and sexual behavior and mating) and pheromone production of the gregarious desert locust, *S. gregaria*.

## 2. MATERIALS AND METHODS

### 2.1. Insects

Locusts, *Schistocerca gregaria* (Forsk.) reared at the International Center of Insect Physiology and Ecology (ICIPE) insectary originating from a stock obtained from the Desert Locust Control Organization for Eastern Africa (DLCO-EA) in Addis Ababa, Ethiopia, were used during the study. The gregarious locusts were reared under crowded conditions in groups of 300-400 individuals in 50 x 50 x 50 cm aluminum cages. Locusts had been reared for more than 70 generation on a diet of grass and wheat bran at 32±2°C, 60 % relative humidity and 12 h photoperiod. Newly emerged adult insects (1-2 days old) and 5<sup>th</sup> instar nymphs (3-5 days old) from the same batch were used in all experiments to eliminate possible variability in physiological status. For each experiment, gregarious males and females in groups of 30 individuals were used under crowded conditions in 20 x 20 x 20 cm aluminum cages in another room under the same conditions as described above.

### 2.2. Treatments

Precocene II (Sigma Chemical Company, U. K.) was dissolved in different concentrations in AnalaR grade acetone for topical applications or in pure olive oil for injections. In topical applications, 5<sup>th</sup> instar nymphs (3-5 days old) or adults (1-2 days old) received a single dose of 50 µg or 150

µg of precocene II (P II) in 5 µl acetone on the dorsal side of the abdomen. Another group of nymphs and adults received three consecutive topical applications, each of 50 µg (a cumulative of 150 µg) of P II per insect. In this case insects were administered daily for three days on the 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> days of the 5<sup>th</sup> instar nymphs or the adults. Control insects received 5 µl acetone in a single dose or triple doses. In another experiment, 5 µl of olive oil containing 50 µg or 150 µg of P II were injected between the second and third sternites on the 4<sup>th</sup> day of the 5<sup>th</sup> instar nymphs and adults. A cumulative dose of 150 µg (3× 50 µg) of P II per insect were injected daily for three days as described above. Control insects received only 5 µl olive oil also in a single dose or triple doses.

### 2.3. Collection and Analyses of Volatiles

The collection and analyses of volatiles were carried out as described [25]. For each treatment, aeration were carried out on groups of three adult males selected randomly and contained in a trapping chamber (10 cm long x 3.5 cm-i.d.) and through a charcoal (120 mg) packed between two glass wool plugs in glass tube (6 cm long x 8 mm i.d.) at 106 ml/min<sup>-1</sup> for 18 h at 32±2°C. Collections were replicated three times. The charcoal was eluted with HPLC grade dichloroethane and preserved at -15°C. Prior to analysis, each sample was concentrated to 100 µl and 250µg of *o*-methyl acetophenone was added as an internal standard. Aliquots (2.5 µl) of the extracts were analyzed by capillary gas chromatography (GC) on a Hewlett-Packard (HP) 5890 series II gas chromatograph equipped with a flame ionization detector (FID) and a HP capillary column (Carbowax 20 M, 50 m x 0.32 mm ID x 0.3 µm) using nitrogen as the carrier gas at flow rate of 0.35 ml/min<sup>-1</sup>. The oven temperature was initially isothermal at 60°C for 10 min, then programmed at 5°C/min<sup>-1</sup> to 180°C, where it was maintained for 5 min and then to 220°C at 10°C /min<sup>-1</sup> to where it was maintained for 15 min. Chromatographic peaks were integrated using a HP 3393 Series II integrator.

### 2.4. Observations on the External Coloration (Body Yellow Color), Male Sexual Behavior and Mating

Observations on the external coloration (body yellow color), male sexual behavior and mating were carried out during the adult stage, four times a day for treated and control insects.

### 2.5. Statistical Analysis

The results were analyzed by ANOVA and Least Significant Difference test (LSD) at P < 0.05 using Statistical Analysis System (SAS) [40].

## 3. RESULTS

### 3.1. The Effects of Topical Application of P II

Topical application of a single dose of 50 µg of P II to young adults (4 days old) was ineffective (P > 0.7) on pheromone production; the onset and quantity of pheromone release were similar to that of the controls. However, topical application of a cumulative dose of 150 µg per insect (3 x 50 µg) on days 3, 4, and 5 after adult emergence, resulted in a

slight effect on the maximum amount of pheromone production ( $P > 0.3$ ). Moreover, there was no significant difference ( $P > 0.7$ ) on the onset of pheromone production between treated and control insects (Fig. 1). In this case, the pheromone production started on day 15 and reached a maximum 20 days after adult emergence (Fig. 1). Generally, topical application of a single dose of 50  $\mu\text{g}$  of P II on day 4 or a cumulative dose of 150  $\mu\text{g}$  per insect ( $3 \times 50 \mu\text{g}$ ) on days 3, 4, and 5 after adult emergence, had no effect on external yellow coloration, sexual behavior and mating in comparison to control insects.

Topical application of a single dose of 50  $\mu\text{g}$  of P II to 5<sup>th</sup> instar nymphs (4 days old) was ineffective ( $P > 0.7$ ) on pheromone production in the subsequent adult males; the onset and quantity of PAN release were similar to that of control insects (Fig. 2). In contrast, a significant effect ( $P < 0.01$ ) was observed in insects treated with a cumulative dose of 150  $\mu\text{g}$  P II ( $3 \times 50 \mu\text{g}$ ) on days 3, 4, and 5 of 5<sup>th</sup> instar nymphs. In this case, pheromone production started on day 20 and reached a maximum 35 days after adult emergence (Fig. 2). Also, treated males had a fading yellow color instead of the bright yellow color characteristic of normal gregarious adult males. Moreover, they started exhibiting sexual behavior and mating between 25-30 days instead of 15-20 days after adult emergence in control insects. Generally, topical application of a single high dose of 150  $\mu\text{g}$  of P II on day 4 to 5<sup>th</sup> instar hoppers or adults had no effect on the onset and quantity of PAN release in comparison to controls.

### 3.2. The Effects of Injection of P II

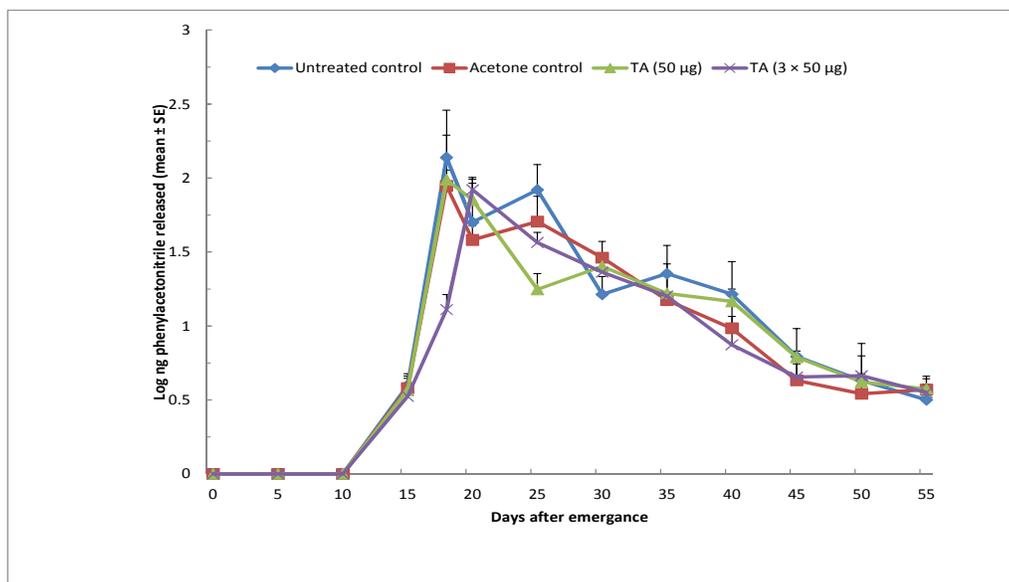
Injection of a single dose of 50 or 150  $\mu\text{g}$  of P II into day 4 adults had no effect on the quantity and onset of pheromone emission (Fig. 3). On the other hand, adults injected with a cumulative dose of 150  $\mu\text{g}$  P II ( $3 \times 50 \mu\text{g}$ ) on days 3, 4, and 5 started pheromone emission on day 18 ( $P < 0.1$ ) and reached a maximum on day 25 ( $P < 0.001$ ) (Fig. 3). In addition, treated males had a fading yellow color instead of the

bright yellow color characteristic of normal gregarious adult males. Moreover, they started exhibiting sexual behavior and mating between 20-25 days instead of 15-20 days after adult emergence in control insects.

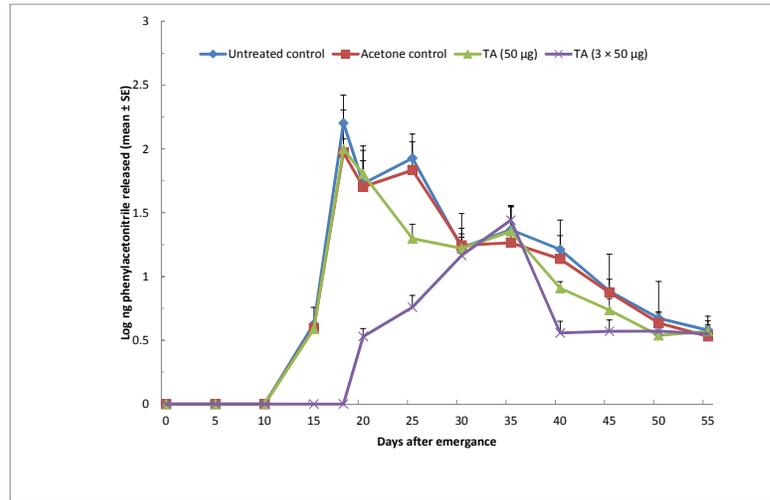
Injection of a single dose of 50  $\mu\text{g}$  of P II into 5<sup>th</sup> instar nymphs (day 4) had no significant effect ( $P > 0.7$ ) on the onset or quantity of pheromone released in the adult stage (Fig. 4). Also, injection of a single dose of 50  $\mu\text{g}$  of P II to 5<sup>th</sup> instar nymphs (4 days old) had no effect on external yellow coloration, sexual behavior and mating in the adult stage in comparison to control insects. On the other hand, high mortality and deformed adults resulted from a cumulative dose of 150  $\mu\text{g}$  P II ( $3 \times 50 \mu\text{g}$ ) or a single high dose 150  $\mu\text{g}$ . Thus, pheromone production by insects treated with these doses is not shown in Fig. (4).

## 4. DISCUSSION

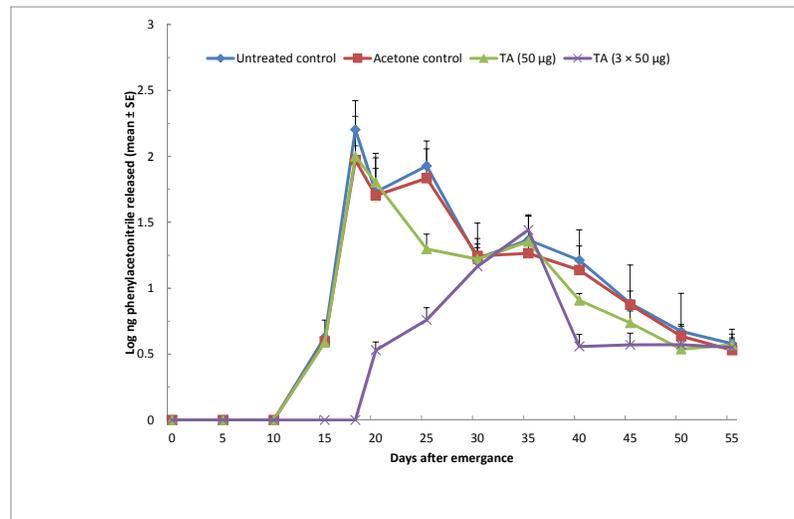
Color differences between gregarious and solitary locusts are probably the best known and most easily recognized visible phase characteristic [41, 42]. In crowded *S. gregaria* newly fledged adults are pinkish. Thereafter, the color changes are sex-dependent. With sexual maturation of crowded males, the color changes to yellow, then to bright yellow. In crowded females, the color changes from beige-brown to yellowish, but never becomes bright yellow. Isolated adults of *S. gregaria* do not show appreciable color changes; both sexes are usually beige-brown. Our results showed that topical application to 5<sup>th</sup> instar nymphs or injection to adults of anti-JH (P II) result in the fading of the yellow color of crowded adult males, *S. gregaria*. The yellowing of crowded adult locusts depends completely on the CA and JH. Allatectomy of young adults or last instar hoppers prevents yellowing, whereas reimplantation of CA or administration of JH reinduces it in *S. gregaria* [20, 21, 43], *L. migratoria* [44, 45] and *Nomadacris sepemfasciata* [46]. Moreover, Fridman-Cohen *et al.* [47] demonstrated that the anti-allatin effect of P II applied to last instar nymphs of



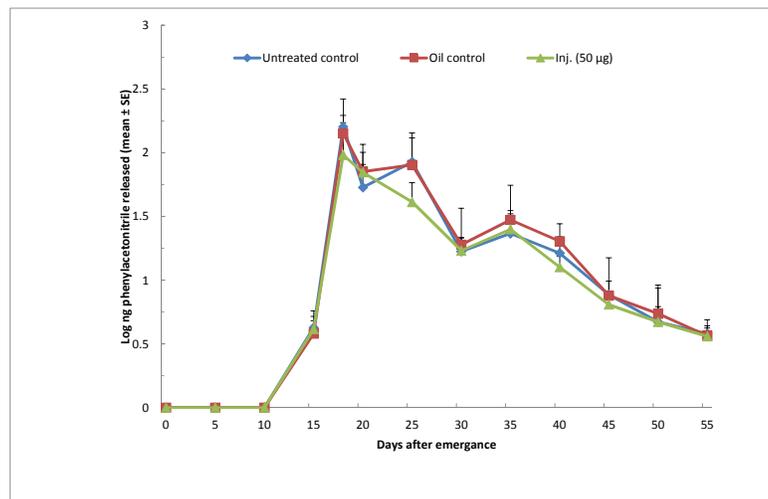
**Fig. (1).** Release of phenylacetone nitrile (mean  $\pm$  SE) at different ages by adult males, when adults were received topical applications (TA) of precocene II (P II).



**Fig. (2).** Release of phenylacetone nitrile (mean ± SE) at different ages by adult males, when 5<sup>th</sup> instar nymphs were received topical applications (TA) of precocene II (P II).



**Fig. (3).** Release of phenylacetone nitrile (mean ± SE) at different ages by adult males, when adults were injected (Inj.) with different concentrations of precocene II (P II).



**Fig. (4).** Release of phenylacetone nitrile (mean ± SE) at different ages by adult males, when 5<sup>th</sup> instar nymphs were injected (Inj.) with different concentrations of precocene II (P II).

*S. gregaria* become overt by sterility and lack of yellow color in the adults. Allatectomy of sexually mature yellow adults, *S. gregaria* results in the fading of the yellow color [48]. Furthermore, implantation of extra CA into isolated adults does not induce yellowing, whereas transfer of isolated adults into newly formed crowds does, even without implantation of extra CA [45].

The results of the present experiments revealed that maturation, sexual behavior and mating are affected by exogenous anti-JH (P II), and coincided with the pheromone production in gregarious adult males, *S. gregaria*. These results confirmed previous finding that the pheromone has a releaser and primer effects [24, 25, 49, 50]. The effect of the CA on mating behavior of adult male locusts and grasshoppers has been investigated by several workers. These glands completely control male sexual behavior in the desert locust, *S. gregaria* [43, 51, 52], in the migratory locust, *L. migratoria* [53] and in the red locust, *Nomadacris sepemfasciata* [46]. However, Norris and Pener [54] reported that the presence of allatectomized males or of allatectomized females inhibit the maturation of young adult males of *S. gregaria*.

Our results showed that anti-JH (P II) plays a role in the regulation of pheromone production in the desert locust, *S. gregaria*. Pheromone production in gregarious adult males, *S. gregaria* was shown to depend on the presence of the CA [20, 43, 54]. Interestingly, Tawfik et al. [32] showed that, the occurrence of maximal CA size (days 7-16) correlates with the onset of emission of the aggregation-maturation pheromone in gregarious adult males, *S. gregaria*. Furthermore, Tawfik and Mohammed [55] showed that at the time of maximal pheromone production the CA cells of gregarious adult male, *S. gregaria* are characterized by thin cytoplasm with only small numbers of mitochondria, smooth endoplasmic reticulum (SER) and ribosomes. Also, the majority of nucleoli were mainly in the form of hollow spheres. Whereas, before and after the maximal pheromone production the CA cells had well developed SER, numerous mitochondria and abundant ribosomes. Also, Odhiambo [56] reported more SER and mitochondria of more complex shapes in CA of adult male, *S. gregaria* developing toward sexual maturity than in those just after adult emergence. In accordance with the previous results, Tawfik et al. [32] found that, the JH titer in gregarious adult males, *S. gregaria* was low on day 10, elevated on day 15 coinciding with the start of the production of the pheromone, and dropped to a relatively low level on day 20 around the time of maximal pheromone production, then rising again by day 25. On the other hand, the effects of juvenile hormone treatment on phase changes and pheromone production in the desert locust, *S. gregaria* were studied [57]. Tawfik et al. [57] administered JH III to 3- to 5-day-old last-instar hoppers and, separately, 3- to 5-day-old young adults of crowded *S. gregaria* males, by three alternative routes, topical application in acetone, injection in olive oil and exposure to vapor ('fumigation'). The authors recorded significant effect of the treatments on the timing and rate of phenylacetonitrile (PAN) release. Furthermore, Ignell et al. [58] investigated the effect of JH on behavioral patterns of adult *S. gregaria* in response to PAN, which is considered to be the most potent adult aggregation pheromone component in this species [58]. They showed that the behavioral response to the major and most

potent adult aggregation pheromone component, PAN, is age- and JH-dependent. In addition, the authors reported that JH influences the responsiveness of olfactory interneurons in the antennal lobe to aggregation pheromone, whereas the responsiveness of antennal receptor neurons is not changed. Old locusts and locusts injected with JH, in contrast to young locusts and locusts deprived of JH through allatectomy, do not display any aggregation behavior, as indicated by long-term behavioral observations.

The results of the present study showed that topical application to 5<sup>th</sup> instar nymphs or injection to adults of anti-JH (P II) inhibits pheromone production in gregarious adult males, *S. gregaria*. It seems likely that P II influences the pheromone production indirectly by inhibiting JH production. Topical treatments with P II inhibited the pheromone production in *Ips paraconfusus* [36]. Precocene II also inhibited or delayed both pheromone production and oocyte growth in a dose-dependent manner in *Blattella germanica* [37]. However, distinct pheromone glands are not known for male desert locust. It has therefore been assumed that specialized epidermal cells produce such volatiles. This assumption was based on histological studies by [59-61] which describe glandular epidermal cells in the epidermis of mature adult male, *S. gregaria*. Thus, another possible mode of action is that P II could act directly at the site of pheromone production.

Generally, the 5<sup>th</sup> instar nymphs exhibited a higher sensitivity to topical application of P II than the adults. In contrast, the adults exhibited a higher sensitivity to injection of P II in comparison to 5<sup>th</sup> instar nymphs. Treated adults exhibit resistance against topical applications of P II in comparison to last instar nymphs. This could reflect better peripheral protecting mechanism(s) against precocene, such as more efficient metabolic detoxification, higher excretion rate and lower penetration rate through the integument. On the other hand, the 5<sup>th</sup> instar nymphs exhibited a higher sensitivity to topical applications of P II. From the point of view of the effects of JH and JH analogs on the last nymphal instar of both *Schistocerca* and *Locusta* may be divided into three distinct intervals [62, 63]. The first one, ranging from ecdysis to the fourth day of the instar, is the period of the action of JH on morphogenesis. The second interval, which encompassed days 4 and 5, represents the sensitive period for the phase changes. In the third interval from day 6 to the next ecdysis, JHs induce no visible effect. Further important endocrinological relationships exist between the occurrence of sensitive period to JH and the titers of ecdysteroids in the body [64]. Moreover, the two phases differ markedly in ecdysteroid titer in the hemolymph during the last two nymphal instars [65].

Several reports have indicated that, in locusts, the gregarious phase is characterized by a lower JH content than in the solitary phase [28, 66-68]. Thus, an experimental elevation of the JH content in gregarious locusts should induce a solitarization. This is in agreement with results by Tawfik et al. [57] that the exogenous JH treatments affect the coloration and timing and rate of PAN release in gregarious adult males of the desert locust. Contrarily, results of the present study showed that JH deficiency caused by anti-JH (P II) affects the pheromone production, coloration, sexual behav-

ior and mating in gregarious adult males, *S. gregaria*. Moreover, Tawfik *et al.* [32] found that the pheromone production coincides with a specific titer of JH in gregarious adult males of the desert locust. Furthermore, as mentioned above the occurrence of maximal CA size (days 7-16) correlates with the onset of emission of the aggregation-maturation pheromone [32]. Also, the ultrastructural study revealed that at the time of maximal pheromone production the CA cells of gregarious adult male, *S. gregaria* contain small number of mitochondria, smooth endoplasmic reticulum (SER) and ribosomes [55]. Whereas, before and after the maximal pheromone production the CA cells had well developed SER, numerous mitochondria and abundant ribosomes. Therefore, phase changes and pheromone production of the desert locust could be regulated by threshold of JH at a critical period.

### CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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