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Dual effect of the antibiotic Cycloheximide on desert locust *Schistocerca gregaria* forskal (Orthoptera: Acrididae)

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Abstract
The desert locust *Schistocerca gregaria* is a dangerous pest devastating agricultural productions and pastures in different countries. The objective of the present study was to assess the dual effect of cycloheximide (RNA and protein synthesis inhibitor), JH-like activity and anti-JH activity, against this pest. Five doses (200, 100, 30, 20 and 10 µg/nymph) were topically applied onto the sternites of newly penultimate (4th) instar nymphs *S. gregaria* in Faculty of Science, Al-Azhar University, Cairo, Egypt. Cycloheximide exhibited a toxic effect on nymphs and adults. LD_{50} was 8.53 µg/nymph. It exerted a potent suppressing action on nymphal growth and developmental rate. As a symptom of the suspended development, some of the treated 4th instar nymphs failed to moult into the next instar but remained as permanent nymphs and died after 4-fold period of the control nymphs. The successfully moulted 5th instar nymphs suffered an impairing action of cycloheximide; since malformed nymphs were produced and died after a few days. Some of the treated nymphs precociously metamorphosed into adultoids, skipping off the 5th instar. These precocious adultoids spent more than one month and eventually died with mating. In addition, cycloheximide induced solitary tendency in the treated 5th instar nymphs, at the lower two doses.

Keywords: Adult, development, gregaria, growth, metamorphosis, morphogenesis, mortality, nymph, solitaria

Introduction
Two phases appeared in the development of the desert locust, *Schistocerca gregaria* (Forskål), solitary and gregarious phases. The gregarious phase is the extremely dangerous for the agricultural crops [1]. The devastation of this insect corresponds to several tons of fresh plant material. Invasions of this locust are the cause of calamity because they can result in up to 100% crop loss [2, 3]. It has been a most serious crop pest in many countries of Africa and Asia [4, 5]. In Africa, *S. gregaria* can devastate the cultures of a whole continent [6-9].
Current locust control operations are mainly based on organophosphorus pesticides [10]. In the outbreak of *S. gregaria* during 2003-2005, 13 million ha were treated with insecticides in different countries [11]. The excessive and indiscriminate uses of conventional insecticides against insect pests usually lead to various problems to human and environment, as well as the development of insect resistance to insecticides [12-16]. Therefore, eco-friendly control agents have received global attention in recent years. These alternative compounds are characterized by lower toxicity to non-target organisms than conventional insecticides and they are effective at low concentrations [17, 18]. Also, they are biodegradable into harmless compounds for avoiding the problems of environmental pollution [19-21].
Juvenile hormone (JH) is necessary for insect development throughout the immature stages [22]. In addition, JHs play crucial roles in several other physiological processes, such as reproduction, diapause, behaviour, polymorphism, migration, metabolism, etc [23-28]. The limited scope of JH analogues (JHAs) in insect pest control demanded the use of alternative chemicals that are environmentally safe. Compounds which can block or reduce the synthesis of authentic JH were considered as better alternatives for impairing hormone-dependent processes of development and reproduction [29, 30].
Anti-JH agents are those compounds inhibiting JH-dependent developmental and reproductive processes in insects. Bede *et al.* [31] demonstrated that the design of JH mimics or anti-JH agents is an effective strategy for insecticide discovery. As reported by many authors [32-34], anti-JH agents are considered as new alternatives of juvenoid-type chemicals to avoid some of

their disadvantages. These chemicals are potentially efficacious for control of the major insect pests where most of the damage is caused by larval stage.

The RNA and protein synthesis inhibitor, cycloheximide (Acti-dione) (3-[(2R)-2-[(1S, 3S, 5S)-3, 5-dimethyl-2-oxocyclohexyl]-2-hydroxyethyl] glutarimide) was originally isolated from *Streptomyces griseus* [35]. As an antibiotic, cycloheximide was earlier applied clinically in the treatment of candidiasis and meningitis. In the agricultural uses, cycloheximide was found as inhibitor of protein synthesis and irreversible inhibitor of multiplication nuclear polyhedrosis virus in the lepidopterous insects [36]. Also, it inhibits the growth, in culture, of many plant pathogenic fungi [37]. Moreover, cycloheximide was found to interfere with the hormonal regulation of developmental processes in insects, such as the migratory locust *Locusta migratoria* [38] and *S. gregaria* [39]. The stimulation of the steroidogenesis in insects is rapidly inhibited by cycloheximide [40]. Cycloheximide inhibited the protein synthesis necessary for the development of eclosion hormone sensitivity in the tobacco horn worm *Manduca sexta* [41]. In insects, also, cycloheximide exhibited an anti-gonadotropic action in insects, such as the tobacco caterpillar *Spodoptera litura* [42] and the mealworm beetle *Tenebrio molitor* [43]. In addition, cycloheximide was recorded as a suppressive agent on the enzyme activities in insects, such as the detoxifying enzyme Glutathione S-transferse in the common cutworm *Spodoptera litura* [44]; phenoloxidase in *Spodoptera exigua* [45] and transhydrogenase in the midgut mitochondria in *M. sexta* 5th instar larvae [46]. The objective of the present study was to assess the dual action of cycloheximide, JH-like activity and anti-JH activity, against *S. gregaria*.

2. Materials and Methods

2.1. Experimental insect

A gregarious stock culture of *Schistocerca gregaria* (Orthoptera: Acrididae) was raised for successive generations in Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, Cairo, Egypt. The present culture was originated by a sample of gregarious nymphs kindly provided by Locust and Grasshopper Division, Plant Protection Research Institute, Giza. The insects were reared and handled under crowded breeding conditions outlined by Hunter-Jones [47] and improved by Ghoneim *et al*. [48]. Newly hatched hoppers were kept in wood cages with wire-gauze sides (40x40x60 cm) and small door. The developing hoppers were transferred into bigger cages (60 x 60 x 70 cm). Each cage was equipped internally with an electric bulb (100 watt) to maintain a continuous photoperiod (12 L: 12 D) as well as an ambient temperature (32±4ºC). The relative humidity varied between 70-80%. For oviposition by adult females, the bottom of cages was covered with sterilized sandy layer (20 cm depth) moistened with water (10-15% humidity). Fresh clean leaves of clover *Trifolium alexandrinum* were provided as a food every day. Care was seriously taken to clean these cages at regular intervals. The feces, dead locusts and food remains were removed daily before introducing fresh food.

2.2. Cycloheximide administration

Cycloheximide was kindly provided by Dr. Heba Hassan, Plant Protection Institute, Egypt. The compound was dissolved in acetone to prepare five doses: 200, 100, 30, 20 and 10 µg/nymph. All doses had been applied for the newly moulted female 4th instar nymphs. Groups of 20 healthy gregarious nymphs were used as replicates. They were topically treated onto the first and second abdominal sternites, using Hamilton microapplicator (NHN 737). A group of 20 healthy gregarious nymphs of the same instar and age were topically treated with 4 µl acetone only and used as controls. All treated and control nymphs were kept individually under the previously mentioned laboratory conditions.

2.3. Criteria of study

Toxicity test

Starting from the day after cycloheximide application, mortalities of the 4th and 5th instar nymphs and mortality of the emerged adult females had been recorded in %. LD_{50} value was calculated for total mortality by Microsoft office Excel, 2007, according to Finny [49].

Growth and development

All treated and control nymphs were weighed individually every day for calculating the nymphal weight gain during the 4th and 5th instars. The mean weight gain ± SD was used as an informative indicator of the nymphal growth. Also, the nymphal duration of each instar was determined in mean days ± SD using the Dempster's equation [50]. The developmental rate was calculated according to the Richard's equation [51]. The suspended development was observed in permanent 4th instar nymphs (%) which died without moulting into last (5th) instar.

Metamorphic and phase transition parameters

All nymphs were observed daily for recording the possible aberrations in metamorphosis, such as precocious adultoids and deranged morphogenesis, such as nymphal malformations (%). All nymphs and adults were observed every day to record the nymphal and/or adult colour change, according to the phase theory of locusts [52].

2.4. Statistical analysis

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction [53] for the test significance of difference between means.

3. Results

3.1. Toxic effect of cycloheximide on *S. gregaria*

After topical application of cycloheximide onto the newly moulted penultimate (4th) instar female nymphs, the toxic effect was recorded by mortality (%) of nymphs and adult females. Data of mortality had been summarized in Table (1). According to these data, mortality of the treated 4th instar nymphs was found almost in a dose-dependent course (05.0, 05.0, 10.0, 60.0 and 70.0% mortality of treated nymphs, at 10, 20, 30, 100 and 200 µg/nymph, respectively, *vs*. 00.0% mortality of control nymphs). Also, the successfully moulted last (5th) instar nymphs were subjected to a lethal action of cycloheximide, but in no certain trend (31.6, 52.6, 66.7, 37.5 and 66.7% mortality of 5th instar nymphs, at 10, 20, 30, 100 and 200 µg/nymph, respectively, *vs*. 00.0% mortality of control nymphs). Cycloheximide exhibited an extended toxic effect on the successfully emerged adult females, since different mortality percentages were recorded, but in no certain trend (15.4, 11.1, 50.0, 20.0 and 50.0% adult mortality, at 10, 20, 30, 100 and 200 µg/nymph, respectively, *vs*. 00.0% mortality of control adults). LD_{50} value was calculated as 8.53 µg/nymph.

3.2. Effect of cycloheximide on growth and developmental of *S. gregaria*

Data of the nymphal body weight gain, duration and developmental rate were assorted in Table (2). Depending on these data, cycloheximide exerted potent suppressing action on the growth of treated 4th instar larvae, since their weight gain was remarkably reduced, in no certain trend (409.9±69.3, 333.1±96.8, 341.9±51.8, 265.1±61.8 and 199.5±54.3 mg, at 10, 20, 30, 100 and 200 µg/nymph, respectively, *vs*. 467.3±66.2 mg of control nymphs). Also, growth of the successfully moulted 5th instar nymphs was considerably inhibited, especially at the higher three doses, since their weight gain values were significantly declined (420.2±22.6, 367.3±64.0 and 328.2±62.1 mg, at 30, 100 and 200 µg/nymph, respectively, *vs*. 469.9±48.2 mg of control nymphs).

In the light of data contained in the same table, cycloheximide exerted a retarding action on the treated 4th instar nymphs, since their durations were conspicuously prolonged, in no certain trend (9.4±0.8, 9.8±0.8, 9.5±1.1, 8.8±0.9 and 8.7±0.8 days, at 10, 20, 30, 100 and 200 µg/nymph, respectively, *vs*. 8.0±0.7 days of control nymphs). As clearly shown in the aforementioned table, the developmental rate was seriously regressed, in no certain trend. Also, the successfully moulted 5th instar nymphs were subjected to a retarding action of cycloheximide, since their durations were significantly prolonged, especially at the higher three doses (11.8±1.5, 11.8±1.3, 11.2±1.5 days, at 30, 100 and 200 µg/nymph, respectively, *vs*. 10.2±1.1 days of control nymphs). In addition, their developmental rate was remarkably regressed.

As obviously shown in Table (3), different percentages of suspended development where the treated 4th instar nymphs failed to moult into the next instar but remained as permanent nymphs. The developmentally interrupted nymphs did not feed and appeared inactive with a deep dark pattern. They survived for 32 days (4-fold of the control congeners) and died without moulting to the next instar. This feature of suspended development appeared in a dose-dependent manner (5, 5, 10, 60 and 70% permanent nymphs, at 10, 20, 30, 100 and 200 µg/nymph, respectively, *vs*. 0% permanent control nymphs).

3.3. Effect of cycloheximide on morphogenesis and metamorphosis of *S. gregaria*

On the basis of data distributed in Table (3), cycloheximide exerted a disruptive action on the morphogenesis and metamorphosis of *S. gregaria*. Two major symptoms could be exiguously observed: deformed 5th instar nymphs and precocious adultoids. Morphogenesis of some of the successfully moulted 5th instar nymphs suffered an impairing action of cycloheximide, since malformed nymphs were produced, in no certain trend (31.6, 52.6, 50.0, 37.5 and 16.7% deformed 5th instar nymphs, at 10, 20, 30, 100 and 200 µg/nymph, respectively, *vs*. 0% deformed control nymphs). These deformed nymphs appeared with thin cuticle, malformed appendages, some attached parts of 4th instar exuvia and a deep dark patch at the site of cycloheximide application. All of these deformed nymphs died after a few days with no capability to metamorphose into adults.

As clearly seen in the previously mentioned table, cycloheximide induced some of the 4th instar nymphs to precociously metamorphose into adultoids, skipping off the 5th nymphal instar. This interesting feature of impaired metamorphosis was only recorded at the lower three doses of the tested compound (10.0, 06.7 and 36.7% precocious adultoids, at 10, 20 and 30 µg/nymph, respectively, *vs*. 0% precocious control locusts). These precocious adultoids appeared diminutive with pink colour of normal gregarious adults. They survived more than one month and eventually perished with no ability to mate.

3.4. Effect of cycloheximide on phase transition in *S. gregaria*

As highlighted by data of Table (3), cycloheximide induced the solitary tendency in the treated 5th instar nymphs after treatment of 4th instar nymphs only with the lower two doses (50 and 30% nymphs with solitary green colour, at 10 and 20 µg/nymph, respectively). This green colour is one of the most important characteristics of solitary *S. gregaria*.

Table 1: Lethal effect (%) of cycloheximide on *S. gregaria* after treatment of the newly moulted penultimate instar larvae.

Dose (µg/nymph)	Nymphal mortalities		Adult mortality	Total Mortality	LD_{50}
	4th instar	5th instar			
200	70.0	66.7	50.0	95.0	8.53
100	60.0	37.5	20.0	80.0	
30	10.0	66.7	50.0	85.0	
20	5.0	52.6	11.1	60.0	
10	5.0	31.6	15.4	45.0	
Control	00.0	00.0	00.0	00.0	

Table 2: Growth and development of *S. gregaria* as affected by topical application of cycloheximide onto the newly moulted penultimate instar nymphs of *S. gregaria*.

Dose (µg/nymph)	4th instar nymphs			5th instar nymphs		
	Weight gain (Mean mg± SD)	Duration (Mean days± SD)	Develop. rate	Weight gain (Mean mg ± SD)	Duration (Mean days± SD)	Develop. rate
200	199.5 ± 54.3 d	8.7 ± 0.8 b	11.5	328.2 ± 62.1 c	11.2 ± 1.5 b	8.9
100	265.1± 61.8 d	8.8 ± 0.9 b	11.4	367.3 ± 64.0 c	11.8 ± 1.3 d	8.5
30	341.9 ± 51.8 d	9.5 ± 1.1 c	10.7	420.2 ± 22.6 c	11.8 ± 1.5 b	8.5
20	333.1 ± 96.8 d	9.8 ± 0.8 d	10.2	423.8 ± 97.1 a	10.6± 1.5 a	8.9
10	409.9 ± 69.3 d	9.4 ± 0.8 d	10.8	441.8 ± 43.8 a	10.2±1.1 a	8.8
Control	467.3 ± 66.2	8.0 ± 0.7	12.5	469.9 ± 48.2	10.2 ± 1.1	10.1

Mean ± SD followed by letter (a): Not significantly different ($P>0.05$), (b): Significantly different ($P<0.05$), (c): Highly significantly different ($P<0.01$), (d): Very highly significantly different ($P<0.001$). Develop: Developmental.

Table 3: Metamorphosis disturbance and phase transition (%) of *S. gregaria* after topical application of cycloheximide onto the newly moulted penultimate instar nymphs

Dose (µg/nymph)	Permanent 4th instar nymphs	Deformed 5th instar nymphs	Precocious adultoids	5th instar nymphs with solitary green colour
200	70.0	16.7	00.0	00
100	60.0	37.5	00.0	00
30	10.0	50.0	36.7	00
20	05.0	52.6	06.7	30
10	05.0	31.6	10.0	50
Control	00.0	00.0	00.0	00

4. Discussion

4.1. Affected survival of *S. gregaria* by cycloheximide

Toxic effects of several anti-juvenile hormone (anti-JH) compounds had been reported against different insect species. For examples, both precocene I (PI) and precocene II (PII) exhibited larvicidal activities against several mosquito species [54, 55]. Also, precocenes exhibited larvicidal effects on the Colorado potato beetle *Leptinotarsa decemlineata* [56]. A toxicological effect of PII was reported by Abdullah [57] against larvae of red palm weevil *Rynchophorus ferrugineus*. Also, PII exhibited larvicidal and pupicidal effects on the grey flesh fly *Parasarcophaga dux* [58]; larvicidal effect on the lepidopterous pest *Pericallia ricini* [59] and larvicidal effect on the Asian tiger mosquito *Aedes albopictus* [60]. After exposure of the newly moulted 2nd or 4th instar nymphs of the grasshopper *Euprepocnemis plorans* to some doses of PII, various mortalities were recorded among the treated nymphs of different instars and the emerged adults [61]. Apart from precocenes, other anti-JH compounds displayed different toxicities against some insects, such as EMD (ethyl (E)-3-methyl-2-dodecenoate) [62] and Fluoromevalonate (tetrahydro-4-fluoromethyl-4-hydroxy-2H-pyran-2-one) [63] against the mulberry silkworm *Bombyx mori*. Results of the present study were, to a great extent, in agreement with the previously reported results, since cycloheximide exhibited a toxic effect on nymphs and adult females of *S. gregaria*. For explication of the nymphicidal effect of cycloheximide, it might 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 [64]. Also, these nymphal deaths might be due to the prevented feeding and continuous starvation of *S. gregaria* nymphs [65]. The adult mortalities can be explained by the retention and distribution of cycloheximide in the locust body as a result of rapid transport *via* the haemolymph to other tissues, and/or by lower detoxification capacity of adults against the tested compound [66].

The reported LD_{50} (or LC_{50}) values of anti-JH compounds are variable in different insects. For examples, LD_{50} of PII against the red cotton stainer *Dysdercus koenigii* had been found as 85.46 and 82.37 mgl^{-1} for 4th and 5th instar nymphs, respectively [67]. After treatment of 4th instar larvae of *A. albopictus* with PI and PII, LC_{50} values were estimated in 41.63 and 43.55 µg/ml, respectively [60]. LC_{50} values of PII and PI against the booklice *Liposcelis bostrychophila* were calculated as 30.4 and 64.0$\mu g/cm^2$, respectively [68]. LC_{50} of PI against the cat flea *Ctenocephalides felis* was 10.97 ppm [69]. LC_{50} values of the anti-JH agent Pitavastatin against the tobacco hornworm *Manduca sexta* and the viviparous cockroach *Diploptera punctata* were estimated in 5.23, and 395.2 µM, respectively [21]. LD_{50} values of PII against *E. plorans* were 0.388 and 17.022 $\mu g/cm^2$ after topical treatment of newly moulted 2nd and 4th instar nymphs, respectively [61]. In the current study, LD_{50} value of cycloheximide against *S. gregaria* was found 8.53 µg/nymph. However, LD_{50} (or LC_{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.

4.2. Growth inhibition in *S. gregaria* by cycloheximide

In current investigation on *S. gregaria*, cycloheximide exerted a potent suppressing action on growth of treated 4th instar larvae and the successfully moulted 5th instar nymphs, since their weight gains were remarkably reduced. These results were, to some extent, in accordance with some of the reported results of inhibited growth of various insects by various anti-JH compounds. Several chromene derivatives inhibited the growth of last instar larvae of *T. molitor* [70]. PI and PII were reported to exhibit inhibitory effects on growth of different mosquitoes [54, 55]. After treatment of 2nd or 4th instar nymphs of *E. plorans* with PII, the nymphal growth had been inhibited [61]. Feeding of *M. sexta* larvae on the anti-JH compounds Fluvastatin, Lovastatin or Pitavastatin-treated diet led to significantly inhibited growth [21]. In the current study, the growth inhibition of *S. gregaria* can be attributed to the blocking action of cycloheximide on release of morphogenic peptides, causing alteration in the ecdysteroid and juvenoid titers [71], deranged tissues and cells undergoing mitosis [72] or an inhibitory action on the protein in haemolymph and fat body [73].

4.3. Retarded development of *S. gregaria* by cycloheximide

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 [74] recorded a prolongation of 5th nymphal instar in the milkweed bug *Oncopeltus fasciatus* after treatment with PI. Treatment of the 4th instar nymphs of *S. gregaria* with PII resulted in prolongation of both 4th and 5th nymphal instars [39]. Treatment of 6th instar larvae of the lawn armyworm *Spodoptera mauritia* with PII resulted in prolongation of duration in last larval instar [75, 76]. The nymphal period of the grasshopper *Aiolopus thalassinus* was prolonged after topical application of PIII onto 5th instar nymphs [66]. 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 [77, 78]. After treatment of 4th instar nymphs of *D. koenigii* with PII, duration of the successfully moulted 5th instar nymphs was prolonged [67]. In addition, prolongation of the larval period in the fall webworm *Hyphantria cunea* was recorded after treatment with FMev [79]. Similar results of prolonged larval duration were reported in *B. mori* by KK-22 (phenylimidazoles) [80, 81].

After treatment of 4th instar larvae of *B. mori* with the synthesized 3-(2-methyl-l-phenyl-l-propenyl) pyridine, the larval period was prolonged [82].

Our results were in corroboration with those previously reported results, since cycloheximide exerted a retarding action on the treated 4th instar nymphs, and the successfully moulted 5th instar nymphs of *S. gregaria*, since durations of both instars were conspicuously prolonged indicating seriously regressed developmental rate. To explicate the prolongation of the nymphal duration and regressed developmental rate in *S. gregaria*, in the present study, after treatment with cycloheximide; this prolongation might be due to the indirect interference of this compound with the neuroendocrine organs responsible for the synthesis and release of tropic hormones, like prothoracicotropic hormone [83]. Also, the recorded prolongation might be attributed to a disturbing action of cycloheximide 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 [32, 62]. In addition, cycloheximide might exhibit a delaying effect on the pupal transformation into adults [64]. In particular, the final step of chitin biosynthesis pathway was inhibited by cycloheximide and the precursor was not converted into chitin leading to a prolongation of developmental duration [84].

In insects, a symptom of suspended development attracts a great attention of some entomologists. This feature is usually expressed in 'permanent nymphs or larvae'. The induction of permanent nymphs or larvae was reported in some insect species as a response to some insect growth regulators (IGRs) or botanicals. Among IGRs, some authors [85-88] 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 and the timing of application onto the 5th instar larvae [89]. 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 [90]. In addition, some botanicals, plant extracts or isolated phytochemicals, had been reported to induce permanent nymphs in various insects, such as *O. fasciatus* (Hemiptera) after injection of the newly moulted last instar nymphs with azadirachtin [91]; *O. fasciatus* and the cotton stainer bug *Dysdercus peruvianus* (Hemiptera) after topical application of *Manilkara subsericea* (Sapotaceae) extracts onto 4th instar nymphs [92]; *S. litura* (Lepidoptera) after treatment of larvae with acetone leaf extract of *Withania somnifera* (Solanaceae) [93]; 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) [94]. Among anti-JH compounds, The dose 20 µg/cm^2 of PII topically applied onto the newly moulted 2nd instar nymphs of *E. plorans* induced some 'permanent nymphs' in 2nd and 4th instars [61]. In addition, El-Gammal *et al*. [95] observed permanent nymphs in *S. gregaria* after exposure of gamma irradiation against the 3rd instar nymphs.

In the present study, topical application of cycloheximide onto the newly moulted 4th instar nymphs of *S. gregaria* suspended the developmental of some treated nymphs which failed to moult into the next instar but remained as permanent nymphs. These nymphs did not feed and appeared inactive with a deep dark pattern. They survived for 4-fold of the control congeners and perished without moulting to the next instar. To explicate the induction of permanent nymphs of *S. gregaria*, cycloheximide 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. The tested compound might disrupt the ecdysteroid metabolism or might alternatively act directly to inhibit the release of ecdysis-triggering hormone [93].

4.4. Disrupted morphogenesis and metamorphosis of *S. gregaria* by cycloheximide

Some anti-JH compounds had been reported to exert adverse actions on the morphogenesis of different holometabolous and hemimetabolous insects, such as some terpenoid imidazole compounds against larvae of *B. mori* [96], PI, PII or PIII against the last instar larvae of *S. ruficornis* [97], PI, PII or ethoxyprecocene against *S. litura* larvae [78], PII against *P. dux* larvae [58], PII against *Musca domestica* maggots [98] and PI against *E. integriceps* larvae [99]. The present results on *S. gregaria* were, to a great extent, in agreement with those reported results, since 5th instar malformed nymphs were produced after topical application of cycloheximide onto 4th instar nymphs. All of these deformed nymphs died after a few days without metamorphosis into adults. The production of deformed nymphs of *S. gregaria*, in the current study, may be explained by a suppressive action of cycloheximide during synthesis and deposition of cuticular protein [38, 100] or blocking the release of morphogenic peptides, causing alteration in both ecdysteroid and juvenoid titers [71].

In the present study, also, cycloheximide induced some of the 4th instar nymphs of *S. gregaria* to precociously metamorphose into adultoids, skipping off the 5th instar. This feature of impaired metamorphosis was observed at only the lower three doses (30, 20 and 10 µg/nymph). This result was, 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 *S. gregaria* to PII (15 µg/cm^2) induced precocious adultoids [85, 101]. Different doses of PI or PII (20-100 µg/insect) induced different degrees of precocious metamorphosis in the Mediterranean splendid grasshopper *Heteracris littoralis* [102]. Exposure of 2nd instar nymphs of *E. plorans* to PII led to a precocious moult to 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 [61]. Among Hemiptera, PII induced precocious metamorphosis in the kissing bugs *Rhodnius prolixus* and *Triatoma dimidiata*[103]. Ayoade *et al*. [104] 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 [56]. In addition, precocious metamorphosis had been induced by precocenes in several insects of Diptera, such as the flesh fly *Neobellieria bullata* [105] and *M. domestica* [98] as well as in Lepidoptera, such as *S. litura* [78] and *P. ricini* [59]. Moreover, other anti-JH compounds induced permanent larvae in various insects, such as FMev against the fall webworm *Hyphantria cunea* [79] and *S. mauritia* [106], ETB [62], KK-42 [107, 108], KK-22 [81, 109] and 3-pyridine derivatives [82] against *B. mori*. Treatment of *N. bullata* larvae with KK-110 and J-2710 resulted in precocious pupation [105].

The production of precocious non-viable adultoids, after treatment of the 4th instar nymphs of *S. gregaria*, in the present study, can be explained by the inhibitory effect of cycloheximide on RNA and protein synthesis, especially the JH-binding protein [110, 111] indicating an anti-JH activity of the tested compound leading to deficiency in JH level. On the molecular basis of JH action, Wilson [112] 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, Erezyilmaz *et al*. [113] 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, the action mechanisms of anti-JH compounds in insects had been deeply discussed by many authors [99, 114-122].

4.5. Induction of solitary tendency in *S. gregaria* by cycloheximide

Prior to the discussion of induction of solitary tendency in *S. gregaria* by cycloheximide, in the present study, it may be important to mention that *S. gregaria* has two phases, solitary and gregarious, which differ considerably in many aspects, including behaviour, physiology and morphology [123, 124]. Several genes or metabolites play crucial roles in the regulation of this phase change. Also, epigenetic mechanisms and non-coding RNAs have been involved in the regulation of phase change in locusts, but the functional roles have not yet been known (for review, see Wang and Kang [125]). On the other hand, the involvement of endocrine factors in the regulation of locust phase transformation has been extensively reviewed by many authors [126-129].

With regard to the change in body color, gregarious locusts display a contrasting pattern of black and orange, with little to no variation in pattern among individuals in the same crowd. Solitary locusts are cryptic and range from green to brown depending on the external environmental factors [130-132]. Several studies have demonstrated that the solitary phase in locusts is characterized by a higher JH level than the gregarious phase [133]. In other words, the higher activity of corpora allata in the solitary phase of *S. gregaria* produce higher titers of JH in haemolymph and a green colouration has been appeared by the cuticle [130, 134]. Injection of cycloheximide into 4th instar nymphs of the migratory locust *Locusta migratoria* led to the inhibition of JH-esterase activity resulting in 5th instar nymphs with a solitary green colour, in response to the high level of JH [38, 135]. As pointed out by Applebaum *et al*. [136], the JH is a key regulator of the induction of green body colour in locusts.

In the present study, cycloheximide induced the solitary tendency in the treated 5th instar nymphs of *S. gregaria* (50 and 30% solitary green colour nymphs), after treatment of 4th instar nymphs with the lower two doses 10 and 20 µg/nymph, respectively. This result was, to a great extent, in agreement with very few reported results of phase transition in *S. gregaria* by some anti-JH compounds [129, 137, 138].

The change of nymph colour in *S. gregaria* to green indicated an increased tendency toward the solitary phase, in the current investigation. This result can be explained by a suppressing action of cycloheximide on the JH esterases (JH-attacking enzymes) and thus JH level was elevated in haemolymph. Such explanation may be substantiated by different studies on the involvement of endocrine factors in the regulation of phase transformation, as previously discussed in this section. From the practical point of view, the elevation of JH level in gregarious locusts should induce a solitarization tendency, and thus the locust invasion can be avoided, since the gregarious phase is responsible for swarming and subsequently the world's most devastating plagues (for reviews, see [127, 139]).

5. Conclusions

Depending on the obtained results, in the present study, the antibiotic cycloheximide exhibited a toxic effect on nymphs and adults of *S. gregaria*, an inhibitory effect on the nymphal growth and development, an impairing effect on morphogenesis and metamorphosis. It induced the nymphs toward the solitary phase which is very important for avoiding the gregarious phase responsible for swarming and invasion of the economic crops. With regard to the dual effect of cycloheximide on *S. gregaria*, it exerted JH-like activity against some of 4th instar nymphs which remained as 'permanent nymphs' beside anti-JH activity against some of 4th instar nymphs inducing to precociously metamorphose into non-viable adultoids. Some safety reports mentioned that the use cycloheximide in agricultural applications is now decreasing due to the recent findings of health risks at the low doses. Therefore, intensive investigation should be conducted in the foreseeable future to assess its hazards to human, domestic animals, beneficial arthropods and environmental systems before recommendation to use cycloheximide in the IPM program against *S. gregaria*,

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7. References

1. Uvarov B. Grasshoppers and Locusts. In: "A Handbook of General Acridology". Centre for Overseas Pest Research, London, UK, 1977, 2.
2. Meinzingen WF. A guide to migrant pest management in Africa. Rome, FAO, 1993, 82-184.
3. FAO (Food Agriculture Organization of the United Nation) La crise du Sahel/la crise alimentaire et nutritionnelle du sahel: l'urgence d'appuyer la résilience des populations vulnérables. www.fao.org/.../sahel/, 2012, 1-5.
4. Steedman A. (ed.) Locust Handbook 3rd edition. Natural Resources Institute, Chatham, UK, 1990.
5. Ceccato P, Cressman K, Giannini A, Trzaska S. The desert locust upsurge in West Africa (2003-2005): Information on the desert locust early warning system and the prospects for seasonal climate forecasting. International Journal of Pest Management. 2007; 53(1):7-13. https://doi.org/10.1080/09670870600968826
6. Lecoq M, Mestre J. Collection Acridologie Opérationnelle N°2. La surveillance des sauteriaux du Sahel. CIRAD/PRIFAS (France), 1988, 32.
7. Sanchez-Zapata JO, Donazar JA, Delgado A, Forero MG, Ceballos O, Hiraldo F. Desert locust outbreaks in the Sahel: resource competition, predation and ecological effects of pest control. Journal of Applied Ecology. 2007; 44(2):323-329. doi: 10.1111/j.1365-2664.2007.01279.x
8. Ammar M, Ben Hamouda A, Kallel S, Moumene K, Ben Hamouda MH. Phase characteristics of the desert locust *Schistocerca gregaria* swarming populations during the

2004 outbreak in Tunisia and that of 2005 in Algeria. Tunisian Journal of Plant Protection. 2009; 4(2):145-156.
9. Latchininsky AV. Locusts and remote sensing: a review. Journal of Applied Remote Sensing, 2013, 7, Article ID: 075099. http://dx.doi.org/10.1117/1.JRS.7.075099
10. Lecoq M. Recent progress in desert and migratory locust management in Africa. Are preventive actions possible? Journal of Orthoptera Research. 2001; 10(2):277-291. https://www.jstor.org/stable/3503745
11. Belaynech YT. Acridid pest management in the developing world: a challenge to the rural population, a dilemma to the international community. Journal of Orthoptera Research. 2005; 14(2):187-195. https://doi.org/10.1665/1082-6467 (2005) 14[187: APMITD] 2.0. CO;2
12. Miles M, Lysandrou M. Evidence for negative cross resistance to insecticides in field collected *Spodoptera littoralis* (Boisd.) from Lebanon inlaboratory bioassays. Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent. 2002; 67(3):665-669.
13. Aydin MH, Gurkan MO. The efficacy of spinosad on different strains of *S. littoralis* (Boisduval) (Lepidoptera: Noctuidae). Turkish Journal of Biology. 2006; 30:5-9.
14. Davies TGE, Field LM, Usherwood PNR, Williamson MS. DDT, pyrethrins and insect sodium channels. IUBMB Life. 2007; 59:151-162.
15. Mosallanejad H, Smagghe G. Biochemical mechanisms of methoxyfenozide resistance in the cotton leafworm *Spodoptera littoralis*. Pest Management Science. 2009; 65:732-736. doi: 10.1002/ps.1753
16. Garriga M, Caballero J. Insights into the structure of urea-like compounds as inhibitors of the juvenile hormone epoxide hydrolase (JHEH) of the tobacco hornworm *Manduca sexta*: Analysis of the binding modes and structure-activity relationships of the inhibitors by docking and CoMFA calculations. Chemosphere. 2011; 82:1604-1613. https://doi.org/10.1016/j.chemosphere.2010.11.048
17. Attathom T. Biotechnology for insect pest control. Proceedings of the Satellite Forum: "Sustainable Agricultural System in Asia". Nagoya, Japan, 2002, 73-84.
18. Gäde G, Goldsworthy GJ. Insect peptide hormones: a selective review of their physiology and potential application for pest control. Pest Management Science. 2003; 59(10):1063-1075. DOI: 10.1002/ps.755
19. Tiryaki D, Temur C. The fate of pesticide in the environment. Journal of Biological and Environmental Sciences. 2010; 4(10):29-32.
20. Walkowiak K, Spochacz M, Rosinski G. Peptidomimetics- A new class of bioinsecticides. Postepy Biologii Komorki. 2015; 42(2):235-254.
21. Li YM, Kai ZP, Huang J, Tobe SS. Lepidopteran HMG-CoA reductase is a potential selective target for pest control. Peer J. 2017; 5:e2881. doi: 10.7717/peerj.2881
22. Staal GB. Anti juvenile hormone agents. Annual Review of Entomology. 1986; 31:391-429.
23. Gilbert LI, Granger NA, Roe RM. The juvenile hormones: historical facts and speculations on future research directions. Insect Biochemistry and Molecular Biology. 2000; 30:617-644. doi:10.1016/S0965-1748 (00)00034-5
24. Mitsuoka T, Takita M, Kanke E, Kawasaki H. Ecdysteroid titer, responsiveness of prothoracic gland to prothoracicotropic hormone (PTTH), and PTTH release of the recessive trimolter strain of *Bombyx mori* in extra-ecdysed larvae by JHA and 20E application. Zoological Science, Japan. 2001; 18(2):235-240.
25. Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS. A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. Science. 2001; 292:107-110. Doi: 10.1126/science. 1057987
26. Tatar M, Priest N, Chien S. Negligible senescence during reproductive diapause in *D. melanogaster*. American Naturalist. 2001b; 158:248-258. doi: 10.1086/321320
27. Truman JW, Riddiford LM. The morphostatic actions of juvenile hormone. Insect Biochemistry and Molecular Biology. 2007; 37:761-770. https://doi.org/10.1016/j.ibmb.2007.05.011
28. Amsalem E, Malka O, Grozinger C, Hefetz A. Exploring the role of juvenile hormone and vitellogenin in reproduction and social behavior in bumble bees. BMC Evolutionary Biology. 2014; 14:45. https://doi.org/10.1186/1471-2148
29. Riddiford LM. Juvenile hormone action: A 2007 perspective. Journal of Insect Physiology. 2008; 54:895-901. https://doi.org/10.1016/j.jinsphys.2008.01.014
30. Denlinger DL, Yocum GD, Rinehart JP. Hormonal control of diapause. In: "Insect Endocrinology" (Lawrence, I.G., Ed.). Academic Press, San Diego, CA, USA, 2012, 430-463.
31. Bede JC, Teal PE, Goodman WG, Tobe SS. Biosynthetic pathway of insect juvenile hormone III in cell suspension cultures of the sedge *Cyperus iria*. Plant Physiology. 2001; 127(2):584-593. doi: 10.1104/pp.010264.
32. Bowers WS. Toxicology of the precocenes. In: "Insecticide Mode of Action" (Coats, J.R. Ed.) New York, Academic Press, USA, 1982, 403-427,
33. El-Ibrashy MT. Juvenile hormone mimics in retrospect and antagonists in prospect. Zeitschrift für Angewandte Entomologie. 1982; 94:217-236.
34. Staal GB. Insect control with growth regulation interfering with the endocrine system. Entomologia Experimentalis et Applicata. 1982; 31:15-23.
35. Baliga BS, Pronczuk AW, Munro HN. Mechanisms of cycloheximide inhibition of protein synthesis in a cell-free system prepared from rat liver. Journal of Biological Chemistry. 1969; 244:4480-4489.
36. Kelly DC, Lescott T. The Effects of inhibitors of nucleic acid and protein synthesis on the replication of *Spodoptera frugiperda* nuclear polyhedrosis virus in cultured *Spodoptera frugiperda* cells. Intervirology. 1976; 7:360-365.
37. Mac Bean C. The Pesticide Manual. 16th ed., British Crop Production Council. 2012; 557.
38. Phillips DR, Loughton BG. The effect of inhibition of RNA and protein synthesis on the development of larvae of *Locusta migratoria*. Insect Biochemistry. 1979; 9(2):241-245.
39. Eid MA, Salem MS, El-Ibrashy MT, Abdel-Hamid M. Effects of precocene II, Cycloheximide and C_{16}-JH on the growth rate and adult morphometrics of *Schistocerca gregaria* Forsk. Bulletin of Entomological Society of Egypt (Economic Series). 1982; 13:95-105.
40. Keightley DA, Lou KJ, Smith WA. Involvement of translation and transcription in insect steroidogenesis.

Molecular and Cellular Endocrinology. 1990; 74:229-237.
41. Morton DB, Truman JW. Effect of cycloheximide on eclosion hormone sensitivity and the developmental appearance of the eclosion hormone and cGMP regulated phosphoproteins in the CNS of the tobacco hornworm, *Manduca sexta*. Journal of Receptor and Signal Transduction Research. 2008; 15:773-86. https://doi.org/10.3109/10799899509079906
42. Sridevi R, Ray A. Protein synthesis in the male accessory reproductive glands of *Spodoptera litura* (Noctuidae: Lepidoptera). International Journal of Tropical Insect Science. 1988; 9(5):635-637. https://doi.org/10.1017/S1742758400005130
43. Soltani-Mazouni N, Soltani N. Protein synthesis in the fat body of *Tenebrio molitor* (L.) during oocyte maturation: Effect of diflubenzuron, cycloheximide and starvation. Journal of Stored Products Research. 1995; 31(2):117-122. https://doi.org/10.1016/0022-474X(94)00046-V
44. Wu M Ch, Lu KH. Juvenile hormone induction of glutathione S-transferase activity in the larval fat body of the common cutworm *Spodoptera litura* (Lepidoptera: Noctuidae). Archives of Insect Biochemistry and Physiology. 2008; 68(4):232-240. https://doi.org/10.1002/arch.20257
45. Shrestha S, Kim Y. Factors Affecting the Activation of Hemolymph Prophenoloxidase of *Spodoptera exigua* (Lepidoptera: Noctuidae). Journal of Asia-Pacific Entomology. 2007; 10(2):131-135.
46. Vandock KP, Drummond CA, Smith SL, Fioravanti CF. Midgut and fat body mitochondrial transhydrogenase activities during larval-pupal development of the tobacco hornworm, *Manduca sexta*. Journal of Insect Physiology. 2010; 56:774-779. doi: 10.1016/j.jinsphys.2010.01.008
47. Hunter-Jones P. Rearing and breeding locusts in the laboratory. Bull. Anti-locust Res. Centre, London, 1961, 12.
48. Ghoneim KS, Tanani MA, Basiouny AL. Influenced survival and development of the desert locust *Schistocerca gregaria* (Acrididae) by the wild plant *Fagonia bruguieri* (Zygophyllaceae). Egyptian Academic Journal of Biological Sciences. 2009; 2(2):147-164.
49. Finney DJ. Probit analysis. 3rd ed. Cambridge, England: Cambridge University Press, 1971, 318.
50. Dempster C. The population dynamic of moraccan locust *Dociostarus marcocanus* in Cyprus. Anti Locust Bull, 1957, 27.
51. Richard AG. Cumulative effects of optimum and suboptimum temperatures on insect development. In: "Influence of Temperature on Biological Systems" (Johnson, FH. Ed.). Ronald Press Comp., New York, USA, 1957, 145-162.
52. Uvarov BP. Grasshoppers and locusts. Commonwealth Institute of Entomology, London, UK. 1928, 352.
53. Moroney MJ. Facts from figures. (3rd Ed.). Penguin Books Ltd., Harmondsworth, Middlesex, 1956, 228.
54. Saxena RC, Sharma M, Kumar ML, Bansal SK, Shrivastava D. Developmental effects of 6, 7-dimethoxy-2,2-dimethyl chromene on the preimaginal stages of *Anopheles stephensi*. Proceedings of Academy of Environmental Biology. 1994; 3:181-184.
55. Yasyukevich VV, Zvantsov AB. Effects of antihormones on the susceptibility of *Anopheles sacharovi* mosquitoes to the malaria causative organism *Plasmodium gallinaceum*. Meditsinskaya Parazitologiya i Parazitarnye Bolezni. 1999; 68:46-48.
56. Farazmand H, Chaika SYu. Effects of Precocene-I and II on the development of Colorado potato beetle, *Leptinotarsa decemlineata* (Col.: Chrysomelidae). Applied Entomology and Phytopathogy. 2008; 76(1):14.
57. Abdullah MAR. Toxicological and histopathological studies of *Boxus chinensis* oil and precocenes II on larvae of the red palm weevil *Rynchophorus ferrugineus* (Oliver) (Coleoptera: Curculionidae). Egyptian Academic Journal of Biological Sciences. 2009; 2(2):45-54.
58. Nassar MM, Hafez ST, Farrag AM, Abahussain MO. Efficacy of BaySir-8514 and precocene II against the grey flesh fly *Parasarcophaga dux* (Thomson) (Diptera: Sarcophagidae). Journal of the Egyptian Society of Parasitology. 1999; 29(3):697-707.
59. Khan IA, Kumar K. Precocene II acts as insect growth regulator in polyphagous pest, *Pericallia ricini* F. (Lepidoptera: Arctiidae). Proceedings of Natural Academy of Science, India. 2000; 70(B):279-285.
60. Liu XC, Liu ZL. Evaluation of larvicidal activity of the essential oil of *Ageratum conyzoides* L. aerial parts and its major constituents against *Aedes albopictus*. Journal of Entomology and Zoology Studies. 2014; 2(4):345-350.
61. Ghoneim K, Basiouny A. Insecticidal and antijuvenile hormone activities of Precocene II against the grasshopper *Euprepocnemis plorans plorans* (Charp.) (Orthoptera: Acrididae). International Journal of Trend in Scientific Research and Development. 2017; 1(6):510-524.
62. Kuwano E, Tanaka Y, Kikuchi M, Eto M. Effects of anti-juvenile hormones and related compounds on development in the larvae of *Bombyx mori*. Journal of Faculty of Agriculture, Kyushu University, Japan. 1988; 33(1, 2):17-28.
63. Shuto A, Kuwano E, Eto M. Synthesis of two optical isomers of the insect anti-juvenile hormone agent fluoromevalonolactone (FMev) and their biological activities. Agricultural and Biological Chemistry. 1988; 52(4):915-919. DOI: 10.1080/00021369.1988.10868759
64. Linton YM, Nisbet AJ, Mordue (Luntz) AJ. The effect of azadirachtin on the testes of the desert locust *Schistocerca gregaria* (Forskal). Journal of Insect Physiology. 1997; 43:1077-1084. https://doi.org/10.1016/S0022-1910(97)00060-7
65. Ghoneim KS, Mohamed HA, Bream AS. Efficacy of the neem seed extract, Neemazal, on growth and development of the Egyptian cotton leafworrn, *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae). Journal of Egyptian German Society of Zoology. 2000; 33:161-179.
66. Osman EE, Rarwash I, El- Samadisi MM. Effect of the anti-moulting agent "Dimilin" on the blood picture and cuticle formation in *Spodopterea littoralis* (Boisd.) larvae. Bulletin of Entomological Society of Egypt (Economic Series). 1984; 14:3-46.
67. Banerjee S, Kalena GP, Banerji A, Singh AP. New synthetic precocenoids as potential insect control agents. Journal of Environmental Biology. 2008; 29(6):951-957.
68. Lu XN, Liu XC, Liu QZ, Liu ZL. Isolation of insecticidal constituents from the essential oil of *Ageratum houstonianum* Mill. against *Liposcelis bostrychophila*. J Chem., 2014. Article ID 645687,6 pp. http://dx.doi.org/10.1155/2014/645687

69. Rust MK, Hemsarth WLH. Intrinsic activity of IGRs against larval cat fleas. Journal of Medical Entomology. 2017; 54(2):418-421. DOI: https://doi.org/10.1093/jme/tjw201
70. Roberto CF, Sosa ME, Favier LS, Penna F, Guerreiro E, Giordano OS *et al*. Growth inhibitory activities of benzofuron and chromene derivatives towards *Tenebrio molitor*. Journal of Natural Products. 1998; 61:1209-1211.
71. Barnby MA, Klocke JA. Effects of azadirachtin on levels of ecdysteroids and prothoracicotropic hormone-like activity in *Heliothis virescens* (Fabr.) larvae. Journal of Insect Physiology. 1990; 36:125-131. https://doi.org/10.1016/0022-1910(90)90183-G
72. Nasiruddin M, Mordue (Luntz) AJ. The protection of barley seedlings from attack by *Schistocerca gregaria* using azadirachtin and related analogues. Entomologia Experimentalis et Applicata. 1994; 70:247-252 .
73. Lange AB, Phillips DR, Loughton BG. The effects of precocene II on early adult development in male *Locusta*. Journal of Insect Physiology. 1983; 29(1):73-81. DOI: 10.1016/0022-1910(83)90108-7
74. Bowers WS, Aldrich JR. *In vivo* inactivation of denervated corpora allata by precocene II in the bug *Oncopeltus fasciatus*. Experientia. 1980; 36:362-364.
75. Mathai S, Nair VSK. Effects of precocene II on last instar larvae of *Spodoptera mauritia* (Lepidoptera: Noctuidae). Current Science. 1983; 52:376-377.
76. Mathai S, Nair VSK. Treatment of larvae with repeated doses of precocene II induces precocious metamorphic changes in *Spodoptera mauritia* (Lepidoptera: Noctuidae) Archives of Insect Biochemistry and Physiology. 1984; 1:199-203. https://doi.org/10.1002/arch.940010210
77. Srivastava S, Kumar K. Precocene I and II induced metamorphosis in a noctuid moth, *Spodoptera litura* Fabr. Proceedings of Natural Academy of Science, India. 1997; 67(B):213-226.
78. Srivastava S, Kumar K. Juvenilizing effects of ethoxyprecocene in a lepidopteran insect. Indian Journal of Experimental Biology. 1999; 37(4):379-383.
79. Farag AI, Varjas L. Precocious metamorphosis and moulting deficiencies induced by an anti-JH compound FMev in the fall webworm, *Hyphantria cunea*. Entomologia Experimentalis et Applicata. 1983; 34:65-70.
80. Kuwano E, Eto M. Terpenoid imidazoles: New anti-juvenile hormones and insecticidal compounds. Proceedings of 10th International Congress of Plant Protection, Brighton, UK. 1983; 1:278.
81. Asano S, Kuwano E, Eto M. Anti-juvenile hormone activity of 1-citronellyl-5-phenylimidazole in the 3rd instar silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). Applied Entomology and Zoology. 1984; 19(2):212-220.
82. Yoshida T, Shiotsuki T, Kuwano E. Synthesis and precocious metamorphosis-inducing activity of 3-(1-alkenyl) pyridines. Journal of Pesticide Science. 2000; 25:253-258. https://doi.org/10.1584/jpestics.25.253
83. Subrahmanyam B, Mueller T, Rembold H. Inhibition of turnover of neurosecretion by azadirachtin in *Locusta migratoria*. Journal of Insect Physiology. 1989; 35:493-497. https://doi.org/10.1016/0022-1910(89)90056-5
84. Djeghader NEH, Aïssaoui L, Amira K, Boudjelida H. Impact of a chitin synthesis inhibitor, Novaluron, on the development and the reproductive performance of mosquito *Culex pipiens*. World Applied Sciences Journal. 2014; 29(7):954-960.
85. Salem MS, El-Ibrashy MT, Abdel-Hamid M. Disruption and abnormalities induced by precocene II, Cycloheximide and/or C 16-JH in the desert locust, *Schistocerca gregaria* Forsk. Bulletin of Entomological Society of Egypt. 1985a; (13):127-136.
86. El-Gammal AM, Taha MA. The morphogenetic effects of Diflubenzuron on the desert locust *Schistocerca gregaria* (Forskal). Journal of Faculty of Education, Ain Shams University, Egypt. 1984; 11:275-286.
87. Ghoneim KS. Anti-JH effects of Cycloheximide on *Schistocerca gregaria* (Orthoptera: Acrididae). Journal of Faculty of Education, Ain Shams University, Egypt. 1988; 13:93-103.
88. Abou El-Ela RG. Morphometric and morphogenetic aberrations induced by the IGR Chlorfluazuron (IKI) and two formulations of Triflumuron in *Schistocerca gregaria* Forsk. Bulletin of Entomological Society of Egypt. 1993; 20:217-227.
89. Gadenne C, Grenier S, Mauchamp B, Plantevin G. Effects of a juvenile hormone mimetic, fenoxycarb, on postembryonic development of the European corn borer, *Ostrinia nubilalis* Hbn. Experientia. 1990; 46:744-747.
90. Ghoneim KS, Ismail IE. Survival, developmental and morphogenic deficiencies of *Parasarcophaga argyrostoma* (Diptera: Sarcophagidae) induced by the chitin biosynthesis inhibitor, Chlorefluazuron (IKI-7899). Journal of Egyptian Society of Parasitology. 1995; 25(2):561-581.
91. Dorn A, Rademacher JM, Sehn E. Effects of azadirachtin on the moulting cycle, endocrine system and ovaries in last instar larvae of the milkweed bug *Oncopeltus fasciatus*. Journal of Insect Physiology. 1986; 32:231-238. https://doi.org/10.1016/0022-1910(86)90063-6
92. Fernandes CP, Xavier A, Pacheco JPF, Santos MG, Mexas R, Ratcliffe NA, *et al*. Laboratory evaluation of the effects of *Manilkara subsericea* (Mart.) Dubard extracts and triterpenes on the development of *Dysdercus peruvianus* and *Oncopeltus fasciatus*. Pest Management Science. 2013; 69:292-301. DOI: 10.1002/ps.3388
93. Gaur R, Kumar K. Insect growth-regulating effects of *Withania somnifera* in a polyphagous pest, *Spodoptera litura*. Phytoparasitica. 2010; 38(3):237-241.
94. Lingampally V, Solanki VR, Kaur A, Raja SS. Andrographolide- an effective insect growth regulator of plant origin against *Tribolium confusum* (Duval). International Journal of Current Research. 2013; 5(1):22-26.
95. El-Gammal AM, Abdelsalam KA, Mourad AK. The neurosecretory activity in the permanent nymphs of *Schistocerca gregaria* (Forskal) as a result of gamma irradiation of the third nymphal instar. Isotope and Radiation Research. 1986; 18(2):147-153.
96. Kuwano E, Takeya T, Eto M. Terpenoid imidazoles: new antijuvenile hormones. Agricultural and Biological Chemistry. 1983; 47:921-923. https://doi.org/10.1080/00021369.1983.10865749
97. Srivastava S, Kumar K. Effect of precocenes on metamorphosis of flesh fly, *Sarcophaga ruficornis* F. Indian Journal of Experimental Biology. 1996; 34:547-553.
98. Gaur R, Kumar K. Effect of precocene on morphogenesis

in housefly, *Musca domestica* Linn. Entomon. 2009; 34:107-110.
99. Amiri A, Bandani AR, Ravan S. Effect of an anti-juvenile hormone agent (Precocene I) on sunn pest, *Eurygaster integriceps* (Hemiptera: Scutelleridae) development and reproduction. African Journal of Biotechnology. 2010; 9(36):5859-5868.
100. Retnakaran A, Granett J, Ennis T. Insect growth regulators. In: "Comprehensive Insect Physiology, Biochemistry and Pharmacology" (Kerkut, G.A., Gilbert, L.I., Eds.). Pergamon, Oxford, UK. 1985; 12:529-601.
101. Salem MS, Eid MA, El-Ibrashy MT, Abdel-Hamid M. Effect of Prococene II and Cycloheximide on flight and coxal muscles respiratory metabolism in the desert locust, *Schistocerca gregaria* Forsk. Bulletin of the Entomological Society of Egypt. 1985b; 13:117-125.
102. Alrubeai HF. The effects of precocenes in grasshopper *Heteracris littoralis* (Orthoptera: Acrididae). International Journal of Tropical Insect Science. 1986; 7(4): 529-531. https://doi.org/10.1017/S1742758400009784
103. Tarrant C, Cupp EW, Bowers WS. The effects of precocene II on reproduction and development of triatomine bugs (Reduviidae: Triatominae). American Journal of Tropical Medicine and Hygiene. 1982; 31(2):416-420. DOI: 10.4269/ajtmh.1982.31.416
104. Ayoade O, Morooka S, Tojo S. Metamorphosis and wing formation in the brown planthopper, *Nilaparvata lugens*, after topical application of Precocene II. Archives of Insect Biochemistry and Physiology. 1996; 32:485-491. https://doi.org/10.1002/(SICI)1520-6327(1996)32:3/4<485::AID-ARCH20>3.0.CO;2-7
105. Darvas B, Kuwano E, Eto M, Tag al-din MH, Timar T. Effects of some anti-juvenile hormone agents (Precocenes-2, J-2710, KK-110) on post embryonic development of *Neobellieria bullata*. Agricultural and Biological Chemistry. 1990; 54(11):3045-3047. https://doi.org/10.1080/00021369.1990.10870450
106. Balamani E, Nair VSK. Effects of anti-juvenile hormone agents and a juvenile hormone analog on neck-ligated post-feeding last instar larvae of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae). International Journal of Tropical Insect Science. 1989; 10(5):583-589. https://doi.org/10.1017/S1742758400021706
107. Kuwano E, Takeya R, Eto M. Synthesis and anti-juvenile hormone activity of 1-substituted-5-[(*E*)-2,6-dimethyl-1,5-heptadienyl] imidazoles. Agricultural and Biological Chemistry. 1985; 49:483-486. https://doi.org/10.1080/00021369.1985.10866750
108. Akai H, Mauchamp B. Suppressive effects of an imidazole derivative, KK-42 on JH levels in hemolymph of *Bombyx* larvae. Journal of Sericultural Science, Japan. 1989; 58:73-74.
109. Asano S, Kuwano E, Eto M. Anti-juvenile hormone activity of imidazole compound (KK-22) and its diminution by the methoprene in the 4th instar silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). Applied Entomology and Zoology. 1986; 21(1):63-69.
110. Hammock B, Nowock J, Goodman W, Stamoudis V, Gilbert LL. The influence of haemolymph-binding protein on juvenile hormone stability and distribution in *Manduca sexta* fat body and imaginal disks *in vitro*. Molecular Cell Endocrinology. 1975; 3:167-184. https://doi.org/10.1016/0303-7207(75)90043-X
111. Sandburge LL, Kramer J, Keydy FJ, Law JH, Oberlander H. Role of juvenile hormone esterases and carrier protein in insect development. Nature, London, UK. 1975; 253:266-267.
112. Wilson TG. The molecular site of action of juvenile hormone and juvenile hormone insecticides during metamorphosis: how these compounds kill insects. Journal of Insect Physiology. 2004; 50(2, 3):111-121. https://doi.org/10.1016/j.jinsphys.2003.12.004
113. Erezyilmaz DF, Riddiford LM, Truman JW. The pupal specifier broad directs progressive morphogenesis in a direct-developing insect. Proceedings of the National Academy of Sciences of USA (PNAS). 2006; 103(18):6925-6930.
114. Staal GB, Henrick CA, Bergot BJ, Cerf DC, Edwards JP, Kramer SJ. Relationship and interactions between JH and anti-JH analogues in Lepidoptera. Int. Conf. Regulation of Insect Development and Behaviour (Karpaz, P.; Sehnal, F.; Zabza, A.; Menn, J.J. and Cymborowski, B., Eds). Worclaw: Technical University Press, 1981, 323-340
115. Hamnett AF, Pratt GE. The absolute configuration of precocene I dihydrodiols produced by metabolism of precocece I by corpora allata of *Locusta migratoria in vitro*. Life Science. 1983; 32:2147-2153. https://doi.org/10.1016/0024-3205(83)90395-8
116. Brooks GT, McCaffery AR. The precocene antijuvenile hormones (Allatotoxins): a case history in insect toxicology. In: "Chromatography and isolation of insect hormones and pheromones" (McCaffery, A.R. and Wilson, I.D., Eds). Plenum Press: New York, USA, 1990, 33-43.
117. Unnithan GC, Andersen JF, Hisane T, Kuwano E, Feyereisen R. Inhibition of juvenile hormone biesynthesis and methyl farnesoate epoxidase activity by 1,5-disubstituted imidazoles in the cockroach *Diploptera punctata*. Pesticide Science. 1995; 43:13-19.
118. Miao Y, Jiang L, Bharathi D. Effects of Jinlu, an anti-juvenile hormone on the growth, ultra structure of the corpora allata and prothoracic gland of silkworm, *Bombyx mori* L. J. Zhejiang University (Science), 2001; 2(3):294-297.
119. Kumar K, Khan IA. Effect of precocene on development of ovarian follicles in flesh fly, *Sarcophaga ruficornis* F. Indian Journal of Experimental Biology. 2004; 42(1):74-80.
120. Chen YR, Chen SC, Chang HW, Sun G, Kou R. Effect of exogenous juvenile hormone III and precocene II on agonistic behavior and the corpora allata *in vitro* activity in the male lobster cockroach *Nauphoeta cinerea* (Dictyoptera: Blaberidae, Oxyhaloinae). Zoological Studies. 2005; 44:409-416.
121. Chen Z, Madden RD, Dillwith JW. Effect of precocene II on fatty acid metabolism in the pea aphid, *Acyrthosiphon pisum*, under cold stress. Journal of Insect Physiology. 2005; 51(4):411-416. https://doi.org/10.1016/j.jinsphys.2005.02.006
122. Minakuchi C, Riddiford LM. Insect juvenile hormone action as a potential target of pest management. Journal of Pesticide Science. 2006; 31(2):77-84.
123. Tawfik AI, Tanaka S, de Loof A, Schoofs L, Baggerman G, Waelkens E *et al*. Identification of the gregarization-associated dark pigmentotropin in locusts through an albino mutant. Proceeding of Natural Academy of

Science, USA. 1999; 96:7083-7087.
124. Gordon SD, Rogers S, Windmill J. Hearing differences of gregarious and solitary locusts (*Schistocerca gregaria*), an example of epigenetic effects. Front. Behav. Neurosci. Conference Abstract: Tenth International Congress of Neuroethology, 2012. doi: 10.3389/conf.fnbeh.2012.27.00122
125. Wang XH, Kang L. Molecular mechanisms of phase change in locusts. Annual Review of Entomology. 2014; 59:225-244. https://doi.org/10.1146/annurev-ento-011613-162019
126. Tawfik AI, Sehnal F. A role for ecdysteroids in the phase polymorphism of the desert locust. Physiological Entomology. 2003; 28:19-24. https://doi.org/10.1046/j.1365-3032.2003.00316.x
127. Pener MP, Simpson SJ. Locust phase polyphenism: an update. Advances in Insect Physiology. 2009; 36:1-286. https://doi.org/10.1016/S0065-2806(08)36001-9
128. Tawfik AI. Hormonal control of the phase polyphenism of the desert locust: a review of current understanding. The Open Entomology Journal. 2012; 6:22-41. DOI: 10.2174/1874407901206010022
129. Tawfik AI, Osir EO, Hassanali A. Effects of anti-juvenile hormone (Precocene II) treatment on phase changes and pheromone production in the desert locust, *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae). The Open Entomology Journal. 2014; 8:10-16.
130. Uvarov BP. Grasshoppers and locusts. In: "A Handbook of General Acridology". The University Press, Cambridge, UK, 1966, I.
131. Pener MP. Locust phase polymorphism and its endocrine relations. Advances in Insect Physiology. 1991; 23:1-79. https://doi.org/10.1016/S0065-2806(08)60091-0
132. Tanaka S. Corazonin and locust phase polyphenism. Applied Entomology and Zoology. 2006; 41:179-193. https://doi.org/10.1303/aez.2006.179
133. Dale JF, Tobe SS. Biosynthesis and titre of juvenile hormone during the first gonotrophic cycle in isolated and crowded *L. migratoria* females. Journal of Insect Physiology. 1986; 32:763-769. https://doi.org/10.1016/0022-1910(86)90079-X
134. Langewald J, Schmutterer H. Colour change in the desert locust, *S. gregaria* (Forsk.), induced by topical treatment with azadirachtin-enriched neem oil in semi-field trails in Benin at different population densities. Journal of Applied Entomology. 1995; 119:221-226.
135. Phillips DR, Loughton BC. Cuticle protein in *Locusta migratoria*. Comparative Biochemistry and Physiology. 1976; 558:129-135.
136. Applebaum SW, Avisar E, Heifetz Y. Juvenile hormone and locust phase. Archives of Insect Biochemistry and Physiology. 1997; 35:375-391. https://doi.org/10.1002/(SICI)1520-6327(1997)35:4<375::AID-ARCH3>3.0.CO;2-R
137. El-Gammal AM. Studies on recent control measures against the desert locust, *Schistocerca gregaria* Forskal. Ph.D. Thesis (Agric.), Cairo University, Egypt, 1983.
138. Ismail SH, Hassanali A, Osir E, Tawfik A. Effects of anti-juvenile hormone (Precocene II) treatment on phase changes and pheromone production in the desert locust, *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae). Journal of insect Physiology. 1997; 43(12):1177-1182. DOI: 10.1016/S0022-1910(97)00079-6
139. Sword GA, Lecoq M, Simpson SJ. Phase polyphenism and preventative locust management. Journal of insect Physiology. 2010; 56:949-957. https://doi.org/10.1016/j.jinsphys.2010.05.005