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Effectiveness of *Punica granatum* Linn. (Lythraceae) extracts on the adult performance of desert locust *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae)

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ABSTRACT

The current investigation was carried out aiming to study the effects of ethanol, petroleum ether and n-butanol extracts from the fruit peel of Punica granatum on several parameters of adult performance of Schistocerca gregaria. Treatment of penultimate (4^{th}) instar nymphs with the highest concentration level (80.0%) of each of the extracts resulted in 100.0% adult mortality. After treatment of last (5^{th}) instar nymphs with the highest concentration level of ethanol or n-butanol extract, 100.0% adult mortality was recorded while petroleum ether extract caused only 50.0% adult mortality. Considering the adult morphogenesis, P. granatum extracts exhibited some impairing effects since various deformed adults were recorded after treatment of penultimate instar nymphs. In connection with the sexual maturity and ovarian maturation period of adult females, treatment of nymphs with P. granatum peel extracts resulted in significantly, or insignificantly, prolonged ovarian maturation period. A weak prohibitory action was exerted by all peel extracts on the reproductive life-time (oviposition period) of adult females after treatment of penultimate or last instar nymphs. With regard to total adult longevity, treatment of penultimate instar nymphs with petroleum ether extract, with an exception of its highest concentration level, exerted an accelerating action while a general delaying effect was exhibited by ethanol and n-butanol extracts. After treatment of last instar nymphs, contradictory effects on the total longevity were detected.

Keywords: ethanol, petroleum ether, n-butanol, survival, morphogenesis, longevity, ovarian maturation.

INTRODUCTION

The extensive use and repeated application of conventional synthetic pesticides for eradication of various pests and harmful insects and to protect agricultural production led to several undesirable consequences, such as chemical pollution of the environmental systems [1-3], production of resistant strains of the pest to many pesticides [4], disruption of natural enemies (predators and parasites) in the biological control system [5, 6] beside the destruction of pollinating insects and growing toxic hazards to man, his livestock and wild life [7, 8]. Consequently, intensive efforts are being made worldwide to find alternative methods of pest control. Botanical insecticides and microbial pesticides are highly effective and less toxic to the environment and ecologically acceptable [9, 10]. Many plants have now been reported for their pesticidal properties of which the most promising one is the neem, *Azadirachta indica* [11]. Others include *Cassia fistula*, *Lantana camara*, *Chrysanthemum coronarium*, *Calotropis procera*, *Punica granatum* and *Murraya koinigii* with antifeedant, repellant and growth regulating effects [11, 12].

Pomegranate (*P. granatum*) is one of the oldest cultivated plants in the world [13] and was a symbol of immortality and love in oriental regions [14]. Also, it is cultivated in Central Asia and the drier parts of Southern Asia [15], as well as in the Mediterranean, tropical and subtropical areas [16]. It was introduced into Latin America, California and Arizona [17]. Botanically, *P. granatum* is included in the family Punicaceae but recently classified in the family Lythraceae [18]. Many chemical constituents had been isolated and identified from flowers and fruits of pomegranate [19]. The bark and stem contain a number of alkaloids [20]. In addition, some other compounds have been reported such as punicalagin, ellagic acid, hydroquinone pyridinium and pelargonidin [21, 22]. Ethanolic, aqueous and chloroform extracts from seeds or peel contain triterpenoids, steroids, glycosides, saponins, alkaloids, flavonoids, tannins, carbohydrate and vitamin C [23]. The peel is, also, a rich source of polyphenols and some anthocyanins as delphinidins and cyanidins [24].

As pointed out by many authors [25-36], pomegranate is of a great interest to research in pharmaceutical and new drug development fields, due to its distinctive bioactivities, such as hypolipidemic, antiviral, antifungal, antineoplastic, anticandidal, anti-inflammatory, antimutagenic antioxidant, antibacterial and antidiarrheal. In the field of pest control, aqueous extract from *P. granatum* fruit rind was more toxic against tape-worms than earthworms and round-worms [37]. Also, extracts from pomegranate bark exhibit molluscicidal activity on the *Lymnaea acuminata* [38, 39]. Also, pomegranate fruit rind is effective on some parasitological parameters of *Schistosoma mansoni* [40]. With regard to the biocontrol of insect pests, the available literature reported some insecticidal effects of *P. granatum* extracts [41, 42]. The n-hexane extracts possessed contact toxicity against *Sitophilus zeamais* and *Tribolium castaneum* [43]. The insecticidal efficacy of pulverized leaves had been recorded against *T. castaneum* [44] and *Rhyzopertha dominica* [45]. Ethanolic extract from leaves and peel was found toxic to *T. castaneum* [46]. Also, extracts exhibited insecticidal activities against *Spodoptera litura* [47], *Anopheles pharoensis* [48], *Culex pipiens* [36] and *Musca domestica* [49, 50].

The desert locust *S. gregaria* (Forskal) (Orthoptera: Acrididae), which is called "teeth of the wind" by Arabs, ranks together with other migratory locusts-among the most important crop pests in Africa [51]. Each individual gregarious locust is able to consume roughly its own weight (about 2 grams, or 0.7 ounces) in foliage daily. Now consider that in the last century alone, there were seven periods of numerous plagues, the longest of which lasted intermittently for 13 year [52]. This partly explains how dense swarms of adults, or marching bands of hoppers, can inflict considerable economic harms during only a short time. Plagues of *S. gregaria* have threatened agricultural production in Africa, the Middle East, and Asia for centuries. No reported works for the effects of *P. granatum* extracts on the reproduction of insects, as well as the biological and physiological processes of *S. gregaria*, have been included in the literature. The present study was designed and carried out aiming to investigate the disruptive effects of different extracts from the *P. granatum* peel on several parameters of the adult performance of *S. gregaria*.

MATERIALS AND METHODS

1. Experimental insect

The desert locust *S. gregaria* was used as an experimental insect in the present study. The culture was originated by a sample of gregarious nymphs from Plant Protection Research Institute, Ministry of Agriculture, Giza. As designed by Hunter-Jones [53] and improved by Ghoneim et al. [54], insects were reared in wood formed cages (60 x 60 x 70 cm). The bottom was furnished with a sandy layer (20 cm depth) and provided with10-15% humidity to be suitable for egg laying. An electric bulb (100 watt) was adjusted to maintain a continuous photoperiod (12 L: 12 D) in each cage as well as in order to maintain an ambient temperature (32±2^{o}C). The insects were reared and handled under the crowded conditions. The feces, dead locusts and food remains were removed daily before introducing the freshly food. Care was seriously taken to clean these cages at regular intervals and the sand was sterilized in drying oven (at 140^{o}C for 24 hours) to avoid contamination with any pathogenic microorganisms. Fresh clean leaves of clover *Trifolium alexandrinum* were provided, as a food for insects, every day.

2. Plant Extraction

A weight of 1.5 Kg *Punica granatum* peel (or rind), which purchased from an Egyptian market, was thoroughly cleaned with tap water for disposing of impurities. The peel was shade dried and then finely grinded by a micromill. The pulverized powder was macerated with ethanol in a stoppered container for a defined period with frequent agitation until soluble matter is dissolved as adopted from Ncube et al. [55]. The ethanol extract was divided into two parts: a part of the ethanol extract was evaporated for obtaining 37 gm dried extract. Another part of the ethanol extract was concentrated into 300 ml by rotary evaporator, and then diluted with 300 ml distilled water. Using a separating funnel, the dilute was fractionalized by petroleum ether (300 ml X 5) and n-butanol (300 ml X 5) giving 29 and 34 gm, respectively. From each of the crude ethanol extract and the fractionalized petroleum ether and n-butanol extracts, the following concentrations were prepared: 80, 40, 20, 10, 5 and 2.5%.

3. Nymphal treatments

The newly moulted 4th (penultimate), or 5th (last) instar nymphs of *S. gregaria* were fed on fresh leaves of *Trifolium alexandrinum* after dipping in the different concentration levels of each extract. After dipping for three minutes, the treated leaves were allowed to dry before offering to nymphs. A day after treatment, all nymphs (treated and control) were provided with untreated fresh food plant. Ten replicates (one nymph/replicate) were used for each concentration. Each individual nymph was isolated in a glass vial provided with a thin layer of sterilized sand as a floor. All vials were located in a large cage having a suitable electric bulb. The nymphs were carefully handled until the adult emergence just after which all parameters of adult performance were recorded.

4. Adult performance parameters

For investigation the adulticidal activity of *P. granatum* extracts on *S. gregaria*, the adult mortality was observed throughout the adult longevity and calculated in percentage. For investigating the morphogenic efficiency, the adult deformities were observed and calculated in percentage according to Vargas and Sehnal [56] as follows:

$$[\text{No. of deformed adults} / \text{No. of larvae}] \times 100$$

The sexual maturity has been taken place through the time interval elapsed between the adult emergence of females and the day of changed body colour (from red, or pink, to yellow). At its day the adult female will be sexually mature. The ovarian maturation period (pre-oviposition period) was calculated (in days±SD) from the day of emergence to the day before laying the first egg. The reproductive life-time (oviposition period) is the time interval during which the adult female oviposit all eggs. It was measures in days±SD. Total adult longevity was measured (in days±SD) including ovarian maturation period, reproductive life-time and post-oviposition period [57].

5. Statistical analysis of data

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

RESULTS

According to data assorted in Table (1), the survival potential of adult *S. gregaria* was tremendously suppressed by a latent adulticidal action of *P. granatum* extracts. Treatment of penultimate (4^{th}) instar nymphs with the highest concentration level (80%) of ethanol, petroleum ether or n-butanol extract resulted in 100% mortality. Treatment of these nymphs with a decreasing range of concentration resulted in decreasing adult mortality. Moreover, the lowest concentration level of petroleum ether extract caused no mortality. As clearly shown in the same table, similar prohibited survival potential of adults was determined after treatment of last (5^{th}) instar nymphs with the highest concentration level of both ethanol and n-butanol extracts, while the highest concentration level of petroleum ether extract caused only 50% adult mortality. Generally, the adult mortality was observed in a dose-dependent course, with few exceptions.

In connection with the impairing effects of *P. granatum* extracts on the morphogenesis program of *S. gregaria*, data distributed in Table (2) exiguously show various percentages of deformed adults. The n-butanol extract was the least potent in this regard since only its higher two concentration levels caused 16.7 and 14.3% of adult deformities while petroleum ether extract exhibited the most potent action because the treatment of penultimate instar nymphs resulted in almost dose-dependent deformities. In addition, various percentages of adult deformities had been observed after treatment with the ethanol extract, but in no certain trend. Several adult malformations were demonstrated in Fig. (1). As clearly shown, some adults emerged with crumbled wings after treatment of penultimate instar nymphs with 20.0 or 10.0% of ethanol extract. After treatment of last instar nymphs with ethanol extract, no affected adult morphology was observed (see Table 2). In contrast, treatment of their congeners with the higher four concentration levels (80.0-10.0%) of petroleum ether and n-butanol extracts resulted in decreasing % of deformities as the concentration was decreased. The most powerful deranging effect on the adult morphogenesis was exhibited by n-butanol extract since all concentration levels caused deformities ranging from 25.0 to 11.1%. Referring to Fig. (1), several deformities had been produced since the newly emerged adults appeared with elongated and curled legs, as well as crumbled wings, after treatment of last instar nymphs with 80, 40 or 20% of n-butanol extract.

With regard to the ovarian maturation period (pre-oviposition period), treatment of penultimate instar nymphs of *S. gregaria* with *P. granatum* extracts, a predominant inhibitory effect was detected since data arranged in Table (3) indicate significantly or insignificantly prolonged ovarian maturation period (pre-oviposition period). As seen in the same table, remarkably prolonged period, and subsequently retarded ovarian maturation or delayed sexual maturity was recorded at concentration level 40% of ethanol extract (23.3±1.5 days vs. 16.6±3.5 days of control adults), as well as at concentration levels 40.0 and 20.0% of petroleum ether extract (20.7±2.1 and 19.3±2.5 days at 40.0 and 20.0%, respectively, in comparison with 15.3±1.3 days of control adults). On the other hand, slightly prolonged ovarian maturation period was determined after treatment with n-butanol extract. Data distributed in the same table unambiguously indicate no powerful inhibitory effect of all extracts from *P. granatum* peel on the ovarian maturation period because insignificantly lengthened pre-oviposition period was estimated after treatment of last instar nymphs, regardless the extract and concentration level. In spite of this glance, nymphal treatments with the highest concentration level of ethanol, petroleum ether and n-butanol extracts resulted in 22.7±1.5 days (vs. 22.0±1.6 days of controls), 24.0±1.4 days (vs. 21.7±2.5 days of controls) and 21.3±3.1 days (vs. 17.7±3.1 days of controls), respectively.

Just a look at the data of Table (4) indicates a slight prohibitory action was exerted by all extracts from *P. granatum* peel on the reproductive life-time (oviposition period) of the adult females after treatment of penultimate instar nymphs of *S. gregaria*. In other words, insignificantly prolonged reproductive life-time was measured, regardless the extract and concentration level. Such prolongation was observed in no certain trend. However, the longest reproductive life-time was calculated in 20.5±3.5 days (vs. 14.7±2.1 days of control congeners) after treatment with ethanol extract at 5.0%, 18.0±2.6 days (vs. 14.5±1.9 days of control congeners) after treatment with petroleum ether extract at 40.0% and 19.7±2.5 days (vs. 15.0±2.0 days of control congeners) after treatment with n-butanol extract at 5.0%. After treatment of last instar nymphs, similar results had been obtained for this time interval, i.e. insignificantly prolonged period was caused by all of *P. granatum* extracts. For some detail, data included in Table (4) obviously show the longest reproductive life-time as 17.6±1.5 days (vs. 13.0±0.8 days of control congeners) after treatment with ethanol extract at 20.0%, 19.0±2.0 days (vs. 16.0±2.6 days of control congeners) after treatment with petroleum ether extract at 40.0% and 17.7±1.2 days (vs. 13.7±1.2 days of control congeners) after treatment with n-butanol extract at 5.0%.

Data of the total adult longevity, as affected by the treatment of penultimate instar nymphs of *S. gregaria* with extracts from *P. granatum* peel are presented in Table (5). The petroleum ether extract, with an exception of its highest concentration level, exerted an accelerating action on the total longevity of adult females, but a general delaying effect was exhibited by ethanol and n-butanol extracts, irrespective of the concentration level. As shown in the same table, the most potent delaying effect on aging was undoubtedly exhibited by n-butanol extract since pronouncedly extended total longevity was estimated, regardless the concentration level. For some detail, the longest total longevity was measured in 56.7±2.1 days (vs. 46.0±2.1 days of control congeners) after treatment with ethanol extract at 20.0% and 54.3±1.2 days (vs. 44.0±1.7 days of control congeners) after treatment with n-butanol extract at 5.0%. On the other hand, the shortest total longevity was measured in 43.7±1.5 days (vs. 50.0±2.9 days of control congeners) after treatment with petroleum ether extract at 5.0%. Depending on the data assorted in the same table, contradictory effects had been exhibited by the extracts on the total longevity because ethanol extract achieved a shortening effect while petroleum ether and n-butanol extracts achieved lengthening effects. In other words, treatment of last instar nymphs of *S. gregaria* with ethanol extract resulted in slightly shortened total longevity indicating accelerated adult aging. The shortest longevity (53.3±3.2 days, compared to 55.8±2.6 days of control adults) was caused by this extract at both 40.0 and 2.5% concentration levels. On the other hand, delayed adult aging was taken place as a response to the action of both petroleum ether and n-butanol extracts. For some detail, the most delaying action was exerted at 40.0% of petroleum ether extract (61.7±4.0 in comparison with 49.0±3.6 days of control adults) and at 5.0% of n-butanol extract (53.7±3.2 in comparison with 44.0±3.5 days of control adults).

DISCUSSION

1. Reduced adult survival potential of *S. gregaria* by *P. granatum* extracts

The available literature contains many reported toxicities of extracts from various plant species on the immature stages of several insect pests [54, 59-68] while the lethal effects of botanicals on adults are very scarcely reported. In the present study, treatment of penultimate (4^{th}) instar nymphs of *S. gregaria* with the highest concentration level (80.0%) of ethanol, petroleum ether or n-butanol extract from *P. granatum* peel resulted in 100.0% adult mortality. Treatment of last (5^{th}) instar nymphs with the highest concentration level of ethanol or n-butanol extract resulted in 100.0% adult mortality but petroleum ether extract caused 50.0% adult mortality. Adult mortality was generally observed in a dose-dependent course, with few exceptions. These adulticidal activity of *P. granatum* peel on *S. gregaria* agree, to a great extent, with those reported lethal activities of different plant extracts on adults of some pests, such as *T. castaneum* [69], *Muscina stabulans* [70] and *M. domestica* [71]. Also, the current results are in consistent with the adulticidal activities of extracts derived from *Rhizophora mucronata* on the present locust [72] and *Ammi visnaga* extracts, or its component khellin on some other pests [73, 74].

The adult mortality, i.e., reduced survival potential, of *S. gregaria* by *P. granatum* extracts, in the present study, may be explicated by a latent prohibitory effect on feeding leading to continuous starvation and subsequently death [61]. It may be, also, attributed to the action of certain active ingredients in the *P. granatum* extracts on the homeostasis leading to increasing loss of body water and subsequently the death [71]. In addition, alkaloids are, among many components, had been identified in the *P. granatum* peel [22, 23]. Certain compounds of these alkaloids affect the maintenance of the adult life through disrupting the enzymatic pattern or hormonal hierarchy [75].

2. Deranged adult morphogenesis of *S. gregaria* by *P. granatum* extracts

As obviously shown in the present study, *P. granatum* extracts exhibited some impairing effects on the adult morphogenesis of *S. gregaria* since various deformed adults were recorded after treatment of penultimate instar nymphs. After treatment of last instar nymphs with ethanol extract, no affected adult morphology was observed. In contrast, treatments of their congeners with the higher four concentration levels of petroleum ether or n-butanol

extract resulted in decreasing % of deformities as the concentration level was decreased. The current results are in agreement with those reported results for extracts from various plants against the same locust. As for examples, adult morphogenic defects were observed after treatment of last instar nymphs with a neem oil [76], after treatment of penultimate instar nymphs with ethanol extract from *Cyprus rotendus* [77], Neemazal (a neem preparation) or *Nigella sativa* extracts [78], as well as some extracts from *Fagonia bruguieri* [79]. Moreover, various malformed moths of *Spodoptera littoralis* were caused by Neemazal [61], acetone and ethanol extracts from *Aristolochia pubescens* impaired the adult morphogenesis of *Aticarsia gemmatalis* [80], many adult deformities in both *Spodoptera frugiperda* and *Tenebrio molitor* were observed after treatment with methanol extract from *Myrtillocactus geometrizans* [81], and azadirachtin (a neem seed kernel extract) caused several adult defects in *Rhynchophorus ferrugineus* [82].

However, the production of adult deformities of *S. gregaria*, in the present study, can be explained by the intervention of the *P. granatum* extracts in the hormonally controlled program of morphogenesis. This may be due to the modification of ecdysteroid titer, which leads to changes in lysosomal enzyme activity causing overt morphological abnormalities [83]. This proposal may be conceivable in the identification of steroids, among other components, in *P. granatum* peel [23, 24].

3. Disturbed ovarian maturation in *S. gregaria* by *P. granatum* extracts

In Orthoptera, the sexual maturity usually needs a time interval elapsed between the day of adult emergence until the day of laying the first egg. During such period, the ovaries (or testes) developed and the adult will be sexually mature. Generally, the pre-oviposition period may be informative for the sexual maturity rate, i.e. the shorter period indicates the faster rate and *vice versa*. Thus, it may acceptable to use the pre-oviposition period in adult females of *S. gregaria* as a good indicator to the ovarian maturation rate. In this regard, several contradictory results had been reported in the literature, since some plant extracts promoted the ovarian maturation, and hastened the sexual maturity, but others prohibited the ovarian maturation, and retarded the sexual maturity. A promoting effect on the ovarian maturation of *S. gregaria* was recorded by certain concentration levels of Neemazal [78] as well as by methanol and petroleum ether extracts from *F. bruguieri* [79]. On the other hand, some extracts from *C. rotendus* completely prevented the sexual maturity and ovarian maturation of the same locust [77] while no effect was exhibited by Margosan-o (a neem preparation) or Jojoba oil on this vital process in *M. domestica* [84].

In the present study, a prevalent disturbing effect had been exhibited on the sexual maturity of *S. gregaria* adult females since slightly or remarkably prolonged ovarian maturation period was determined after treatment of penultimate or last instar nymphs with the *P. granatum* extracts. A similar delayed maturation period, to some extent, was reported for the same locust by some concentration levels of Neemazal of *N. sativa* extracts [78]. In addition, delayed sexual maturity was caused in *M. domestica* by an aqueous extract from *Hyoscyamus muticus* [85], in *Euprepocnemis plorans* by Margosan-o [86] and in *Spilostethus pandrus* by azadirachtin [87]. An appreciable interpretation of the prolonged ovarian maturation period in *S. gregaria* by the *P. granatum* extracts, in the present study, is still obscure but some active compounds in these extracts may interfere with the hormonal regulation of this physiological process.

4. Influenced reproductive life-time of *S. gregaria* by *P. granatum* extracts

As reported in the literature, treatments of some insects with extracts from various plants resulted in shortened reproductive life-time (oviposition period) of the adult females. With regard to *S. gregaria*, treatment of 2^{nd}-4^{th} instar nymphs with ethanol extract from *Melia volkensii* shortened the reproductive life-time [88]. A similar result was reported after nymphal treatments with *F. bruguieri* [89] or *N. sativa* extracts [78]. Also, shortened reproductive life-time of some other insects was caused by several botanicals, such as *M. domestica* by an aqueous extract from *H. muticus* [85] and Margosan-o or Jojoba oil [84], and *Chrysomya chloropyga* by some extracts from Nigerian plants [90]. In contrast to those reported results, the current investigation recorded an insignificant prolongation in the reproductive life-time of *S. gregaria* after treatment nymphs with *P. granatum* extracts, regardless the extract, concentration level and the time of treatment. The shortened reproductive life-time may reveal an enhancing effect on the adult females to lay their eggs quickly, but the prolonged period, as in the present study, cannot be interpreted now!! Therefore, further investigation should be carried out to disclose the mode of action of certain chemical in these extracts on this important event.

5. Delayed total adult longevity of *S. gregaria* by *P. granatum* extracts

Several neem products pronouncedly affected the total adult longevity of some insect pests, such as *S. litura* [91, 92], *M. stabulans* [70], *M. domestica* [71], *Clavigralla scutellavis* [64]. *Chrysomya megacephala* [68]. Also, the adult longevity of *S. littoralis* was shortened by larval treatments with extracts from *Melia azedarach* [93, 94]. Considering the present experimental locust, *S. gregaria*, Neemazal treatments of penultimate instar nymphs resulted in remarkably shortened adult longevity but a reversed effect was recorded after treatment of last instar

nymphs [95]. Also, adult longevity of this locust was shortened or prolonged by the action of *N. sativa*, depending on the time of treatment and concentration level [95]. Similar results had been reported for the same locust by extracts from *F. bruguieri* [79].

After the attainment of sexual maturity, insects often show degenerative changes in some tissues and organs which can be called 'senility' or 'aging'. In insects, the affected adult longevity can be considered an informative indicator of the adult aging, i.e. prolongation of longevity may denote a delay of aging and *vice versa*. In the present study, contradictory results of shortened or prolonged total longevity i.e. accelerated or retarded aging, of *S. gregaria* by the *P. granatum* extracts had been recorded, depending on the extract, concentration level and time of treatment. For some detail, treatment of penultimate instar nymphs with petroleum ether extract, with an exception of its highest concentration level, exerted an accelerating action the adult aging while a general delaying effect was exhibited by ethanol and n-butanol extracts. After treatment of last instar nymphs, contradictory effects on the total longevity were detected because ethanol extract achieved a shortening effect while petroleum ether extract and n-butanol extract exhibited lengthening effects. Retardation of adult aging of S. gregaria, in the present study, may be attributed to the antioxidant properties of pomegranate peel [17, 26, 28, 96-98]. On the other hand, the shortening of adult longevity, i.e. the accelerated aging, in *S. gregaria* may be attributed to certain chemicals in the pomegranate peel extracts of a hormonal activity because there is a close relation between certain hormones and adult longevity. This suggestion can be appreciated in the light of reported results in *Drosophila*. In this fly, representatives of peptide hormone, lipophilic hormones and bioactive amines have been shown to modulate longevity by manipulations that directly decrease hormone production [99], through inactivating mutations in hormone receptors or their downstream targets [100, 101] or by polymorphic alterations in the genes required for hormone biosynthesis [102]. At least one of the *Drosophila* insulin-linked peptides expressed in the median neurosecretory cells (which produce the prothoracicotropic hormone) is likely to contribute to the endocrine regulation of longevity [103].

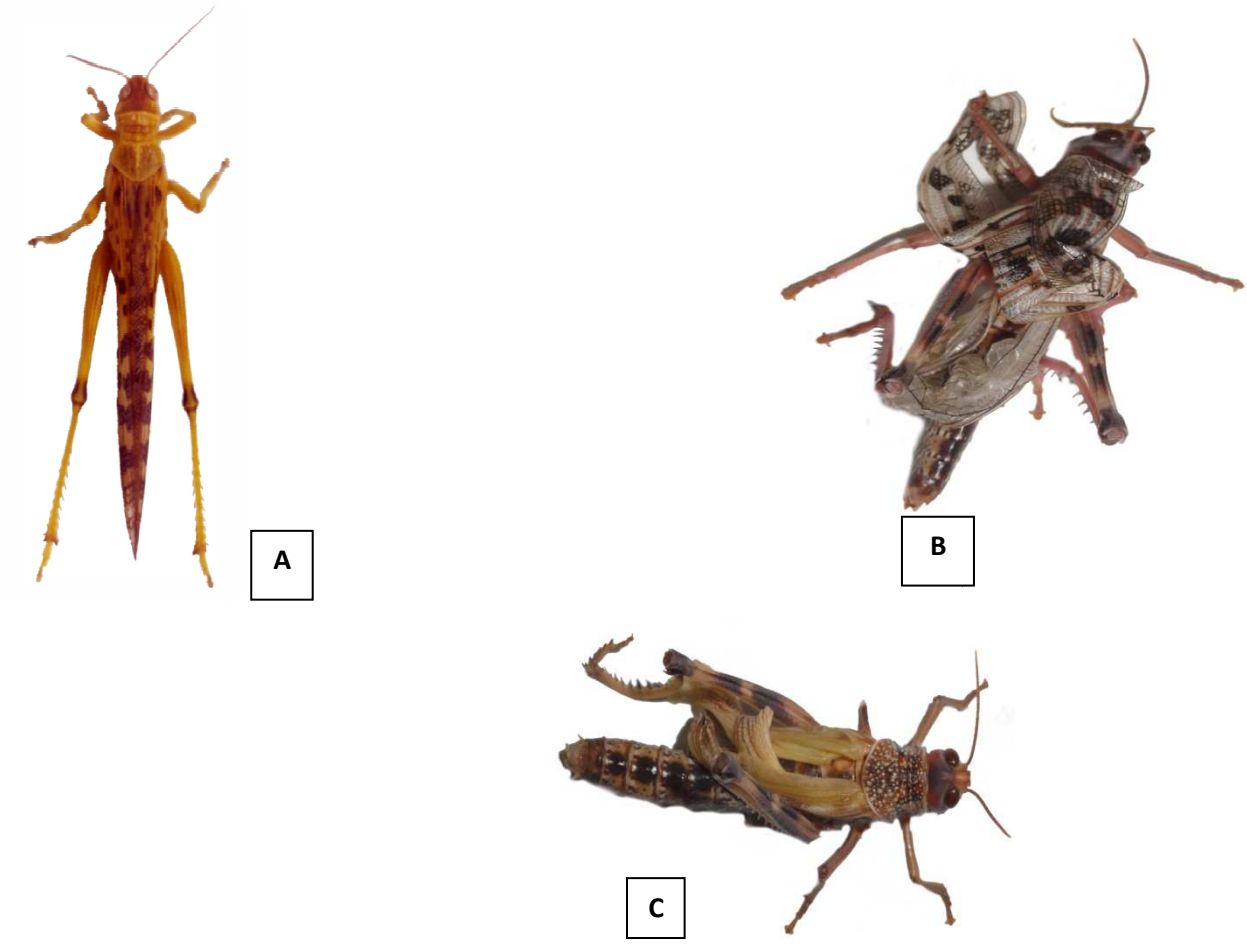

Fig. (1): Different adult malformations of *Schistocerca gregaria* were produced as a result of the nymphal treatments with different extracts of *Punica granatum*. (A) Normal adult. (B) Adult with crumbled wings, after treatment of the newly moulted penultimate instar nymphs with 20% and 10% of ethanol extract. (C) Adult with elongated and curled legs with crumbled wings, after treatment of the newly moulted last instar nymphs with 80%, 40% and 20% of n-butanol extract.

Table 1: Adulticidal activity of *Punica granatum* extracts on *Schistocerca gregaria*

Solvent	Conc. (%)	After treatment of 4th instar nymphs	After treatment of 5th instar nymphs
Ethanol	80.0	100.0	100.0
	40.0	42.9	022.2
	20.0	33.3	11.1
	10.0	12.5	11.1
	05.0	12.5	10.0
	02.5	11.1	00.0
	Controls	00.0	00.0
Petroleum ether	80.0	100.0	50.0
	40.0	25.0	40.0
	20.0	33.3	25.0
	10.0	16.7	30.0
	05.0	16.7	22.2
	02.5	00.0	11.1
	Controls	00.0	10.0
n-butanol	80.0	100.0	100.0
	40.0	37.5	25.0
	20.0	25.0	28.0
	10.0	25.0	25.0
	05.0	12.5	10.0
	02.5	11.1	22.2
	Controls	00.0	00.0

Conc.: Concentration level.

Table (2): Affected adult morphogenesis of *Schistocerca gregaria*, as indicated by deformed adult %, by nymphal treatments with *Punica granatum* extracts.

Solvent	Conc. (%)	After treatment of 4th instar nymphs	After treatment of 5th instar nymphs
Ethanol	80.0	-	-
	40.0	28.6	00.0
	20.0	16.7	00.0
	10.0	00.0	00.0
	05.0	12.5	00.0
	02.5	00.0	00.0
	Controls	00.0	00.0
Petroleum ether	80.0	-	25.0
	40.0	25.0	40.0
	20.0	20.0	25.0
	10.0	16.7	20.0
	05.0	16.7	00.0
	02.5	12.5	00.0
	Controls	00.0	00.0
n-butanol	80.0	-	-
	40.0	16.7	25.0
	20.0	14.3	14.3
	10.0	00.0	12.5
	05.0	00.0	11.1
	02.5	00.0	11.1
	Controls	00.0	00.0

: adult females died just after emergence.-Conc. : See footnote of Table (1),

Conc. : See footnote of Table (1), - : adult females died just after emergence.

Table (3): Influenced ovarian maturation period (Mean days±SD) of *Schistocerca gregaria* by nymphal treatments with *Punica granatum* extracts.

Solvent	Conc. (%)	After treatment of 4th instar nymphs	After treatment of 5th instar nymphs
Ethanol	40.0	23.3 ± 1.5 c	22.7 ± 1.5 a
	20.0	21.5 ± 3.5 a	24.3 ± 1.5 a
	10.0	20.0 ± 2.0 a	24.7 ± 1.5 a
	05.0	20.5 ± 2.1 a	23.7 ± 1.5 a
	02.5	18.7 ± 1.5 a	22.3 ± 2.8 a
	Controls	16.6 ± 3.5	22.0 ± 1.8
Petroleum ether	80.0	-	24.0 ± 1.4 a
	40.0	20.7 ± 2.1 c	27.3 ± 1.5 a
	20.0	19.3 ± 2.5 b	26.3 ± 2.1 a
	10.0	18.0 ± 2.6 a	25.0 ± 1.4 a
	05.0	16.3 ± 2.6 a	22.3 ± 2.5 a
	02.5	16.0 ± 3.1 a	24.7 ± 2.1 a
	Controls	15.3 ± 1.3	21.7 ± 2.5
n-butanol	40.0	23.7 ± 1.5 a	21.3 ± 3.1 a
	20.0	22.3 ± 3.2 a	21.0 ± 3.0 a
	10.0	22.8 ± 2.1 a	22.3 ± 2.5 a
	05.0	22.7 ± 1.2 a	21.7 ± 1.5 a
	02.5	18.9 ± 3.0 a	20.5 ± 2.6 a
	Controls	18.7 ± 1.5	17.7 ± 3.1

Conc. :See footnote of Table (1), -: See footnote of Table (2), Mean ±SD followed by letter (a): is 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).

Table (4): Disturbed reproductive life-time (Mean days±SD) of *Schistocerca gregaria* by nymphal treatments with *Punica granatum* extracts

Solvent	Conc. (%)	After treatment of 4th instar nymphs	After treatment of 5th instar nymphs
Ethanol	40.0	15.7 ± 2.5 a	15.0 ± 2.2 a
	20.0	19.0 ± 2.0 a	17.6 ± 1.5 a
	10.0	20.3 ± 1.5 a	14.0 ± 1.3 a
	05.0	20.5 ± 3.5 a	17.0 ± 2.0 a
	02.5	17.0 ± 2.1 a	14.3 ± 0.8 a
	Controls	14.7 ± 2.1	13.0 ± 0.8
Petroleum ether	80.0	-	18.0 ± 1.4 b
	40.0	18.0 ± 2.6 a	19.0 ± 2.0 b
	20.0	15.3 ± 2.5 a	18.3 ± 2.5 a
	10.0	15.3 ± 2.5 a	18.0 ± 1.4 a
	05.0	17.0 ± 4.0 a	18.0 ± 1.0 a
	02.5	16.3 ± 2.1 a	17.7 ± 3.2 a
	Controls	14.5 ± 1.9	16.0 ± 2.6
n-butanol	40.0	16.0 ± 3.0 a	15.7 ± 1.7 a
	20.0	16.0 ± 3.6 a	15.0 ± 1.2 a
	10.0	18.5 ± 1.9 a	16.0 ± 2.6 a
	05.0	19.7 ± 2.5 a	17.7 ± 1.2 a
	02.5	16.0 ± 3.0 a	16.3 ± 2.5 a
	Controls	15.0 ± 2.0	13.7 ± 1.2

Conc.: See footnote of Table (1), -: See footnote of Table (2). a, b: See footnote of Table (3).

Table (5): Disturbed total adult longevity (Mean days±SD) of *Schistocerca gregaria* by nymphal treatments with *Punica granatum* extracts

Solvent	Conc. (%)	After treatment of 4th instar nymphs	After treatment of 5th instar nymphs
Ethanol	40.0	51.3 ± 8.5 a	53.3 ± 3.2 a
	20.0	56.7 ± 2.1 a	54.3 ± 3.5 a
	10.0	55.0 ± 4.4 a	53.7 ± 6.8 a
	05.0	52.0 ± 2.8 a	54.7 ± 1.5 a
	02.5	50.3 ± 7.1 a	53.3 ± 6.6 a
	Controls	46 ± 2.1	55.8 ± 2.6
Petroleum ether	80.0	-	61.5 ± 0.7 b
	40.0	50.3 ± 1.2 a	61.7 ± 4.0 b
	20.0	46.3 ± 4.2 a	53.7 ± 7.5 a
	10.0	45.3 ± 1.5 a	57.0 ± 1.4 a
	05.0	43.7 ± 5.0 a	53.3 ± 2.1 a
	02.5	44.3 ± 5.0 a	54.3 ± 4.2 a
	Controls	50.8 ± 2.9	49.0 ± 3.6
n-butanol	40.0	50.3 ± 1.2 c	50.3 ± 9.1 a
	20.0	53.7 ± 1.5 d	51.0 ± 7.5 a
	10.0	54.0 ± 5.6 c	52.3 ± 8.0 a
	05.0	54.3 ± 1.2 c	53.7 ± 3.2 a
	02.5	45.0 ± 3.5 b	51.3 ± 1.5 a
	Controls	44.0 ± 1.7	44.0 ± 3.5

Conc.: See footnote of Table (1). -: See footnote of Table (2). a, b, c, d: See footnote of Table (3).

CONCLUSION

The current results unambiguously revealed several slight or considerable effects of the *P. granatum* peel extracts on various parameters of the adult performance of *S. gregaria*, such as survival, morphogenesis, ovarian maturation, reproductive life-time and total longevity. Although these results suggest the feasible use *P. granatum* extracts, as a complementary measure in the integrated control of this destructive locust, further investigation should be conducted to ascertain the active ingredient (s) contained in these extracts and their exact mode of action. In the view of literature available to us, the present study can be considered as the first report of assessment of *P. granatum* extracts on adult performance of *S. gregaria*.

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