ISSN No: 2349-2864

Entomology and Applied Science Letters, 2014, 1, 1:1-10

Disruptive effects of pomegranate *Punica granatum* Linn. (Lythraceae) extracts on the reproductive potential of desert locust *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae)

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ABSTRACT

The present study aims to investigate the disruptive effects of ethanol, petroleum ether and n-butanol extracts from the fruit peel of Punica granatum on the reproductive potential of Schistocerca gregaria. After treatment of penultimate or last instar nymphs, a predominant inhibitory effect of all extracts on the number of egg pods/♀ was exhibited. Remarkably decreased number of eggs/pod was caused by ethanol extract at the highest concentration level (40.0%) and n-butanol extract at the higher three concentration levels (40.0, 20.0 and 10.0%). The adult females had been enforced to lay eggs in a slow rate, regardless the extract, concentration level or the time of treatment. Treatment of penultimate instar nymphs with ethanol extract or petroleum ether extract led to slightly reduced fecundity while n-butanol extract caused a drastic reduction in fecundity, especially at the higher three concentration levels. After treatment of last instar nymphs, slightly reduced fecundity was generally recorded but remarkably reduced fecundity was caused at the highest concentration level. A prevalent suppressing effect on the fertility was explored after treatment of penultimate instar nymphs with all extracts. Detrimentally impaired fertility was observed after treatment with ethanol extract or petroleum ether extract. Moreover, unexceptionally deranged fertility was recorded after treatment of last instar nymphs, irrespective of the extract. The embryonic developmental rate had been generally subjected to an extended inhibitory effect since the incubation period was slightly or substantially prolonged, regardless the time of treatment.

Keywords: ethanol, petroleum ether, n-butanol, oviposition fecundity, fertility, embryonic development.

INTRODUCTION

The desert locust, *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae), is a dreaded pest in afro-Asian regions, due to its potential to form huge swarms, which invade complete agricultural areas causing tremendous economical damage [1, 2]. Damage caused by this pest is a consequence of its polyphagous behaviour, high density of the population, and the nature to aggregate and swarm. A single swarm can comprise many millions of locusts, and be more than 100 Km long and several Km wide [3].

Although the use of synthetic insecticides to control insect pests has lead to several adverse effects, including water and soil contamination, insect resistance and toxicity to non-target species [4, 5], these toxic chemicals are still used for controlling this dangerous pest at a large scale. Therefore, there is an urgent need to develop safe, convenient, environmental and low-cost alternatives. Many investigators and institutions are searching for safe alternatives in various countries. The natural products, such as plant extracts, form promising non-conventional pesticides against the destructive pests for crops and health [6-15]. In recent years interest in screening plants for various insecticidal activities has increased significantly and many potent compounds have been isolated and identified [16]. Jacobson [17] suggested that the most promising botanicals were to be found in the families Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae, and Canellaceae. However, screening programs have not been limited to these families but other families have been screened for various insecticidal activities. With regard to *S. gregaria*, some plants, such as *Calotropis procera* [18], *Calotropis gigantean* [19], *Peganum harmala* [20], *Azadirichta indica*, *Melia volkensii* [21], *Cestrum parqui* [22] and *Nerium oleander* [23-25] were classified as plants with a toxic, repellent or deterrent effect on locusts due to some secondary compounds contained in these plants [26]. In addition, several plant species affect differentially the development, fertility, and behaviour of the desert locust [18].

Pomegranate (*Punica granatum* Linn.) is one of the oldest cultivated plants in the world [27] and was a symbol of immortality and love in oriental regions [28]. Also, it is cultivated in Central Asia and the drier parts of Southern Asia [29], as well as in the Mediterranean, tropical and subtropical areas [30]. It was introduced into Latin America,

California and Arizona [31]. Botanically, *P. granatum* is included in the family Punicaceae but recently classified in the family Lythraceae [32]. It is a small tree, much branched, showy flowers are found on the branch tips or in clusters, calyx red, fleshy with pointed sepals, petals red [33]. The fruit has a leathery skin, reddish or yellowish green wall. It contains many seeds (arils) separated by white, numerous pericarp, and each is surrounded by small amounts of tart, red juice [34]. Many chemical constituents had been isolated and identified from flowers and fruits of pomegranate [35]. The bark and stem contain a number of alkaloids [36]. In addition, some other compounds have been reported such as punicalagin, ellagic acid, hydroquinone pyridinium and pelargonidin [37, 38]. Ethanolic, aqueous and chloroform extracts from seeds or peel contain triterpenoids, steroids, glycosides, saponins, alkaloids, flavonoids, tannins, carbohydrate and vitamin C [34]. The peel is, also, a rich source of polyphenols and some anthocyanins as delphinidins and cyanidins [39].

From the medical point of view, ancient historical uses have been reported for pomegranate. Also, it is used in several systems of modern medicine for a variety of ailments, such as cancer, cardiovascular disease, diabetes, dental conditions, Alzheimer's disease, male infertility, arthritis infant brain, ischemia, obesity and protection from ultraviolet radiation [40, 41]. In general, 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 and antidiarrheal [42-53]. Antioxidant and antibacterial properties of pomegranate peel have been also reported [31, 54-57].

In the field of pest control, aqueous extract from *P. granatum* fruit rind was more toxic against tape-worms than earthworms and round-worms owing to isopelletierine in the extract [36]. Also, extracts from pomegranate bark exhibit molluscicidal activity on the *Lymnaea acuminata* [58]. Sublethal concentrations of the active fractions of *P. granatum* bark significantly inhibited the enzyme activity in the nervous tissue of the same snail [59]. Also, pomegranate fruit rind is effective on some parasitological parameters of *Schistosoma mansoni* [60]. With regard to the biocontrol of insect pests, the available literature shows insecticidal effects of *P. granatum* extracts [61, 62] but, unfortunately, few examples had been reported. The n-hexane extracts possessed contact toxicity against *Sitophilus zeamais* and *Tribolium castaneum* [63]. The insecticidal efficacy of pulverized leaves had been recorded against *T. castaneum* [64] and *Rhyzopertha dominica* [65]. Ethanolic extract from leaves and peel was found toxic to *T. castaneum* [66]. *P. granatum* extracts exhibited insecticidal activities against *Spodoptera litura* [67], *Anopheles pharoensis* [68] and *Culex pipiens* [53]. Considering the house fly *Musca domestica*, aqueous extract from leaves inhibited the growth of ovarian follicles beside its serious effect on the histological structure of midgut [69]. Some of larvae failed to develop into adult stage and the successfully developed larvae appeared as deformed pupae or adults, as well as pupal-adult intermediates were observed [70]. No reported works for the effects of *P. granatum* extracts on the reproduction of insects, as well as the biological and physiological processes of the desert locust *S. gregaria*, have been included in the available literature. The current study represents a link in an extended research on the effects of *P. granatum* extracts on different biological and physiological processes in the desert locust *S. gregaria*. It was carried out aiming to investigate the disruptive effects of various extracts from the fruit peel on several reproductive criteria of this locust.

MATERIALS AND METHODS

Desert locust

The desert locust *Schistocerca gregaria* (Forskal)(Orthoptera: Acrididae) was used as an experimental insect in the present study. The present culture was originated by a sample of gregarious nymphs from Plant Protection Research Institute, Ministry of Agriculture, Giza. As designed by Hunter-Jones [71] and improved by Ghoneim et al. [14], 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.

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*. [72]. 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%.

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.

In a preliminary experiment, treatments of penultimate or last instar nymphs with the highest concentration level (80%) resulted in complete mortality of the newly emerged adults. Thus, only the adult females from nymphs treated with 40, 20, 10, 5 and 2.5% concentration levels of all extracts had been used for investigating the reproductive criteria.

Reproductive criteria

The successfully emerged adult females were kept separately in cages (30 x 30 x 30 cm, with three sides of glass and the top of wire gauze, the fourth side was made of wood and provided with a small door). For the copulation, each treated female was confined with two normal adult males, provided from the main culture. Plastic cylindrical cups (10 X 8 cm) were filled with sifted, sterilized and moistened sand, as an oviposition site. Water was added to the sand at a rate of 15% by volume [71]. Just after copulation, females were allowed to dig in the moistened sand floor for laying the egg pods. At the end of the reproductive lifetime, all egg pods and eggs were counted and transferred into Petri dishes provided with moistened cotton pad in an incubator until the egg hatching. Meanwhile, the cotton pads received an antifungal material.

After examining all pods, constructed and laid in the oviposition site for each female, the average number of egg pods per female was calculated. Each of the completely laid egg pods was examined for counting the number of eggs. All egg pods were undergone to this procedure for calculating the average number of eggs per pod. The oviposition rate was calculated as follows:

Number of laid eggs per ♀/reproductive lifetime (in days).

All eggs laid by each adult female were counted. The oviposited eggs of all replicates were used to determine the fecundity by calculating the average number of eggs per female.

In respect to the incubation period, and subsequently the rate of embryonic development, the oviposited eggs were kept in cups covered with muslin cloth and tied with rubber band under favourable laboratory conditions in a cage of 32 ^{o}C and moistened sandy bottom. Just after the oviposition, eggs were observed until hatching.

Fertility was usually expressed in the hatchability and was calculated by the hatching of the laid eggs. On the other hand, the sterility index was calculated according to Toppozada *et al*. [73] as follows:

$$\text{Sterility Index} = 100 - [(a\ b / A\ B) \times 100]$$

Where: a = the number of eggs laid per female in the treatment. b = percentage of hatching in the treatment. A = the number of eggs laid per female in the controls. B = percentage of hatching in the controls.

Statistical analysis of data

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction [74] for the test significance of difference between means.

RESULTS

Effects on the oviposition efficiency of *S. gregaria*

After treatment of penultimate (4th) instar nymphs of *S. gregaria* with ethanol, petroleum ether and n-butanol extracts from peel of *P. granatum*, data of the egg pods/♀ had been assorted in Table (1). A predominant inhibitory effect on this oviposition criterion was exhibited by the present extracts. The decreasing number was statistically

insignificant except at the highest concentration level of ethanol extract by which this number was considerably regressed (2.0±0.0 vs. 2.7±0.6 egg pods/control ♀).

According to the data arranged in the same table, treatment of last (5^{th}) instar nymphs resulted in a generally decreased number of egg pods/♀, regardless the extract or concentration level. For some detail, significant decrements in the number of egg pods/♀ were determined after treatment with ethanol extract at both 40.0 and 10.0% (1.7±0.6 and 2.0±0.0, respectively, vs. 2.8±0.5 egg pods/control ♀). Similar considerably dropped number of the egg pods/♀ was caused by petroleum ether extract at both 40.0 and 10.0% (2.0±0.0 vs. 2.3±0.6 egg pods/control ♀). In addition, pronouncedly dropped number of the egg pods/♀ was caused by n-butanol extract at its higher two concentration levels (1.6±0.6 and 1.7±0.6, respectively, in comparison with 2.7±0.6 egg pods/control ♀).

Another important parameter of the oviposition efficiency is the number of eggs/pod. As clearly shown the previously mentioned table, treatment of penultimate instar nymphs with *P. granatum* extracts resulted in significantly or insignificantly decreased number of eggs/pod. The remarkably decreased number of eggs/pod was caused by ethanol extract at its highest concentration level (41.0±2.0 vs. 48.5±3.9 eggs/pod by control congeners) and by n-butanol extract at its higher three concentration levels (41.7±2.0, 42.3±0.9 and 42.6±2.1 egg/pod at 40.0, 20.0 and 10.0%, respectively, vs. 48.1±3.4 eggs/pod by control congeners).

Similar prohibitory effect of the present extracts had been exhibited on this oviposition process after treatment of last instar nymphs. The petroleum ether extract and n-butanol extract exerted a pronouncedly suppressing action on this number, irrespective of the concentration level (For detail, see Table 1).

As a response to *P. granatum* extracts, treatment of penultimate instar nymphs enforced the adult females to lay eggs in a slow rate. For some detail, the oviposition rate slightly regressed by petroleum ether extract but remarkably regressed by ethanol extract, especially at its higher two concentration levels (4.5±1.9 and 5.7±0.7 at 40.0 and 20.0%, respectively, vs. 9.0±1.9 of control congeners). Moreover, n-butanol extract exhibited the most potent action on this rate because treatment with the majority of concentration levels led to tremendously regressed oviposition rate (for detail, see Table 1).

After treatment of last instar nymphs with the same extracts, adult females had been prohibited to oviposit in a normal rate. A prominent inhibitory effect of extracts was detected since pronouncedly regressed rate was caused by ethanol extract at its higher three concentration levels (4.7±1.4, 6.6±1.4 and 5.6±1.9, respectively, compared to 9.6±1.6 of controls), petroleum ether extract at its higher three concentration levels (4.3±0.3, 4.9±0.9 and 4.9±1.0, respectively, compared to 4.8±2.1 of controls) and n-butanol extract at its higher two concentration levels (4.2±1.7 and 4.3±1.7, in comparison with 8.6±1.5 of controls).

Effects on fecundity and fertility of *S. gregaria*

Just a look at data included in Table (2) indicates a major reducing action of *P. granatum* extracts on the adult fecundity of *S. gregaria*. However, treatment of penultimate instar nymphs with ethanol extract or petroleum ether extract resulted in slightly reduced fecundity while n-butanol extract caused a drastic reduction in the fecundity, especially at its higher three concentration levels (083.3±21.0, 098.3±22.2 and 085.3±21.1 eggs/♀ at 40.0, 20.0 10.0%, respectively, compared to 127.0±21.4 eggs/ control♀).

A similar reducing action of *P. granatum* extracts was exerted on the adult fecundity after treatment of last instar nymphs. Slightly reduced fecundity was generally caused but such effect was found stronger by all extracts at the highest concentration level (080.0±27.2 eggs/♀ by ethanol, 080.3±12.1 eggs/♀ by petroleum ether extract and 073.7±22.2 eggs/♀ by n-butanol extract, in comparison with a range 111.3±28.4 – 139.5±22.3 eggs/control ♀).

In the light of data distributed in Table (3), a predominant prohibitory effect on the fertility of *S. gregaria* was explored after treatment of penultimate instar nymphs with all extracts from *P. granatum* peel. For some detail, insignificantly reduced fertility was estimated after treatment with ethanol extract. In contrast, the fertility was detrimentally impaired by both ethanol and petroleum ether extracts, at the majority of concentration levels. The most powerful reducing effect was detected after treatment with n-butanol extract at the highest concentration level (81.0±3.4 hatched eggs vs.92.6±0.4 hatched eggs of controls) and the maximal sterility index (42.4) was calculated. On the other hand, the least powerful reducing effect was exhibited by petroleum ether extract at its lowest concentration level (88.3±2.4 hatched eggs vs. 91.6±1.0 hatched eggs of controls) and the minimal sterility index (8.1) was calculated.

Data arranged in the aforementioned table unexceptionally show deranged fertility of eggs deposited by adult females after treatment of last instar nymphs with *P. granatum* extracts. For some detail, n-butanol extract exhibited

an insignificantly prohibitory effect on fertility while petroleum ether extract exhibited strong prohibitory effect at all concentration levels, as well as ethanol extract exhibited a similar effect at the higher three concentration levels. The maximal sterility index was calculated in 47.8 (73.8±7.4 hatched eggs vs. 91.5±2.0 hatched eggs of controls) while the minimal index was calculated in 08.4 (87.4±1.3 hatched eggs vs. 91.5±2.0 hatched eggs of controls), at the highest concentration level and lowest concentration level, respectively, of petroleum ether extract.

Effects on the embryonic development of *S. gregaria*

After treatment of penultimate instar nymphs of *S. gregaria* with *P. granatum* extracts, data of egg incubation period are listed in Table (4). Generally, the embryonic development had been subjected to an extended inhibitory effect of all extracts since the incubation period was significantly, or insignificantly, prolonged. For some detail, treatment with n-butanol extract resulted in insignificant prolongation in the incubation period, and subsequently slightly prohibited embryonic developmental rate was detected. On the contrary, considerably prolonged incubation period was determined after treatment with ethanol extract or petroleum ether extract. The longest incubation period was recorded after treatment with ethanol extract at its highest concentration level (19.8±1.7 days vs. 14.3±0.4 days of controls) indicating the slowest embryonic developmental rate.

To a great extent, similar prohibitory action was exerted on the embryonic development after treatment of last instar nymphs since the incubation period was significantly prolonged by ethanol extract and petroleum ether extract but slightly prolonged by n-butanol extract. The slowest embryonic developmental rate can be denoted by the longest incubation period (19.4±0.1 days vs. 13.3±0.3 days of controls) after treatment with petroleum ether extract at its highest concentration level.

DISCUSSION

Impaired oviposition efficiency of *S. gregaria* by *P. granatum* extracts

The available literature reports reduced number of egg pods/♀ and decreased number of eggs/pod after treatment of few insect pests with extracts from various plant species. Considerably reduced numbers had been recorded for *S. gregaria* after treatment with a neem kernel suspension [75], a methanolic fruit extract from *Melia azedarach* [76] and *Fagonia bruguieri* extracts [77]. Also, decreased numbers were determined after treatment of penultimate instar nymphs of *Euprepocnemis plorans* with Margosan-o (a neem preparation)[78]. In agreement with those reported results, the present study on *S. gregaria*, after treatment with ethanol, petroleum ether and n-butanol extracts from *P. granatum* peel, recorded decreasing number of egg pods/♀ and number of eggs/pod. For some detail, all extracts exhibited a predominant inhibitory effect on the number of egg pods/♀ after treatment of penultimate instar nymphs. A similar effect was exhibited after treatment of last instar nymphs, regardless the extract or concentration level. Number of egg/pod is another important parameter of the oviposition efficiency. The petroleum ether extract and n-butanol extract exerted the strongest suppressing action of this number. In addition, the adult females had been enforced to lay eggs in a slow rate, as a response to *P. granatum* extracts, irrespective of the concentration level or time of treatment.

We right now have no unambiguous interpretation of the reduced number of eggs/pod or the regressed oviposition rate in *S. gregaria* by the action of *P. granatum* extracts, in the present study. However, the dropped number of egg pods/♀ may be attributed to a prohibitory effect of certain ingredient (s) in these extracts on the accessory glands which produce the pod matrix or on some hormonal regulation of the pod construction. This suggestion may be conceived in the light of isolated or identified chemical constituents from the pomegranate peel [34, 39].

Deranged reproductivity of *S. gregaria* by *P. granatum* extracts

Adult fecundity and fertility are very important reproductive criteria for investigating the effects of botanicals on the reproductive potential of insects. In the current study, treatment of penultimate instar nymphs of *S. gregaria* with ethanol extract or petroleum ether extract from *P. granatum* peel resulted in an insignificantly reduced fecundity while n-butanol extract caused a tremendous reduction in fecundity, especially at the higher concentration levels. After treatment of last instar nymphs, remarkably reduced fecundity was generally caused by all extracts, at the highest concentration level. With regard to fertility, a prevalent prohibitory effect was detected after treatment of penultimate instar nymphs with all extracts. Also, unexceptionally hindered fertility of eggs deposited by females was recorded after treatment of last instar nymphs. To a great extent, similar results of reduced fecundity and fertility had been reported for several insect pests by different neem preparations or azadirachtin derivatives, such as *Ceratitis capitata* [79], *Corcyra cephalonica* [80], *Pieris brassica* [81], *Liriomyza trifolii* [82], *Anopheles stephensi* and *Anopheles culicificacies* [83], *S. litura* [84-86], *Heliothis armigera* [87], *Spodoptera littoralis* [88], *Cosmopolites sordidus* [89], *Chrysomya megacephala* [90], *Muscina stabulans* [91], *M. domestica* [12], etc.

In addition to neem preparations, extracts from several plant species had been reported for deranging the fecundity and/or fertility of various insects, such as *S. littoralis* by extracts from *Abrus precatorius* [92]; *Boussingaultion gracilis* [93]; *Curcuma longa*, *Nicandra physaloides* and *Citrullus colocynthis* [94]; *Schimis terebinlhifolius* [95]; *M. azedarach* and *Lopus termis* [96], etc. On the desert locust *S. gregaria*, reduced fecundity and fertility had been estimated after nymphal treatments with *Fagonia brugieri* extracts [77] or *Nigella sativa* [97]. Also, Shekari et al. [98] reported similar reducing effect on fecundity and fertility of *Xanthogaleruca luteola* by the methanolic extract from *Artemisia annura*.

Table (1): Effects of *Punica granatum* extracts on some parameters of the oviposition efficiency (Mean ±SD) of *Schistocerca gregaria*

Solvent	Conc. (%)	Number of egg pods / female		Number of eggs / pod		Oviposition rate	
		After treatment of 4th instar nymphs	After treatment of 5th instar nymphs	After treatment of 4th instar nymphs	After treatment of 5th instar nymphs	After treatment of 4th instar nymphs	After treatment of 5th instar nymphs
Ethanol	40.0	2.0 ± 0.0 b	1.7 ± 0.6 b	46.2 ± 3.0 a	48.3 ± 4.3 a	4.5 ± 1.9 c	4.7 ± 1.4 c
	20.0	2.3 ± 0.6 a	2.3 ± 0.6 a	46.4 ± 3.3 a	51.6 ± 0.4 a	5.7 ± 0.7 b	6.6 ± 1.4 b
	10.0	2.3 ± 0.6 a	2.0 ± 0.0 b	46.1 ± 2.5 a	51.8 ± 5.6 a	6.1 ± 1.6 a	5.6 ± 1.9 b
	05.0	2.3 ± 0.6 a	2.3 ± 0.6 a	46.3 ± 2.0 a	49.4 ± 2.1 a	6.3 ± 1.9 a	7.0 ± 2.5 a
	02.5	2.3 ± 0.6 a	2.3 ± 1.0 a	43.5 ± 3.1 a	52.0 ± 5.9 a	5.7 ± 2.3 a	7.5 ± 2.4 a
	Controls	2.7 ± 0.6	2.8 ± 0.5	48.4 ± 2.9	55.9 ± 1.4	9.0 ± 1.9	9.6 ± 1.6
Petroleum ether	40.0	2.3 ± 0.6 a	2.0 ± 0.0 b	41.4 ± 2.0 b	40.2 ± 2.3 d	5.5 ± 0.9 a	4.3 ± 0.3 c
	20.0	2.3 ± 0.6 a	2.3 ± 0.6 a	47.1 ± 4.7 a	41.2 ± 3.7 c	6.0 ± 0.5 a	4.9 ± 0.9 b
	10.0	2.3 ± 0.6 a	2.0 ± 0.0 b	43.6 ± 1.9 a	42.5 ± 1.4 c	5.9 ± 2.1 a	4.9 ± 1.0 b
	05.0	2.0 ± 0.0 a	2.3 ± 0.6 a	45.3 ± 3.2 a	43.3 ± 1.3 c	5.7 ± 0.3 a	6.5 ± 2.3 a
	02.5	2.3 ± 0.6 a	2.3 ± 0.6 a	44.7 ± 2.7 a	47.0 ± 1.7 a	6.3 ± 1.7 a	7.7 ± 1.3 a
	Controls	2.3 ± 0.5	2.3 ± 0.6	48.5 ± 3.9	47.7 ± 1.4	7.5 ± 1.4	8.4 ± 2.1
n-butanol	40.0	2.0 ± 0.0 a	1.6 ± 0.6 b	41.7 ± 2.0 b	44.8 ± 2.8 b	4.0 ± 1.2 c	4.2 ± 1.7 b
	20.0	2.3 ± 0.6 a	1.7 ± 0.6 b	42.3 ± 0.9 b	44.2 ± 3.5 b	5.6 ± 0.8 b	4.3 ± 1.7 b
	10.0	2.0 ± 0.0 a	2.3 ± 0.6 a	42.6 ± 2.1 b	44.3 ± 3.7 b	4.3 ± 1.4 c	6.2 ± 0.8 a
	05.0	2.3 ± 0.6 a	2.3 ± 0.6 a	44.2 ± 1.6 a	44.3 ± 0.9 b	5.8 ± 1.0 b	6.2 ± 1.4 a
	02.5	2.3 ± 0.6 a	2.5 ± 0.6 a	46.2 ± 1.6 a	45.4 ± 2.9 b	6.7 ± 1.2 a	6.4 ± 1.6 a
	Controls	2.7 ± 0.6	2.5 ± 0.6	48.1 ± 3.4	48.4 ± 2.5	8.6 ± 1.0	8.6±1.5

Conc.: Concentration level. 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).

Table (2): Effects of *Punica granatum* extracts on the fecundity (Mean eggs±SD) of *Schistocerca gregaria*

Solvent	Conc. (%)	After treatment of 4th instar nymphs	After treatment of 5th instar nymphs
Ethanol	40.0	091.7 ± 45.8 a	080.0 ± 27.2 b
	20.0	111.7 ± 19.4 a	120.3 ± 29.2 a
	10.0	107.7 ± 27.6 a	100.0 ± 41.5 a
	05.0	108.3 ± 29.4 a	116.0 ± 33.8 a
	02.5	102.3 ± 31.2 a	113.5 ± 42.6 a
	Controls	129.0 ± 28.2	139.5 ± 22.3
Petroleum ether	40.0	096.7 ± 24.0 a	080.3 ± 12.1 b
	20.0	103.0 ± 17.4 a	095.0 ± 16.1 a
	10.0	098.5 ± 24.6 a	085.0 ± 15.8 a
	05.0	090.7 ± 16.4 a	100.3 ± 22.3 a
	02.5	103.3 ± 18.8 a	107.0 ± 23.8 a
	Controls	103.3 ± 18.8 a	111.3 ± 28.4
n-butanol	40.0	083.3 ± 21.0 b	073.7 ± 22.2 b
	20.0	098.3 ± 22.2 b	072.3 ± 21.2 a
	10.0	085.3 ± 21.1 b	102.0 ± 16.6 a
	05.0	103.3 ± 18.6 a	103.7 ± 28.0 a
	02.5	107.3 ± 24.1 a	112.3 ± 19.5 a
	Controls	127.0 ± 21.4	128.3 ± 23.1

Conc., a, b, c: See footnote of Table (1).

The reduced fecundity and prohibited fertility of *S. gregaria* by the extracts from *P. granatum* peel, in the current investigation, may be due to a juvenile hormone activity of certain active ingredient (s) contained in these extracts which have deleterious effects on the oogenesis, vitellogenin synthesis or vitellogenesis, via the disturbance of authentic hormone [99]. Also, the considerable derangement of these reproductive events can be attributed to the effects of *P. granatum* extracts on the vitelline envelope formation or the function of follicle cells [100, 101]. However, the intervention of the present extracts, or some of their chemical components, in the vitellogenin synthesis or vitellogenesis may be indirectly through the disruption of gonadotropic hormone production ot its function during the ovarian maturation. With special reference on the prohibited fertility of *S. gregaria*, the *P.*

granatum peel extracts, used in the present study, may seriously affect the survival of developing embryos at certain stages causing death as recorded in the decreasing hatching percentage. In any case, the exact mode of action of the present extracts, or some of their constituents, is still obscure and needs further investigation to be clearly understood.

Table (3): Effects of *Punica granatum* extracts on the fertility (Mean ±SD) and sterility index (%) of *Schistocerca gregaria*

Solvent	Conc. (%)	After treatment of 4th instar nymphs		After treatment of 5th instar nymphs	
		Fertility	Sterility index	Fertility	Sterility index
Ethanol	40.0	86.1 ± 1.6 a	34.0	82.7 ± 4.4 b	47.7
	20.0	84.0 ± 4.3 a	21.5	78.0 ± 3.4 b	25.0
	10.0	84.1 ± 3.2 a	24.3	83.4 ± 0.9 b	34.1
	05.0	89.7 ± 0.2 a	18.8	88.5 ± 2.2 a	18.9
	02.5	86.1 ± 2.7 a	26.2	87.7 ± 1.9 a	21.3
	Controls	92.7 ± 1.2	- - -	90.7 ± 1.6	- - -
Petroleum ether	40.0	83.6 ± 2.5 c	18.5	73.8 ± 7.4 c	41.8
	20.0	83.6 ± 2.5 c	10.1	79.8 ± 0.6 c	25.6
	10.0	85.0 ± 2.1 c	15.6	77.1 ± 3.3 c	35.6
	05.0	85.5 ± 3.0 b	21.8	84.9 ± 1.0 c	16.4
	02.5	88.3 ± 2.4 a	8.1	87.4 ± 1.3 c	08.4
	Controls	91.6 ± 1.0	- - -	91.5 ± 2.0	- - -
n-butanol	40.0	81.0 ± 3.4 c	42.4	84.1 ± 3.8 a	47.7
	20.0	79.5 ± 1.9 c	33.5	86.6 ± 3.6 a	47.1
	10.0	84.6 ± 1.7 c	38.6	87.9 ± 1.3 a	24.3
	05.0	88.7 ± 0.3 c	22.1	89.5 ± 1.6 a	21.6
	02.5	89.1 ± 2.5 a	18.7	89.6 ± 1.9 a	06.5
	Controls	92.6 ± 0.4	- - -	92.3 ± 1.0	- - -

Conc., a, b, c : See footnote of Table (1).

Table (4): Effects of *Punica granatum* extracts on the incubation period (Mean days ±SD) of *Schistocerca gregaria*

Solvent	Conc. (%)	After treatment of 4th instar nymphs	After treatment of 5th instar nymphs
Ethanol	40.0	19.8 ± 1.7 c	16.8 ± 1.0 b
	20.0	19.7 ± 1.5 c	18.1 ± 0.6 c
	10.0	17.9 ± 2.3 c	17.6 ± 2.3 b
	05.0	17.4 ± 0.7 c	17.7 ± 1.2 b
	02.5	18.2 ± 1.4 c	15.0 ± 1.4 a
	Controls	14.3 ± 0.4	14.6 ± 0.8
Petroleum ether	40.0	17.5 ± 1.3 c	18.5 ± 0.7 d
	20.0	17.1 ± 1.8 b	19.4 ± 0.1 d
	10.0	17.1 ± 1.8 a	17.8 ± 3.2 b
	05.0	16.8 ± 0.3 a	15.9 ± 2.2 a
	02.5	16.9 ± 2.3 a	14.0 ± 1.7 a
	Controls	16.9 ± 2.3	13.3 ± 0.3
n-butanol	40.0	17.8 ± 0.3 a	17.8 ± 2.5 a
	20.0	17.6 ± 2.4 a	17.3 ± 2.5 a
	10.0	17.7 ± 1.9 a	16.6 ± 1.9 a
	05.0	17.7 ± 0.8 a	16.7 ± 2.3 a
	02.5	16.0 ± 1.3 a	17.7 ± 1.3 a
	Controls	14.7 ± 1.1	15.0 ± 0.3

Conc.: a, b, c: See footnote of Table (1). (d): Very highly significantly different (P<0.001).

Retarded embryonic development in *S. gregaria* by *P. granatum* extracts

It is of an interest to refer the incubation period in insects can be used as a good indicator for the embryonic developmental rate, i.e., longer period may reveal slower rate and *vice versa*. In the present study, the embryonic developmental rate of *S. gregaria* had been generally subjected to an extended retarding effect of *P. granatum* extracts since the incubation period was slightly or pronouncedly lengthened after treatment of penultimate instar nymphs. A similar disruptive action was almost exerted on this vital process after treatment of last instar nymphs with these extracts. The current results are concomitant with some of the reported results of prolonged incubation period, and consequently retarded embryonic developmental rate in various insects by extracts from different plant species. As for examples, prolonged incubation period in *E. plorans* was recorded after treatment of 4th instar nymphs with Margosan-o [78], regressed embryonic developmental rate in *S. gregaria* was caused by nymphal treatments with *F. bruguieri* extracts [77], slightly prolonged incubation period in the same locust was reported after nymphal treatments with Neemazal (a neem preparation) or some extracts from *N. sativa* [97].

To explicate the prolonged incubation period, and consequently retarded embryonic developmental rate, by the action of plants extracts is unfortunately very scarce in the available literature. However, the retarded embryonic development in *S. gregaria* by the *P. granatum* peel extracts, as clearly shown in the current investigation, may be due to the effect of these extracts on the ecdysteroids responsible for the regulation of embryogenesis at certain developmental stages, especially those ecdysteroids originating from the ovaries of the adult females [102].

CONCLUSION

As obviously shown in the present study, extracts from *P. granatum* peel slightly or deleteriously affected the oviposition efficiency, fecundity, fertility and the embryonic development of *S. gregaria*. The current results have indicating the *P. granatum* peel extracts may be a promising alternative to synthetic insecticides for control this destructive locust. Alkaloids, triterpenoids, steroids, glycosides, saponins, flavonoids, polyphenols, anthocyanins and some other chemical constituents had been isolated from peel of *P. granatum* [34, 39]. Therefore, more investigation should be carried out to ascertain the active ingredient (s) responsible for these disruptive effects on the reproductive potential of *S. gregaria* and for shedding some light on their mode of action.

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