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# Inhibitory Effects of *Nigella Sativa* (Ranunculaceae) Extracts on the Reproduction of Desert Locust *Schistocerca Gregaria* (Orthoptera: Acrididae)

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**Abstract:** *The present study was carried out to investigate the disruptive effects of methanolic, petroleum ether and n-butanol extracts of N. sativa seeds, at 30.0, 15.0, 7.5, 3.7 & 1.8%, on the oviposition efficiency and other parameters of reproductive capacity of S. gregaria. The average number of egg pods/female slightly or remarkably decreased, depending on the extract, concentration and time of treatment. The n-butanol extract was the most potent inhibitory one on the average number of eggs/pod. Treatment of penultimate or last instar nymphs with N. sativa resulted in remarkably or slightly regressed oviposition rate, depending on the extract and concentration. Various reducing effects of N. sativa extracts on fecundity were determined. However, the strongest reducing effect was exhibited at the higher two concentrations, irrespective of the extract. After treatment of penultimate instar nymphs, the most tremendously reduced fertility was recorded by n-butanol extract. After treatment of last instar nymphs, fertility was affected, significantly or insignificantly, depending on the extract and its concentration. Treatment of penultimate instar nymphs resulted in slightly prolonged incubation period by methanolic extract indicating a slow down embryonic rate. A similar inhibitory effect on this rate could be almost obtained by petroleum ether extract while the strongest inhibitory effect was exhibited by n-butanol extract, at the majority of its concentrations. After treatment of last instar nymphs, the petroleum ether extract pronouncedly affected embryonic developmental rate during a prolonged incubation period.*

**Keywords:** *embryonic development, fecundity, fertility, incubation period, oviposition efficiency, oviposition rate.*

## 1. INTRODUCTION

Plagues of the desert locust *Schistocerca gregaria* (Forskal) have threatened agricultural production in Africa, the Middle East and Asia [1, 2]. Damage is caused as a consequence of its polyphagous behaviour, high population density, and the nature to aggregate and swarm [3]. Current locust control is mainly based on organophosphorus pesticides as a result of the banning of organochlorines [4]. 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 [5, 6], 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 [7-20]. In recent years interest in screening plants for various insecticidal activities has increased significantly and many potent compounds have been isolated and identified [21]. Jacobson [22] 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 *Peganum harmala* [23], *Calotropis procera* [24], *Calotropis gigantean* [25], *Azadirichta indica, Melia volkensii* [26], *Cestrum parqui* [27] and *Nerium oleander* [28, 29] were classified as plants with a toxic, repellent or deterrent effect on locusts due to some secondary compounds contained in these plants [30]. In addition, several plant species affect differentially the development, fertility, and behaviour of the desert locust [24].

Among the most important medicinal crops in Egypt is *Nigella sativa* (Ranunculaceae) which is commonly called as known as black seed or black cumin [31] and "Habbat al-barakah" (the seed of

blessing) in Arabic. Seeds of *N. sativa* and their oil have a long history of folklore usage in various systems of medicines. Sharma *et al*. [32] reviewed the medicinal, pharmacological, traditional value and folk remedies of this herb. Also, different pharmaceutic properties and medical uses had been reported [33-38]. In pest control, Deshpande *et al*. [39] reported that oleic and linoleic acid as insecticidal components from *N. sativa* which were found to be toxic to *Callosobruchus chinensis*. Similar results were obtained [40, 41]. The *N. sativa* extracts exhibited toxic effects on *Spodoptera littoralis* [42] and *S. gregaria* [20] in addition to disrupted growth, development and metamorphosis of the latter insect [20]. Also, Ahmad *et al*. [43] studied the insecticidal activity of *N. sativa* extracts against the larvae of *Trogoderma granarium* under laboratory conditions. Recently, Khan *et al.* [44] reported disturbing effects of the acetone seed extract on biology and invasion of the stored product pest *Tribolium castaneum*. The current study was carried out aiming to investigate the disruptive effects of various extracts of *N. sativa* seeds on the oviposition efficiency and other parameters of reproductive capacity of the destructive pest *S. gregaria*.

## 2. MATERIALS AND METHODS

### 2.1. 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 [45] and improved by Ghoneim *et al*. [14], insects were reared in wood formed cages (60 x 60 x 70 cm). 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 ($32\pm2^{o}$C). The insects were reared and handled under the crowded conditions. The feces, dead locusts and food remains were removed daily. Fresh clean leaves of clover *Trifolium alexandrinum* were provided, as a food for insects, every day.

### 2.2. Plant Extraction

Samples of *Nigella sativa* (Ranunculaceae) seeds were purchased from an Egyptian market. The samples were air-dried, powdered and kept in tightly closed amber coloured glass containers for protecting from light, at low temperature. Dried and pulverized powder of *N. sativa* (2 kg) was exhaustively separately extracted with methanol (1.7 Lx3). The combined alcohol extracts were concentrated to 400 ml, diluted with 400 ml of water and the next successively extracted with petroleum ether (5x400 ml) was concentrated to dryness under reduced pressure giving (11 and 90 g), and n-butanol (5x400 ml) extracts were concentrated to dryness under reduced pressure giving (75 and 55 g).

### 2.3. Nymphal treatments

The newly moulted $4^{th}$ (penultimate), or $5^{th}$ (last) instar nymphs of *S. gregaria* were fed on fresh leaves of *T. alexandrinum* after dipping in the different concentrations 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.

### 2.4. Reproductive parameters

Each of the successfully emerged adult females 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. Just after copulation, females were allowed to dig in the moistened sand floor for laying the egg pods. At the end of the reproductive life-time, 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 eggs in replicates were used to determine the fecundity by calculating the average number of eggs per female. 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*. [46] as follows:

Sterility Index = 100 - [(a b / A B) X 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.

In respect of the incubation period (mean day±SD), 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 ºC and moistened sandy bottom. Just after the oviposition, eggs were observed until hatching.

### 2.5. Statistical analysis of data

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction [47] for the test significance of difference between means.

## 3. RESULTS

### 3.1. Effects of *N. sativa* seed extracts on the oviposition efficiency

After treatment of penultimate ($4^{th}$) instar nymphs of *S. gregaria* with methanolic, petroleum ether and n-butanol extracts of *N. sativa* seeds, data of the average number of egg pods/♀ had been assorted in Table (1). Generally, it could be slightly depressed at the higher concentrations of all extracts. Significantly decreasing egg pods/♀ was recorded only at the highest concentration of n-butanol extract (1.3±0.6 compared to 2.8±0.5 egg pods/control females). Only at the higher two concentrations of petroleum ether extract and the highest concentration of n-butanol extract, average number of egg pods/♀ remarkably decreased (1.3±0.6 vs. 7.2±0.6 egg pods/ control females) after treatment of last ($5^{th}$) instar nymphs.

Another informative parameter of the oviposition efficiency is the average number of eggs/pod. As obviously demonstrated in the previously mentioned table, n-butanol extract was the most potent because it pronouncedly prohibited this parameter, regardless the concentration, after treatment of penultimate instar nymphs. Only at the highest concentration of methanolic extract (43.2±2.8 vs. 49.7±2.8 eggs/pod of control congeners) and the lower two concentrations of petroleum ether extract (43.4±2.4 and 42.8±3.3 vs. 49.7±2.8 eggs/pod of control congeners). After treatment of last instar nymphs, methanolic and n-butanol extracts exhibited predominantly inhibiting effects on the number of eggs/pod, except at the lowest concentration. On the other hand, petroleum ether extract exerted a similar inhibitory action only at the highest concentration (41.2±2.2 vs. 50.2±2.5 eggs/pod of control congeners).

Considering the oviposition rate, treatment of penultimate instar nymphs with *N. sativa* resulted in remarkably or slightly regressed rate, depending on the extract and concentration. For some detail, treatment with only the highest concentration of petroleum ether extract (10.4±0.8 vs. 7.8±0.8 of controls) and the higher two concentrations of n-butanol extract (3.6±1.4 and 6.6±0.8, respectively, vs. 12.3±1.0 of controls) resulted in drastically depressed oviposition rate (Table 1). After treatment of last instar nymphs with the same extracts, adult females had been retarded to lay eggs in normal rate, especially at the highest concentration of n-butanol extract (4.4±0.9 vs. 9.2±0.8 of controls), the higher four concentrations of methanolic extract (5.5±0.6, 5.9±0.4, 6.1±0.6 and 6.0±0.3, respectively, in comparison with 7.8±0.8 of controls) and the higher three concentrations of petroleum ether extract (4.5±1.0, 5.3±0.3 and 5.0±0.7, respectively, in comparison with 7.0±0.7 of controls).

**Table1.** *Effects of N. sativa extracts on some parameters of the oviposition efficiency of S. 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 |
| Methanol | 30.0 | 1.7 ± 0.6 a | 2.3 ± 0.6 a | 43.2 ± 2.8 b | 41.6 ± 2.6 b | 6.5 ± 1.2 a | 5.5 ± 0.6 b |
| | 15.0 | 2.0 ± 1.0 a | 2.3 ± 0.6 a | 49.6 ± 3.2 a | 43.8 ± 2.0 b | 6.6 ± 1.3 a | 5.9 ± 0.4 b |
| | 07.5 | 2.7 ± 0.6 a | 2.3 ± 0.6 a | 44.2 ± 3.1 a | 34.3 ± 3.4 b | 8.4 ± 0.9 a | 6.1 ± 0.6 b |
| | 03.7 | 2.7 ± 0.6 a | 2.7 ± 0.6 a | 42.5 ± 3.2 b | 39.4 ± 2.3 c | 6.7 ± 0.6 a | 6.0 ± 0.3 b |
| | 01.8 | 2.7 ± 0.6 a | 2.7 ± 0.6 a | 45.8 ± 2.8 a | 44.1 ± 3.9 a | 7.4 ± 0.6 a | 6.7 ± 0.5 a |
| | Controls | 2.7 ± 0.6 | 2.7 ± 0.6 | 49.7 ± 2.8 | 49.7 ± 2.8 | 7.8 ± 0.8 | 7.8 ± 0.8 |
| Petroleum ether | 30.0 | --- | 1.3 ± 0.6 b | --- | 41.2 ± 2.2 c | --- | 4.5 ± 1.0 b |
| | 15.0 | 1.7 ± 0.6 a | 1.3 ± 0.6 b | 45.8 ± 2.3 a | 46.9 ± 3.3 a | 10.4 ± 0.8 b | 5.3 ± 0.3 b |
| | 07.5 | 2.3 ± 0.6 a | 1.7 ± 0.6 a | 44.9 ± 3.2 a | 50.8 ± 2.7 a | 8.8±0.8 a | 5.0 ± 0.7 b |
| | 03.7 | 2.7 ± 0.6 a | 2.3 ± 0.6 a | 43.4 ± 2.4 b | 48.3 ± 3.1 a | 7.5 ± 0.6 a | 6.2 ± 0.7 a |
| | 01.8 | 2.7 ± 0.6 a | 2.3 ± 0.6 a | 42.8 ± 3.3 b | 48.2 ± 3.0 a | 6.9 ± 1.0 a | 6.4 ± 0.8 a |
| | Controls | 2.7 ± 0.6 | 2.7 ± 0.6 | 49.7 ± 2.8 | 50.2 ± 2.5 | 7.8 ± 0.8 | 7.0 ± 0.7 |
| n-butanol | 30.0 | --- | 1.3 ± 0.6 b | --- | 32.7 ± 2.9 d | --- | 4.4 ± 0.9 c |
| | 15.0 | 1.3 ± 0.6 b | 2.7 ± 0.6 a | 22.5 ± 3.7 d | 34.2 ± 2.4 d | 3.6 ± 1.4 d | 7.9 ± 0.9 a |
| | 07.5 | 2.5 ± 0.7 a | 2.7 ± 0.6 a | 23.8 ± 2.9 d | 45.5 ± 3.0 b | 6.6 ± 0.8 c | 9.0 ± 0.6 a |
| | 03.7 | 2.3 ± 0.6 a | 2.7 ± 0.6 a | 50.4 ± 2.1 c | 46.1 ± 2.8 b | 11.7 ± 0.7 a | 8.3 ± 1.0 a |
| | 01.8 | 2.7 ± 0.6 a | 2.7 ± 0.6 a | 52.8 ± 1.6 b | 49.6 ± 2.9 a | 13.4 ± 1.0 a | 8.6 ± 0.6 a |
| | Controls | 2.8 ± 0.5 | 2.7 ± 0.6 | 61.1 ± 2.5 | 54.7 ± 2.8 | 12.3 ± 1.0 | 9.2 ± 0.8 |

**Conc:** *Concentration level. 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). -: No reproductive adult females were produced.*

### 3.2. Effects of *N. sativa* seed extracts on fecundity and fertility

After treatment of penultimate instar nymphs with *N. sativa* seed extracts, data of adult female fecundity were arranged in Table (2). Fecundity was predominantly reduced by all extracts, especially at the highest concentration of methanolic extract (69.7±18.2 eggs/♀, vs. 130.7±18.3 eggs/♀ of controls) and petroleum ether extract (76.3 ±17.2 eggs/♀, vs. 130.7 ±18.3 eggs/♀ of controls) as well as at the majority of n-butanol extract concentrations (28.0 ±12.2, 57.0 ±19.8 and 113.7 ±19.3 eggs/♀ at 15.0, 7.5 and 3.7 %, respectively, vs. 168.3 ±21.9 eggs/♀ of controls). After treatment of last instar nymphs with *N. sativa* extracts, data contained in the same table prevail various reduction effects of *N. sativa* extracts on fecundity. Moreover, the stronger reducing effects were exhibited at the higher two concentrations of all extracts (95.3 ±11.8 and 100.1 ±10.1 eggs/♀ by methanolic extract, vs. 130.7 ±18.3 eggs/♀ of controls; 52.3 ±23.1 and 59.7 ±29.0 eggs/♀ by petroleum ether extract, vs. 133.0 ±24.2 eggs/♀ of controls; 41.3 ±19.9 and 89.7 ±16.9 eggs/♀ by n-butanol extract vs. 145.0 ±28.8 eggs/♀ of controls).

Concerning the egg fertility, data of Table (3) clearly reveal an inhibitory effect especially after treatment of penultimate instar nymphs with the highest concentration of both methanolic extract (84.7 ±0.6 vs. 87.4 ±0.9 % of controls) and petroleum ether extract (84.0 ±1.3 vs. 87.4 ±0.9 % of controls). In addition, the most tremendously reducing action on fertility was exerted by n-butanol extract (76.9 ±1.8, 79.9 ±3.4 and 87.9 ±2.3 %, at 15.0, 7.5 and 3.7 %, vs. 92.7 ±2.5 % of controls). The calculated sterility index often supported these data (for details, see Table 3). After treatment of last instar nymphs, fertility was significantly or insignificantly affected, depending on the extract and concentration. Only slightly reduced fertility was caused by methanolic extract while both petroleum ether and n-butanol extracts prohibited it, especially at the higher three or two concentrations, respectively (76.0 ±2.1, 76.6 ±2.0 and 80.1 ±1.7 % at 30.0, 15.0 and 7.5 % of petroleum ether extract,

vs. 87.6 ±0.5 % of controls; 81.3 ±2.5 and 84.7 ±1.7 % at 30.0 and 15.0 % of n-butanol extract, vs. 88.5 ±0.2 % of controls). Data of sterility index supported the present results of reduced fertility which was calculated in an almost dose-dependent course (for details, see Table 3).

**Table 2.** *Effects of N. sativa extracts on fecundity (Mean eggs±SD) of S. gregaria*

| Solvent | Conc. (%) | After treatment of 4th instar nymphs | After treatment of 5th instar nymphs |
|---|---|---|---|
| Methanol | 30.0 | 69.7 ± 18.2 b | 95.3 ± 11.8 b |
| | 15.0 | 92.0 ± 29.5 a | 100.1 ± 10.1 b |
| | 07.5 | 117.3 ± 23.4 a | 102.3 ± 19.3 a |
| | 03.7 | 112.3 ± 18.9 a | 103.7 ± 16.0 a |
| | 01.8 | 121.3 ± 21.5 a | 116.0 ± 16.5 a |
| | Controls | 130.7 ± 18.3 | 130.7 ± 18.3 |
| Petroleum ether | 30.0 | --- | 52.3 ± 23.1 b |
| | 15.0 | 76.3 ± 17.2 b | 59.7 ± 29.0 b |
| | 07.5 | 101.0 ± 18.7 a | 84.7 ± 25.7 a |
| | 03.7 | 114.7 ± 20.8 a | 109.0 ± 13.9 a |
| | 01.8 | 113.0 ± 21.0 a | 108.7 ± 24.6 a |
| | Controls | 130.7 ± 18.3 | 133.0 ± 24.2 |
| n-butanol | 30.0 | --- | 41.3 ± 19.9 c |
| | 15.0 | 28.0 ± 12.2 d | 89.7 ± 16.9 b |
| | 07.5 | 57.0 ± 19.8 d | 120.3 ± 20.1 a |
| | 03.7 | 113.7 ± 19.3 b | 122.0 ± 17.4 a |
| | 01.8 | 156.3 ± 24.7 a | 131.3 ± 20.1 a |
| | Controls | 168.3 ± 21.9 | 145.0 ± 28.8 |

Conc., a, b, c, d, -: see footnote of Table (1).

**Table 3.** *Effects of N. sativa extracts on fertility (Mean ±SD) and sterility index (%) of S. gregaria*

| Solvent | Conc. (%) | After treatment of 4th instar nymphs | | After treatment of 5th instar nymphs | |
|---|---|---|---|---|---|
| | | Fertility (%) | Sterility index (%) | Fertility (%) | Sterility index (%) |
| Methanol | 30.0 | 84.7 ± 0.6 b | 48.3 | 86.6 ± 0.6 a | 27.8 |
| | 15.0 | 87.0 ± 1.4 a | 29.9 | 86.5 ± 0.3 a | 23.4 |
| | 07.5 | 86.9 ± 0.1 a | 10.8 | 86.1 ± 0.6 a | 22.9 |
| | 03.7 | 87.0 ± 0.4 a | 14.5 | 86.6 ± 1.5 a | 21.4 |
| | 01.8 | 87.4 ± 1.2 a | 7.2 | 87.6 ± 2.0 a | 11.0 |
| | Controls | 87.4 ± 0.9 | --- | 87.4 ± 0.9 | --- |
| Petroleum ether | 30.0 | --- | --- | 76.0 ± 2.1 d | 65.7 |
| | 15.0 | 84.0 ± 1.3 b | 43.9 | 76.6 ± 2.0 d | 60.7 |
| | 07.5 | 86.5 ± 0.8 a | 23.5 | 80.1 ± 1.7 c | 41.8 |
| | 03.7 | 86.7 ± 0.7 a | 12.9 | 87.2 ± 0.6 a | 18.6 |
| | 01.8 | 86.8 ± 0.3 a | 14.1 | 87.2 ± 1.1 a | 18.6 |
| | Controls | 87.4 ± 0.9 | --- | 87.6 ± 0.5 | --- |
| n-butanol | 30.0 | --- | --- | 81.3 ± 2.5 c | 73.8 |
| | 15.0 | 76.9 ± 1.8 d | 86.2 | 84.7 ± 1.7 b | 40.8 |
| | 07.5 | 79.9 ± 3.4 c | 71.0 | 86.2 ± 2.3 a | 19.2 |

| | | | | | |
|---|---|---|---|---|---|
| | 03.7 | 87.9 ± 2.3 b | 35.9 | 87.9 ± 2.0 a | 16.4 |
| | 01.8 | 88.9 ± 1.8 a | 10.9 | 87.4 ± 1.4 a | 10.6 |
| | Controls | 92.7 ± 2.5 | --- | 88.5 ± 0.2 | --- |

Conc., a, b, c, d, -: see footnote of Table (1).

### 3.3. Effects of *N. sativa* seed extracts on embryonic development

The incubation period can be used as a good indicator for detecting the embryonic developmental rate. In the light of data distributed in Table (4**)**, treatment of penultimate instar nymphs with *N. sativa* extracts resulted in slightly prolonged incubation period by methanolic extract, i.e. the embryonic development rate was slower down as the concentration increased. Similar inhibitory effect on this rate could be almost exhibited by petroleum ether extract (16.2 ±1.0 days at 15.0 %, vs. 13.7 ±0.3 days of controls). The strongest inhibitory effect was exhibited by n-butanol extract at the majority of its concentrations (14.5 ±1.3, 13.2 ±1.6 and 13.6 ±0.8 days at 15.0, 7.5 and 3.7 %, vs. 10.2±1.0 days of controls). Depending on data of the aforementioned table, insignificantly prolonged incubation period of eggs, and subsequently slightly regressed embryonic rate, was recorded after treatment of last instar nymphs with methanolic or n-butanol extract of *N. sativa* seeds. Moreover, petroleum ether extract adversely inhibited the embryonic developmental rate because the incubation period was remarkably prolonged, especially at the higher three concentrations (18.3±1.5, 17.3±1.5 and 15.0±0.5 days, respectively, vs. 13.1±0.8 days of controls).

**Table 4.** *Effects of N. sativa extracts on the incubation period (Mean days ±SD) of S. gregaria*

| Solvent | Conc. (%) | After treatment of 4th instar nymphs | After treatment of 5th instar nymphs |
|---|---|---|---|
| Methanol | 30.0 | 15.0 ± 0.9 a | 14.1 ± 0.6 a |
| | 15.0 | 14.2 ± 0.3 a | 13.7 ± 0.3 a |
| | 07.5 | 13.9 ± 0.6 a | 13.7 ± 0.3 a |
| | 03.7 | 13.8 ± 0.4 a | 13.8 ± 0.2 a |
| | 01.8 | 13.9 ± 0.4 a | 13.7 ± 0.5 a |
| | Controls | 13.7 ± 0.3 | 13.7 ± 0.3 |
| Petroleum ether | 30.0 | --- | 18.3 ± 1.5 c |
| | 15.0 | 16.2 ± 1.0 b | 17.3 ± 1.5 b |
| | 07.5 | 13.8 ± 0.5 a | 15.0 ± 0.5 b |
| | 03.7 | 13.9 ± 0.5 a | 13.2 ± 1.0 a |
| | 01.8 | 13.9 ± 0.2 a | 13.3 ± 0.3 a |
| | Controls | 13.7 ± 0.3 | 13.1 ± 0.8 |
| n-butanol | 30.0 | --- | 16.5 ± 0.5 a |
| | 15.0 | 14.5 ± 1.3 c | 14.7 ± 0.3 a |
| | 07.5 | 13.2 ± 1.6 b | 14.4 ± 0.5 a |
| | 03.7 | 13.6 ± 0.8 c | 14.3 ± 0.3 a |
| | 01.8 | 11.9 ± 1.2 a | 14.4 ± 0.1 a |
| | Controls | 10.2 ± 1.0 | 14.2 ± 0.3 |

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

## 4. DISCUSSION

### 4.1. Deranged oviposition efficiency of *S. gregaria*

As reported in the available literature, parameters of oviposition efficiency, such as the number of egg pods/female, number of eggs/pod and/or oviposition rate, of few insect species had been reduced by extracts of different plant species. Considerably reduced oviposition efficiency had been recorded for *S. gregaria* after treatment with a neem kernel suspension [48], a methanolic fruit extract of *Melia azedarach* [49], *Fagonia bruguieri* extracts [50] and *Punica granatum* peel extracts [17]. Also, significantly reduced oviposition efficiency of the same locust was determined after treatment of

solitary and gregarious females, during the oviposition period, with extracts of froth or eggs [51]. In agreement with those reported results, the present study obviously show various degrees of reduction in the number of egg pods/♀ and eggs/pod of *S. gregaria* after treatment of nymphs with *Nigella sativa* seed extracts. As well as the oviposition rate was remarkably or slightly regressed, depending on the extract, concentration and time of treatment.

Unfortunately, this reduced oviposition efficiency of *S. gregaria* cannot be exactly interpreted right now because further investigation should be carried out to explore the active ingredient(s) in these extracts responsible for these effects. However, some of their chemical constituents may exhibit prohibitory effects on the accessory glands which produce the pod matrix or on some hormonal regulation of the pod construction as well as of the oviposition rate. This suggestion may be conceived in the light of identified chemical components of *N. sativa*, such as conjugated linoleic acid, thymoquinone, nigellone, melanthin, nigilline, damascenine, tannins, flavonoids, saponins, alkaloids, and anethole 4-terpinole [32, 52-55]. In addition, some of these constituents may exert a reverse action to those exerted by ecdysteroid agonists which stimulate the neurosecretory cells to release a myotropic ovulation hormone [56, 57].

### 4.2. Reduced fecundity and fertility of *S. gregaria*

Adult fecundity and fertility (egg hatchability or viability) are very important reproductive parameters for investigating the effects of botanicals on the reproductive potential of insects. Various neem preparations or azadirachtin derivatives had been reported to reduce fecundity and fertility of several insects, such as *Ceratitis capitata* [58], *Corcyra cephalonica* [59], *Cnaphalocrocis medinalis* [60], *Pieris brassica* [61], *Liriomyza trifolii* [62], *Anopheles stephensi* [63], *Heliothis armigera* [64], *Spodoptera littoralis* [65], *Cosmopolites sordidus* [66], *Muscina stabulans* [67], *Spodoptera litura* [68], *Musca domestica* [12], *Chrysomya megacephala* [69] and *Earias vittella* [70]. In addition, fecundity and/or fertility of various insects had been reduced by extracts of several plant species, such as *S. littoralis* by *Abrus precatorius* [71], *Boussingaultion gracilis* [72], *Curcuma longa* [73], *Schimis terebinlhifolius* [74] and *Lopus termis* [75]. Also, similar reducing effect was recorded on fecundity and fertility of *Xanthogaleruca luteola* by the methanolic extract of *Artemisia annura* [76], *Anopheles aegypti* by extracts of *Pengamia pinnata* [77] and *Helicoverpa armigera* by methanolic leaf extract and essential oil of *Artemisia annua* [78]. Results of the present study on *S. gregaria* agree, to a great extent, with those reported results since prohibited adult fecundity was recorded after treatment of penultimate instar nymphs with all *N. sativa* seed extracts. After treatment of last instar nymphs, various reducing effects of *N. sativa* extracts on fecundity were determined. Concerning the egg fertility, a reducing effect, especially at the highest concentration of both methanolic extract and petroleum ether extract applied on the penultimate instar nymphs, was recorded. After treatment of last instar nymphs, fertility was affected, significantly or insignificantly, depending on the extract and its concentration. Similar reduced fecundity and fertility of the same locust were also observed after nymphal treatment with extracts of *F. bruguieri* [50], *Jatropha curcas* oil [79], hexane and ethanol extracts of froth or eggs of the same locust [51] and *P. granatum* peel extracts [17].

The reduced fecundity of *S. gregaria* by the seed extracts of *N. sativa*, in the current investigation, may be due to a juvenile hormone activity of certain active ingredient(s) in these extracts which have deleterious effects on the oogenesis, vitellogenin synthesis or vitellogenesis, *via* the disturbance of the authentic hormone [80]. However, the intervention of these extracts, or some of their chemical components, in the vitellogenin synthesis or vitellogenesis may be indirectly through the disruption of gonadotropic hormone production or its function during the ovarian maturation. Also, the considerable derangement of this reproductive event can be attributed to the effects of *N. sativa* seed extracts on the vitelline envelope formation or the function of follicle cells [81, 82] or an adverse effect on the morphogenesis of ovipositor of adult females, ovarian growth and/or synthesis and metabolism of proteinaceous constituents during the oogenesis [56, 83]. In addition, it may be acceptable to suggest that the reduced reproductive output of *S. gregaria*, in the current work, may be due to an inhibitory effect of *N. sativa* seed extracts on synthesis of both DNA and RNA, suboptimal nutrition owing to reduced feeding, altered mating behaviour as a result of sublethal intoxication, or a combination of factors.

On the other hand, the prohibited fertility of *S. gregaria* after nymphal treatment with *N. sativa* seed extracts, in the present study, can be explicated by a serious lethal action of these extracts on the

developing embryos at certain stages causing death. Another interpretation may be accepted since the developing embryos suffered morphological deformation and incomplete development of some body parts after treatment of the same locust with azadirachtin [84]. Also, the reduction in fertility may be due to the penetration of residual amounts of some *N. sativa* seed contents in mothers into their eggs and disturbance of embryonic cuticle synthesis. So, the fully mature embryos had weak chitinous mouth parts that were insufficiently rigid to perforate the surrounding vitellin membrane and free from the eggs [85, 86]. It is well known that the maturation of insect eggs depends basically on the vitellogenins, precursor materials of vitellins, including proteins, lipids and carbohydrates, all of which are necessarily required for the embryonic development [87, 88]. These materials are synthesized primarily by fat body during the immature stages [89] or by the ovary *in situ* [90]. Wherever the site of synthesis of these materials, *N. sativa* seed extracts, or some of their chemicals, may disturb the production and/or accumulation of these metabolites in adult females leading to reduction of fertility in *S. gregaria*, in the present study. It is important to point out that some insect growth regulators have disruptive effects on the insect reproduction by the perturbation of gene expression in the hierarchy cascade of vitellogenesis and/or choriogenesis [91]. Some of *N. sativa* chemical components may interfere with the gene expression resulting in a reduction of the developing embryos, in the present study. However, the exact mode of action is still obscure and needs further investigation to be clearly understood.

### 4.3. Retarded embryonic development in *S. gregaria*

In the majority of insects, incubation period 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 on *S. gregaria*, treatment of penultimate instar nymphs with methanolic extract of *N. sativa* seeds resulted in slightly prolonged incubation period indicating a slow embryonic rate. A similar inhibitory effect could be almost obtained by petroleum ether extract while the strongest inhibitory effect was exhibited by n-butanol extract, at the majority of concentrations. After treatment of last instar nymphs with all extracts, the embryonic developmental rate was slightly or pronouncedly regressed. These results are concomitant with some of the reported results of prolonged incubation period and retarded embryonic developmental rate in various insects by extracts of different plants, such as *Euprepocnemis plorans* by Margosan-O (a neem preparation)[92], *S. gregaria* by *F. bruguieri* extracts [50], the same locust by Neemazal (a neem preparation) [84, 93] and *P. granatum* peel extracts [17]. The retarded embryonic development in *S. gregaria* by the *N. sativa* seed 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 stages, especially those ecdysteroids originating from the ovaries of the adult females [88].

## 5. CONCLUSION

As clearly shown in the present study, seed extracts of *N. sativa* have inhibitory effects on the oviposition efficiency and other parameters of reproductive potential of the desert locust *S. gregaria*. Therefore, *N. sativa* possesses potential secondary metabolites that may be useful for control this destructive pest. However, further investigation should be carried out to explore the active ingredient(s) responsible for these disruptive effects on the reproductive events and their mode of action.

## REFERENCES

[1]. Showler, A.T., Desert locust control, public health, and environmental sustainability in North Africa. In: "The North African environment at risk" (Swearingen W.D. and Bencherifa A., eds.). West view Press, Boulder, CO., USA, 217-239 (1995).

[2]. Ceccato P., Cressman K., Giannini A. and 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. Int. J. Pest Manage. 53(1), 7-13(2007).

[3]. Lindsey R., Locusts. http://earth. Observatory. NASA. Gov/ Observatory/(2002).

[4]. Lecoq M., Recent progress in desert and migratory locust management in Africa. Are preventive actions possible? J.Orthoptera Res. 10, 277-291(2001).

[5]. Zettler J.L. and Cuperus G.W., Pesticide resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrchidae) in wheat. J. Econ. Entomol. 83, 1677-1681(1990).

[6]. Donahay E., Zalach D. and Rindner M., Comparison of the sensitivity of the development stages of three strains of the red four beetle (Coleoptera: Tenebrionidae) to modified atmospheres. J. Econ. Entomol. 85, 1450-1452(1992).

[7]. Abahussain M.O., The effect of *Calotropis procera* (AIT) (Asclepiadacea) on *Culex pipiens* and *Anopheles multicolor* (Diptera Culicidae).J. Egypt. Ger. Soc. Zool. 30, 205-214(1999).

[8]. Al-Dali A.G., Abdel-Ghaffar A. and Ghoneim K.S., Efficacy of the neem seed extract, Margosan-0, on the developmental and morphogenesis of *Muscina stabulans* (Diptera: Muscidae). J. Egypt. Ger. Soc. Zool. 42, 85-100(2003).

[9]. Kassem M.A., Mohammad T.A. and Bream A.S., Influence of the bioinsecticides, NeemAzal, on main body metabolites of the 3rd larval instar of the house fly *Musca domestica* (Diptera: Muscidae). Afr. J. Biochem. Res. 5(9), 272-276(2011).

[10]. Ghoneim K.S., Mohamed H.A. and Bream A.S., Efficacy of the neem seed extract NeemAzal on the growth and development of the Egyptian cotton leafworm, *Spodoptera littoralis* Boisd (Lepidoptera: Noctuidae). J. Egypt. Ger. Soc. Zool. 33(E), 161-179(2000).

[11]. Ghoneim K.S., Abdel-Ghaffar A.A., Amer M.S., Bream A.S., Al-Dali A.G. and Hamadah Kh.Sh., Effects of certain insect growth regulators and plant extracts on carbohydrate metabolism in the house fly, *Musca domestica* (Diptera: Muscidae). Egypt. J. Biomed. Sci. 21, 206-219(2006).

[12]. Ghoneim K.S., Amer M.S., Bream A.S., Al-Dali A.G. and Hamadah Kh.Sh., Effectiveness of Margosan-O and Jojoba on some reproductive aspects of the house fly, *Musca domestica* (Diptera: Muscidae). Int. J. Agric. Biol. 9(2), 338-341(2007).

[13]. Ghoneim K.S., Abdel-Ghaffar A.A. and Tanani M.A., Changes of acid phosphatases activity by the plant extracts, Margosan-O and Jojoba, during the pupal stage of *Musca domestica* (Diptera: Muscidae). J. Egypt. Acad. Sci. Environ. Develop. 9(1), 47-55(2008).

[14]. Ghoneim K.S., Tanani M.A. and Basiouny A.L., Influenced survival and development of the desert locust *Schistocerca gregaria* (Acrididae) by the wild plant *Fagonia bruguieri* (Zygophyllaceae). Egypt. Acad. J. Biol. Sci. 2(2), 147-164(2009).

[15]. Ghoneim K., Amer M., Al-Daly A., Mohammad A., Khadrawy F. and Mahmoud M., Disrupted survival, growth and development of desert locust *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae) by extracts from toothpick weed *Ammi visnaga* Lamarck (Apiaceae). Int. J. Biosci. 5(1), 397-414(2014).

[16]. Ghoneim K., Amer M., Al-Daly A., Mohammad A., Khadrawy F. and Mahmoud M.A., Effectiveness of *Punica granatum* Linn. (Lythraceae) extracts on the adult performance of desert locust *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae). Entomol. App. Sci. Letters 1(2), 9-19(2014).

[17]. Ghoneim K., Al-Daly A., Amer M., Mohammad A., Khadrawy F. and Mahmoud M.A., Disruptive effects of pomegranate *Punica granatum* Linn. (Lythraceae) extracts on the reproductive potential of desert locust *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae). Entomol.App. Sci. Letters 1(1), 1-10(2014).

[18]. Ghoneim K., Mohammad A.A., Al-Daly A.G., Amer M.S., Khadrawy F. and Mahmoud M.A., Metabolic responsiveness of desert locust *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae) to the khella plant *Ammi visnaga* L. (Apiaceae) extracts. International J. Adv. Life Sci. 7(2), 204-216(2014).

[19]. Ghoneim K., Hamadah Kh., Amer M., El-Hela A. and Mohammad A., Qualitative and quantitative changes in the haemogram of desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) by extracts of *Nigella sativa* (Ranunculaceae). J. Adv. Biol. 7(2), 1275-1292(2015).

[20]. Hamadah Kh.Sh., Ghoneim K.S., El-Hela A.A., Amer S.M. and Mohammad A.A.1., Disturbed survival, growth and development of the desert locust *Schistocerca gregaria* by different extracts of *Azadirachta indica* (Meliaceae) and *Nigella sativa* (Ranunculaceae). Egypt. Acad. J. Biolog. Sci. 6(2), 01-21(2013).

[21]. Weinzierl R.A., Botanical insecticides, soaps, and oils. In: "Biological and Biotechnological Control of Insect Pests"(Rechcigl J.E. and Rechcigl N.A., eds.). Lewis Publishers, pp. 101-121(1998).

[22]. Jacobson M., Botanical pesticides, past, present and future. In: "Insecticides of plant origin" (Arnason J.T., ed.). Proc. Amr. Chemical Soc. Washington, DC, 1-10(1989).

[23]. Abbassi K., Mergaoui L., Atay-Kadiri Z., Stambouli A. and Ghaout S., Effets des extraits de *Peganum harmala* (Zygophyllaceae) sur le criquet pèlerin (*Schistocerca gregaria* Forskål, 1775). Zoologica Baetica 13/14, 203-217(2003).

[24]. Abbassi K., Atay-Kadiri Z. and Ghaout S., Activité biologique des feuilles de *Calotropis procera* sur le criquet pèlerin (*Schistocerca gregaria* Forsk. 1775). Zoologica Baetica 15, 153-166(2004).

[25]. Pari K., Rao P.J., Devacumar C. and Rastogi J.N., A novel insect antifeedant nonprotein amino acid from *Calotropis gigantea*. J. Natural Products 61, 102-104(1998).

[26]. Rembold H., *Melia volkensii*: a natural insecticide against desert locust. In: "New strategies in locust control"(Krall S., Peveling R. and Diallo D.B., eds.). Birkhäuser Verlag, Basel pp. 185-191(1997).

[27]. Barbouche N., Hajjem B., Lognay G. and Ammar M., Contribution à l'étude l'activité biologique d'extraits de feuilles de *Cestrum parqui* L'Herit. (Solanaceae) sur le criquet pèlerin *Schistocerca gregaria* (Forsk.). Biotechnol. Agron. Soc. Environ. 5, 85-90(2001).

[28]. Barbosa R.R., Fonthenel-Neto J.D. and Soto-Blanco B., Toxicity in goats caused by oleander (*Nerium oleander*). Res. Veter. Sci. 85, 279-281(2008).

[29]. Bagari M., Bouhaimi A., Ghaout S. and Chihrane J., The toxic effects of *Nerium oleander* on larvae of the desert locust *Schistocerca gregaria* (Forskål, 1775) (Ortoptera, Acrididae). Zool. baetica 24, 193-203(2013).

[30]. Bruneton J., Plantes toxiques. Végétaux dangereux pour l'homme et les animaux. Lavoisier, Paris, 529 pp(1996).

[31]. Rayan H.Z., Wagih H.M. and Atwa M.M., Efficacy of Black Seed oil from *Nigella sativa* against murine infection with cysts of Me49 strain of *Toxoplasma gondii*. Parasitologists United J. 4(2), 165-176(2011).

[32]. Sharma N.K., Ahirwar D., Jhade D. and Gupta S., Medicinal and phamacological potential of *Nigella sativa*: a review. Ethnobotanical Rev. 13, 946-955(2009).

[33]. Perveen T., Haider S., Kanwal S. and Haleem D.J., Repeated administration of *Nigella sativa* decreases 5-HT turnover and produces anxiolytic effects in rats. Pak. J. Pharmaceut. Sci. 22, 139-144(2009).

[34]. Fahmy H.M., Noor N.A., Mohammed F.F., Elsayed A.A. and Radwan N.M., *Nigella sativa* as an anti-inflammatory and promising remyelinating agent in the cortex and hippocampus of experimental autoimmune encephalomyelitis-induced rats. J. Basic Appl. Zool. 67(5), 182-195(2014).

[35]. Üstün K., Taysi S., Sezer U., Demir E., Baysal E., Demir T., Sarıçiçek E., Alkiş H., Şenyurt S.Z., Tarakçıoğlu M. and Aksoy N., Radio-protective effects of *Nigella sativa* oil on oxidative stress in tongue tissue of rats. Oral Diseases 20(1), 109-113(2014).

[36]. Ping N.C., Hashim N.H. and Adli D.S.H., Effects of *Nigella sativa* (*Habbatus sauda*) oil and nicotine chronic treatments on sperm parameters and testis histological features of rats. Evidence-Based Complemen. Alter. Medicine, Article ID 218293, 7 pp. (2014).

[37]. Shariatifar A., Riazi M., Ebnolelm M. and Jahromy M.H., Effects of *Nigella sativa* L. seed extract on fatigue, blood biochemical parameters and thyroid function in male mice. Chinese Medicine 5, 16-21(2014).

[38]. Mahdavi R., Heshmati J. and Namazi N., Effects of black seeds (*Nigella sativa*) on male infertility: A systematic review. J.Herbal Medicine 03,(2015).

[39]. Deshpande R.S., Adhikary P.R. and Tipnis H.P., Stored grain pest control agents from *Nigella sativa* and *Pogostemon heyneanus*. Bull. Grain Technol. 12(3), 232-234(1974).

[40]. Adebowale, K.O. and Adedire C.O., Chemical composition and insecticidal properties of the underutilized *Jatropha curcas* seed oil. Afr. J. Biotechnol. 5(10), 901-906(2006).

[41]. Adabie-Gomez D.A., Monford K.G., Agyir-Yawson A., Owusu-Biney A. and Osae M., Evaluation of four local plant species for insecticidal activity against *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) and *Callosobruchus maculates* (F) (Coleoptera: Bruchidae). Ghana J. Agric. Sci. 39, 147-154(2006).

[42]. Abd ELatif M.E., Abd El-Nabi L.M.A., Hussein E.H. and Abd El-Hafez Z.A., Effect of two methods of *Nigella* and *Arugula* oils extraction and its efficacy on *Spodoptera littoralis* (Boisd.). J. Agric. Res. Kafrelsheikh Univ. 35(4), 1069-1081(2009).

[43]. Ahmad F., Sagheer M., Hammad A., Rahman S.M.M. and Ul-Hasan M., Insecticidal activity of some plant extracts against *Trogoderma granarium* (E.). The Agriculturists 11(1), 103-111(2013).

[44]. [44]Khan F.Z.A., Sagheer M., ul-Hasan M., ul-Hassan M.N., Farhan, M. and Abdul Rahman, Bioactivity of *Nigella sativa, Syzygium aromaticum* and *Trachyspermum ammi* extracts against *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae). J.Entomol. Zool. Studies 2(3), 103-105(2014).

[45]. Hunter-Jones P., Rearing and breeding locusts in the laboratory. Bull. Anti-locust Res. Center London, 12 pp(1961).

[46]. Toppozada A., Abd-allah S. and El-Defrawi M.E., Chemosterilization of larvae and adults of the Egyptian cotton leafworm, *Prodenia litura* by apholate, metepa and hempa. J. Econ. Entomol. 59, 1125-1128(1966).

[47]. Moroney M.J., Facts from Figures. 3rd ed., Pinguin Book Ltd. Harmondsworth, Middlesex, 228 pp(1956).

[48]. Brajendra S., Singh A.P. and Singh B., Effects of neem kernel suspension on the hatchability of eggs of the desert locust *Schistocerca gregaria* Forsk. J. Adv. Zool. 8(1), 52-54(1987).

[49]. Schmidt G.H. and Assembe-Tsoungui S., Effect of oral application of *Melia* fruit extract on growth, development and gregarization of the desert locust *Schistocerca gregaria* (Forskal, 1775) (Caelifera, Acrididae). Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz. 109(1), 38-56(2002).

[50]. Basiouny A.L.I., Reproductive responsiveness of *Schistocerca gregaria* (Orthoptera: Acrididae) to different extracts from the wild plant *Fagonia bruguieri*. J.Biol. Pharm. Sci. 6(1), 178-193(2008).

[51]. Eid M.A., El-Maasarawy S.A.S., Elshabrawy H.A. and Elsayed G., Do egg pods in the desert locust *Schistocerca gregaria* display as oogenesis limiting factor? II- Effects of females treated during the ovipositing period. Pak. J. Biol. Sci. 7(4), 589-592(2004).

[52]. Burits M. and Bucar F., Antioxidant activity of *Nigella sativa* essential oil. Phytother. Res. 14(5), 323-328(2000).

[53]. Al-Ghamdi M.S., The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*. J. Ethnopharmacol., 76(1):45-48(2001).

[54]. Ali B.H. and Blunden G., Pharmacological and toxicological properties of *Nigella sativa*. Phytother. Res., 17(4): 299-305(2003).

[55]. Ali M.A., Sayeed M.A., Alam M.S., Yeasmin M.S., Khan A.M. and Muhamad, I.I., Characteristics of oils and nutrient contents of *Nigella sativa* Linn. and *Trigonella foenum-graecum* seeds. Bull. Chem. Soc. Ethiop. 26, 55-64(2012).

[56]. Smagghe G., Vinuela E., Budia F. and Degheele D., *In vivo* and *in vitro* effects of the nonsteroidal ecdysteroid agonist tebufenozide on cuticle formation in *Spodoptera exigua*: an ultrastructural approach. Arch. Insect Biochem. Physiol. 32, 121-134(1996).

[57]. Parween S., Faruki S.I. and Begum M., Impairment of reproduction in the red flour beetle, *Tribolium castaneum* (Herbst.)(Coleoptera: Tenebrionidae) due to larval feeding on triflumuron-treated diet. J.App.Entomol. 125, 1-4(2001).

[58]. Steffens R.J. and Schmutterer H., The effect of a crude methanolic neem (*Azadirachta indica*) seed kernel extract on metamorphosis and quality of adults of the Mediterranean fruit fly, *Ceratitis capitata* Wied. (Diptera: Tephritidae). Z Angew Entomol. 94, 98-103(1982).

[59]. Pathak P.H. and Krishna S.S., Neem seed oil, a capable ingredient to check rice moth reproduction (Lepidoptera, Galleriidae). Z angew. Entomol. 100, 33-35(1985).

[60]. Dorn A., Rademacher J.M. and Sehn E., Effects of azadirachtin of the moulting cycle, endocrine system, and ovaries in last instar larvae of the milkweed bug, *Oncopeltus fasciatus*. J. Insect Physiol. 32, 31-38(1986).

[61]. Singh K., Sharma P.L. and Singh K.L., Evaluation of antifeedant and repellent qualities of various neem (*Azadirachta indica*) formulations against *Pieris brassicae* Linn. larvae on cabbage and cauliflower. Res. Devel. Reporter 4(1), 76-78(1987).

[62]. Parkman P. and Pienkowski R.L., Sublethal effects of neem seed extract on adults of *Lyriomiza trifolii* (Diptera: Agromyzidae). J. Econ. Entomol. 83, 1246-1249(1990).

[63]. Dhar R., Dawar H., Garg S., Basir S.F. and Talwar G.P., Effect of volatiles from neem and other natural products on gonotrophic cycle and oviposition of *Anopheles stephensi* and *An. culicifacies* (Diptera: Culicidae). J. Med. Entomol. 33, 195-201(1996).

[64]. Gu Y-F. and Zheng H-X., Study on the effects of extracts from *Melia azedarach* on development and fecundity of *Heliothis armigera*. Acta Agriculturae Universitatis Henanensis 32(4), 403-406(1998).

[65]. Mohamed H.A., Bream A.S. and Ghoneim K.S., Developmental and reproductive responses of the house fly *Musca domestica* (Diptera: Muscidae) to the neem seed extract, NeemAzal. J. Egypt. Ger. Soc. Zool. 31, 235-249(2000).

[66]. [66]Musabyimana T., Saxena R.C., Kairu E.W., Ogol C.P.K.O., Khan Z.R., Effects of neem seed derivatives on behavioral and physiological responses of *Cosmopolites sordidus* (Coleoptera: Curculionidae). J. Econ. Entomol. 94, 449-454(2001).

[67]. Ghoneim K.S. and Al-Dali A.G., Survival and reproductive responses of *Muscina stabulans* Fallen (Muscidae: Diptera) to the neem seed extract, Margosan-O. Proc. 2nd Int. Conf., Plant Prot. Res. Inst. Cairo, Egypt, 21-24 Dec. (2002).

[68]. Senthil Nathan S., Kalaivani K. and Chung P.G., The effects of azadirachtin and nucleopolyhedrovirus on midgut enzymatic profile of *Spodoptera litura* Fab. (Lepidoptera: Noctuidae), Pestic. Biochem. Physiol. 83, 46-57(2005).

[69]. Siriwattanarungsee S., Sukontason K.L., Olson J.K., Chailapakul O. and Sukontason K., Efficacy of neemextract against the blowfly and housefly. Parasitol. Res. 103, 535-544(2008).

[70]. Bhardwaj A.K. and Ansari B.A., Effect of Nimbecidine and Neemazal on the developmental programming of cotton pest, *Earias vittella*. J.Entomol. Zool. Studies 3(1), 38-42(2015).

[71]. Dimetry N.Z. and Abdalla E.F., Bioactivity of *Abrus precatorius* seeds extracts against the cow pea aphid, *Aphis oraccivora* Koch. Bull. Entomol. Soc. Egypt (Econ. Ser.) 17, 159-162(1988).

[72]. Badawy H.M.A., Biological effect of *Bussingaultia gracilis* acetone extract on the larvae of cotton leafworm *Spodoptera littoralis* (Biosd.). Bull. Entomol. Soc. Egypt (Econ. Ser.) 22, 45-54(1995).

[73]. Mogahad M.I., Sharaby A.F. and El-Gengaihi S.M., Utilization of Egyptian plant as natural pesticides against *Spodoptera littoralis* (Boisd.). Bull. Entomol. Soc. Egypt (Econ. Ser.) 24, 107-114(1997).

[74]. Shaurub E.S.H., Ahmed Z.A. and El-naggar S.E.M., Impacts of pyriproxyfen and extract of *Schinus terebinthifolius* Raddi on development, reproduction and reproductive organs in *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). J. Egypt Ger. Soc. Zool. 27(E), 57-82(1998).

[75]. Hassan H.A., Biological and biochemical studies on the effects of some botanical extracts on cotton leafwom *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). M.Sc. Thesis, Fac. Sci., Ain Shams Univ., Cairo, Egypt (2002).

[76]. Shekari M., Sendi J.J., Etebari K., Zibaee A. and Shadparvar A., Effects of *Artemisia annua* L. (Asteracea) on nutritional physiology and enzyme activities of elm leaf beetle, *Xanthogaleruca luteola* Mull. (Coleoptera: Chrysomellidae). Pestic. Biochem. Physiol. 91, 66-74(2008).

[77]. Anithasuganthi S., Thiraviaraj S. and Dhanasekaran S., Mosquitocidal activities of *Pongamia pinnata* Linn against *Aedes aegypti* (Linn.), *Anopheles stephensi* Liston and *Culex quinquefasciatus* (Say) (Diptera: Culicidae). Int. J.Current Innov. Res. 1(11), 51-57(2014).

[78]. Anshul N., Kalra A. and Singh D., Biological effect of sweet wormwood, *Artemisia annua* methanol extracts and essential oil against *Helicoverpa armigera* Hub. (Lepidoptera: Noctuidae). J. Entomol. Zool. Studies 2(6), 304-307(2014).

[79]. Bashir E.M. and El Shafie H.A.F., Insecticidal and Antifeedant Efficacy of *Jatropha* oil extract against the desert locust, *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae). Agricultural. Biol. J. of North Amer. 4(3), 260-267(2013).

[80]. Di Ilio V., Cristofaro M., Marchini D., Nobili, P. and Dallai R., Effects of a neem compound on the fecundity and longevity of *Ceratitis capitata* (Diptera: Tephritidae). J. Econ. Entomol. 92, 76- 82(1999).

[81]. Lucantoni L., Giusti F., Cristofaro M., Pasqualini L., Esposito F., Lupetti P. and Habluetzel A., Effects of a neem extract on blood feeding, oviposition and oocyte ultrastructure in *Anopheles stephensi* Liston (Diptera: Culicidae). Tissue Cell 38, 361-371(2006).

[82]. Khan M., Hossain M.A. and Islam M.S., Effects of neem leaf dust and a commercial formulation of a neem compound on the longevity, fecundity and ovarian development of the melon fly, *Bactocera cucurbitae* (Coquillett) and the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). Pak. J. Biol. Sci. 10, 3656-3661(2007).

[83]. Salem H., Smagghe G. and Degheele D., Effects of tebufenozide on oocyte growth in *Plodia interpunctella*. Medical. Faculty. Landbouww. Gent University 62(1), 9-13(1997).

[84]. Ghazawy N.A., Awad H.H. and Abdel Rahman K.M., Effects of azadirachtin on embryological development of the desert locust *Schistocerca gregaria* Forskål (Orthoptera: Acrididae). J. Orthoptera Res. 19(2), 327-332(2010).

[85]. Sallam M.H., Effect of Diflubenzuron on embryonic development of the acridid, *Heteracris littoralis*. J. Egypt. Ger. Soc. Zool. 30(E), 17-26(1999).

[86]. Sammour E.A., Kandil M.A. and Abdel-Aziz N.F., The reproductive potential and fate of chlorfluazuron and lufenuron against cotton leafworm, *Spodoptera littoralis* (Boisd).Amer. Euras. J. Agric. Environ. Sci. 4(1), 62-67(2008).

[87]. Kanost M.R., Kawooya J.K., Law J.H., Rayn R.O., Van, Heusden M.C. and Ziegler R., Insects haemolymph proteins. In: "Advances in insect physiology" (Evans P.D. and Wigglesworth V.B., eds.), Academic, London, UK 22, 299-396(1990).

[88]. Chapman R.F., The insects Structure and Function. The 4th edition, Cambridge Univ. Press (1998).

[89]. Telfer W.H., Egg formation in Lepidoptera. J. Insect Sci. 9, 50(2009).

[90]. Indrasith L., Sasaki S.T., Yaginuma T. and Yamashita O., The occurrence of premature form of egg-specific protein in vitellogenic follicles of *Bombyx mori*. J.Comp. Physiol. 158, 1-7(1988).

[91]. Sun X., Song Q. and Barrett B., Effect of ecdysone agonists on vitellogenesis and the expression of EcR and USP in codling moth (*Cydia pomonella*). Arch.Insect Biochem. Physiol. 52, 115-129(2003).

[92]. Abdel-Ghaffar A.A., Morphogenic, embryonic and physiological effects induced by some insect growth regulators (IGRs) in berseem hopper, *Euprepocnemis plorans* Charp. (Orthoptera: Acrididae). Ph.D. Thesis, Al-Azhar Univ., Fac. Sci., Cairo, Egypt (1998).

[93]. Hamadah Kh.Sh., Some developmental, haematological and enzymatic effects of certain plant extracts on the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae). Ph.D. Thesis, Al-Azhar, University, Cairo, Egypt (2009).

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