EFFICACY OF *NIGELLA SATIVA* (RANUNCULACEAE) EXTRACTS ON ADULT PERFORMANCE AND PHASE TRANSITION OF THE DESERT LOCUST *SCHISTOCERCA GREGARIA* (ORTHOPTERA: ACRIDIDAE).

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[Ghoneim, K., Hamadah, K., El-Hela, A., Mohammad, A.-H. & Amer, M. 2016. Efficacy of *Nigella sativa* (Ranunculaceae) extracts on adult performance and phase transition of the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae). Munis Entomology & Zoology, 11 (2): 287-302**]**

ABSTRACT: The current work was carried out to investigate the effects of methanolic, petroleum ether and n-butanol extracts (30.0, 15.0, 7.5, 3.7 and 1.8%) of *Nigella sativa* seeds on several parameters of the adult performance and phase transition of *Schistocerca gregaria*. The n-butanol extract exhibited the most potent adulticidal activity followed with petroleum ether and methanolic extract, respectively, after treatment of penultimate (4^{th}) instar nymphs. After treatment of last (5^{th}) instar nymphs, methanolic extract exhibited the least adulticidal activity. Also, treatment of penultimate instar nymphs with *N. sativa* extracts resulted in blocked adult emergence in a dose-dependent course. Whereas no effect was exhibited by n-butanol extract on adult emergence after treatment of last instar nymphs, various degrees of restrained process was determined at some concentrations of other extracts. All *N. sativa* extracts (only at the higher two concentrations) caused adult deformities after treatment of the penultimate instar nymphs. After treatment of the last instar nymphs, n-butanol extract halted the adult morphogenesis only at the higher two concentrations but other extracts impaired it at all concentrations. In connection with the phase transition, treatment of penultimate instar nymphs with n-butanol extract (at 15.0 %) resulted in a solitarious tendency of *S. gregaria* adults as appeared with deeply green colour. The ovarian maturation in adult females was pronouncedly or slightly prohibited by *N. sativa* extracts during prolonged duration, depending on the concentration. Also, the reproductive life-time (oviposition period) was affected. Total adult longevity was shortened or prolonged, i.e. adult aging was accelerated or delayed, depending on extracts, concentration level and time of treatment.

KEY WORDS: emergence, longevity, methanol, morphogenesis, mortality, n-butanol, petroleum ether, solitarization

The desert locust *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae) ranks together with other migratory locusts-amongst the most important crop pests in Africa. Damage caused by the desert locust is a consequence of its polyphagous behaviour, high density of the population, and the nature to aggregate and swarm. Each individual gregarious locust is able to consume roughly its own weight (about 2 grams) in foliage daily (Youdeowei, 1988; Lindsey, 2002; Lecoq, 2005). In the last century alone, there were seven periods of numerous plagues, the longest of which lasted intermittently for 13 years (Lindsey, 2002). Current locust control operations are mainly based on organophosphorus pesticides as a result of the banning of organochlorines (Lecoq, 2001). The widespread use of such synthetic pesticides has considerable drawbacks, such as the development of insect resistance to insecticides, increased costs, handling hazards, concerns about insecticide residues, and great threats to

both human and environmental health (Garriga & Caballero, 2011). Therefore, many institutions have intensified their efforts in the search for integrated locust control measures. Much attention has been devoted to use plant extracts or plant constituents that have insecticidal effects (Schmutterer, 1990a,b; Krall & Wilps, 1994) because they are generally pest-specific, relatively harmless to non-target organisms and they are biodegradable and consequently harmless to the environment (Rembold, 1984; Isman, 2008).

Nigella plants are widely distributed in countries which border the Mediterranean Sea, central Europe and western Asia (Hedrick, 1972). There are many species classified in the genus *Nigella* (Ranunculaceae) (Bailey, 1978; Atta, 2003). Among the most important medicinal crops in Egypt is *Nigella sativa* which is commonly called as known as black seed or black cumin (Rayan et al., 2011) 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. (2009) reviewed the medicinal, pharmacological, traditional value and folk remedies of this herb. In pest control, Deshpande et al. (1974) 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 (Adebowale & Adeire, 2006; Adabie-Gomez et al., 2006). The *N. sativa* extracts exhibited toxic effects on *Spodoptera littoralis* (Abd ELatif et al., 2009) and *S. gregaria* (Hamadah et al., 2013) in addition to disrupted growth, development (Hamadah et al., 2013) and larval haemogram (Ghoneim et al., 2015) of the latter insect. Also, Ahmad et al. (2013) studied the insecticidal activity of *N. sativa* extracts against the larvae of *Trogoderma granarium* under laboratory conditions. Recently, Khan et al. (2014) reported disturbing effects of the acetone seed extract on biology and invasion of the stored product pest *Tribolium castaneum*. The present work was carried out to investigate the effects of different extracts of *N. sativa* on the adult performance of *S. gregaria* including emergence, survival, morphogenesis and longevity. In addition, possible effect of the present plant extracts on phase transition of *S. gregaria* was studied.

MATERIALS AND METHODS

Experimental insect

The desert locust *S. gregaria* was used as an experimental insect in the present study. The insects were reared and handled under the crowded conditions of Hunter-Jones (1961). Depending on the improvements of Ghoneim et al. (2009) Insects were reared in wooden formed cages provided with electric bulbs (150 watt) adjusted to a photoperiod of 12L:12D and to maintain an ambient temperature of 32±2°C. Fresh clean leaves of *Trifolium alexandrinum* (Egyptian clover), in winter, and the leaves of leguminous plant *Sesbania aegyptiaca,* in summer, were used for feeding insects in the stock culture. On the other hand, *T. alexandrinum* leaves only were offered as food for insects of the experimental work.

Plant extracts

Samples of *N. sativa* 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).

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 *N. sativa* seed 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 and solitarization tendency were recorded.

Adult performance parameters

Adult emergence was recorded in percentage. For investigation the adulticidal activity of *N. sativa* 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 & Sehnal (1973) as follows:

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

The ovarian maturation period, reproductive life-time, post-oviposition period and total adult longevity was measured in days±SD (Norris, 1954).

The solitarization tendency of the adults appeared with green colour and other solitary features. The phase transition was estimated in percentage.

Statistical analysis of Data

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) for the test significance of difference between means.

RESULTS

Effect of *N. sativa* extracts on adult survival

Depending on data assorted in Table 1, the survival potential of adult *S. gregaria* was affected by a latent adulticidal activity of *N. sativa* seed extracts. Treatment of penultimate (4th) instar nymphs with the highest concentration of methanolic extract resulted in 20% mortality. The same extract, at other concentrations, failed to cause adult mortality. Both petroleum ether and n-butanol extracts exhibited various adulticidal activities since different mortality percentages were recorded, regardless the concentration. Moreover, n-butanol extract was the most effective on the adult survival followed with petroleum ether and methanolic extracts, respectively. As clearly seen in the same table, a similar adulticidal activity of *N. sativa* extracts could be exhibited after treatment of last instar nymphs. Furthermore, mortality was dose-dependent by both petroleum ether and n-butanol extracts. Methanolic extract was the least toxic one (10.0% mortality at the highest concentration vs. 0.0% mortality of control adults).

Effect of *N. sativa* extracts on adult emergence

Data of Table 2 clearly reveal some effects of *N. sativa* extracts on the nymphal metamorphosis into adults after treatment of penultimate instar nymphs because the adult emergence decreased as the concentration was increased. As for example, the adult emergence was determined as 62.5 and 20.0 (compared to 88.9% of control congeners) at the highest concentration of

methanolic and petroleum ether extracts, respectively. No adults emerged after treatment with the highest concentration of n-butanol extract but the sublethal concentration led to only 50.0% of adult emergence (compared to 90.0% of control congeners). Whereas no effect was displayed by n-butanol extract on the adult emergence after treatment of last instar nymphs, restrained emergence was observed after treatment with the higher two concentrations of petroleum ether extract (60 and 40%, respectively, vs. 90% emergence of control adults). Also, treatment with methanolic extract, at 30.0 and 3.7%, resulted in 90.0% adult emergence (compared to 100% emergence of adult controls, Table 2).

Effect of *N. sativa* extracts on adult morphogenesis

In connection with the impaired adult morphogenesis program of *S. gregaria* by *N. sativa* seed extracts, data distributed in the previously cited table obviously show various percentages of deformed adults. After treatment of the penultimate instar nymphs, only 20% adult deformities were recorded at the highest concentration of methanolic extract but it failed to affect the morphogenesis at other lower concentrations. Petroleum ether and n-butanol extracts were more potent because different percentages of adult malformations were observed almost proportionally to the concentration (50.0, 14.2,14.2 and 12.5 at 30.0, 15.0, 7.5 and 3.7 % of petroleum ether extract as well as 40.0, 33.3, 33.3 and 25.0 at 15.0, 7.5, 3.7 and 1.8 of n-butanol extract). The adult deformities could be, generally, assorted in the following features: Adults with curled legs and coiled incompletely developed short antennae. Adults with crumpled wings and transparent posterior area and coiled antennae (Fig.1). Adult failure to completely get rid the last nymphal exuvia, where the nymphal exuvia remained as attached parts to the adult body (Fig. 2). After treatment of the last instar nymphs with methanolic extract, no deranging action could be exerted on the adult morphogenesis (Table 2). In contrast, treatment with petroleum ether or n-butanol extracts resulted in serious adult deformities. At the highest concentration of each, the strongest action was exerted on morphogenesis (33.3 % adult deformities at 30 % of petroleum ether extract and 22.2 % adult deformities at 30 % of n-butanol extract (compared to no adult deformities of control adults). Referring to Figs 1 and 2, features of adult impaired morphogenesis program can be observed and described as previously mentioned.

Effects of *N. sativa* extracts on phase transition

After treatment of penultimate instar nymphs with only n-butanol extract of *N. sativa*, an important solitarization affect was exhibited because 50 % of the deformed adults appeared with some characteristics of the solitary phase (such as deeply green colour of the body) at 15 % of n-butanol extract (Table 2 and Fig. 3). No solitarization effect was recorded after treatment of last instar nymphs, regardless the extract or concentration.

Effects of *N. sativa* extracts on adult longevity

It may be conceivable to mention that the maturation period (preoviposition period) is an important indicator for the ovarian maturation rate, i.e, longer period usually indicate a slower rate and *vice versa*. After treatment of penultimate instar nymphs with *N. sativa* seed extracts, data arranged in Table 3 exiguously show that methanolic extract pronouncedly prohibited the ovarian maturation of *S. gregaria* during remarkably lengthened duration, especially at the higher three concentrations (31.3±1.5, 28.7±1.5 and 28.7±0.6 days at 30.0, 15.0 and 7.5 % vs. 22.0±1.7 days of controls). On the other hand, both petroleum ether and n-butanol extracts slightly prohibited it, during insignificantly prolonged duration, regardless the concentration.

After treatment of last instar nymphs, data of the same table clearly indicate a major prolonging effect of *N. sativa* seed extracts on the ovarian maturation period which may be informative to delayed sexual maturity owing to regressed ovarian maturation rate, especially at the higher concentrations. However, methanolic extract pronouncedly prohibited such vital process at the higher three concentrations (25.7±1.1, 26.0±1.3 and 26.0±1.0 days, at 30.0, 15.0 and 7.5%, vs. 22.0±1.7 days of control congeners). Only at the higher two concentrations of petroleum ether extract and the highest concentration of n-butanol extract, the ovarian maturation period was significantly prolonged indicating remarkably delayed sexual maturity (Table 3).

Considering the reproductive life-time (oviposition period**)**, data assorted in Table 4 show general enforcing action of N. sativa extracts on the adult females to quickly lay eggs during shortened period, after treatment of penultimate instar nymphs. Such action was exerted during significantly or insignificantly shortened period, depending on the concentration of methanolic extract and petroleum ether extract. Moreover, n-butanol extract exerted stronger enforcing action on this process at the majority of concentrations (at least $P<0.05$: 7.7±1.5, 8.7±0.6, 9.7±1.5 and 11.7±1.2 days at 15.0, 7.5, 3.7 and 1.8 %, compared to 13.7±2.1 days of controls). After treatment of last instar nymphs, a prohibiting effect was appreciated for adult females by methanolic extract because they lasted insignificantly prolonged reproductive life time. A reverse result was recorded for both petroleum ether and n-butanol extracts because adult females had been enhanced to lay eggs during shortened time intervals (11.7±1.6 and 11.3±1.5, $p<0.01$, at 30.0 and 15.0 % of petroleum ether extract, vs. 23.7±1.2 days of controls, as well as 9.3±1.0 and 11.3±1.5, $p<0.05$ at least, at 30.0 and 15.0 % of n-butanol extract, vs. 15.7±1.5 days of controls, Table 4).

The total adult longevity can be used as an informative indicator of the adult aging, i.e. the prolonged longevity denotes the delaying of adult aging and *vice versa*. Data of total adult longevity, as affected by the *N. sativa* extracts, were listed in Table 5. After treatment of penultimate instar nymphs, both methanolic and petroleum ether extracts caused a slight prolongation in the total longevity, irrespective of concentration. In contrast, n-butanol extract exhibited a pronounced shortening effect on longevity because all treated adult females reached the death point after remarkably shorter duration than that of control adult females, at all concentrations (39.7±3.5, 43.0±3.6, 43.0±3.5 and 48.7±2.5 days at concentrations 15.0, 7.5, 3.7 and 1.8 %, vs. 58.8±4.6 days of controls).

After treatment of the last instar nymphs, data of aforementioned table obviously revealed a shortening effect of both petroleum ether and n-butanol extracts on the total longevity which was obviously observed at the higher two concentrations (30.0 and 15.0 %, respectively). In other words, petroleum ether and n-butanol extracts led to an accelerated aging of the adults ending in death (45.0±1.0 and 45.7±3.1 days, compared to 53.0±2.6 days of controls, for petroleum ether extract and 38.7±2.3 and 48.0±2.6 days, compared to 53.0±1.0 days of controls, for n-butanol extract). On the contrary, methanolic extract did not exert a similar action but reversely delayed the adult aging during slightly prolonged longevity.

DISCUSSION

Blocked adult emergence of *S. gregaria*

Complete or partial blockage of adult emergence was reported for different insects by various botanicals such as the blocked emergence of *Musca domestica*

(Naqvi et al., 2007) and *Rhynchophorus ferrugineus* by azadirachtin (Abdel-Ghaffar et al., 2008), *Tribolium castaneum* by the methanolic extracts of *Centaurium erythreae* and *Pteridium aquilinum* (Jbilou et al., 2008), *S. gregaria* by extracts of *Fagonia bruguieri* (Aly et al., 2010) and *Ammi visnaga* (Ghoneim et al., 2014a) as well as *Earias vittella* by Neemazal T/S and Nimbecidine (Bhardwaj & Ansari, 2015).

In the present study, treatment of penultimate (4^{th}) instar nymphs of *S. gregaria* with *N. sativa* seed extracts resulted in blocked adult emergence in a dose-dependent course. Whereas no effect was exhibited by n-butanol extract on adult emergence after treatment of last (5^{th}) instar nymphs, various degrees of restrained emergence was determined at the higher two concentrations of petroleum ether extract (30.0, 15.0%) and at 30.0 and 3.7% of methanolic extract. Since the eclosion hormone, a blood-born factor arising from the central nervous system (Truman & Riddiford, 1970) triggers eclosion in a wide range of insect orders including Orthoptera (Truman, 1981), the *N. sativa* extracts probably prevented this hormone from being released at the appropriate time. Hence, the eclosion hormone appears to be affected by a certain active ingredient(s) contained in the *N. sativa* extracts. However, the exact mode of action needs further investigation.

Affected adult survival of *S. gregaria*

The available literature contains many reported toxicities of extracts from various plant species on the immature stages of several insect pests (Nicol and Schmutterer, 1991; Osman, 1993; Ghoneim et al., 2000, 2009; 2014a; von Elling et al., 2002; Athanassiour et al., 2005; Senthil Nathan et al., 2006, 2007; Siriwattanarungsee et al., 2008; Tripathy et al., 2011; Janakan & Ramakrishnan, 2014a,b) while the lethal effects of botanicals on adults are relatively scarce. In the present study, n-butanol extract of *N. sativa* seeds exhibited the most potent adulticidal activity followed with petroleum ether and methanolic extract, respectively, after treatment of penultimate instar nymphs of *S. gregaria*. The methanolic extract exhibited the least mortal effect after treatment of last instar nymphs. These results agree, to some extent, with those reported adulticidal activities of different plant species on some pests, such as *T. castaneum* (Naqvi & Perveen, 1991), *Muscina stabulans* (Ghoneim & Al-Dali, 2002) and *M. domestica* (Amer et al., 2004). Also, the current results are in consistent with the adulticidal activities of extracts derived from *Rhizophora mucronata* (Kabaru & Gichia, 2001), *Fagonia bruguieri* (Aly et al., 2010) and *Punica granatum* (Ghoneim et al., 2014b) on the same locust.

The adult mortality, i.e., reduced survival potential, of *S. gregaria* by *N. sativa* extracts, in the present study, may be explicated by a latent prohibitory effect on feeding leading to continuous starvation and subsequently death (Ghoneim et al., 2000). It may be, also, attributed to the action of certain active ingredients in the *N. sativa* seed extracts on the homeostasis leading to increasing loss of body water and subsequently death (Amer et al., 2004), since *N. sativa* contains conjugated linoleic acid, thymoquinone, nigellone (dithymoquinone), melanthin, nigilline, damascenine, tannins, flavonoids, saponins, alkaloids, proteins, lipids, dithymoquinone carvacol and anethole 4-terpinole (Bruits & Bucar, 2000; Al-Ghamdi, 2001; Ali & Blunden, 2003; Sharma et al., 2009; Ali et al., 2012).

Deranged adult morphogenesis of *S. gregaria*

In the present work, all *N. sativa* extracts (only at the higher two concentrations) caused adult deformities after treatment of the penultimate instar nymphs. After treatment of the last instar nymphs, n-butanol extract halted the adult morphogenesis only at the higher two concentrations but other extracts

impaired it at all concentrations. These 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 (Schmutterer et al., 1993), after treatment of penultimate instar nymphs with ethanol extract of *Cyprus rotendus* (El-Sokkary, 2003), Neemazal (a neem preparation) (Hamadah et al., 2013), some extracts of *F. bruguieri* (Aly et al., 2010) as well as some extracts of *P. granatum* peel (Ghoneim et al., 2014b). Moreover, various malformed moths of *Spodoptera littoralis* were caused by Neemazal (Ghoneim et al., 2000), acetone and ethanol extracts of *Aristolochia pubescens* impaired the adult morphogenesis of *Aticarsia gemmatalis* (Nascimento et al., 2004), as well as many adult deformities in both *Spodoptera frugiperda* and *Tenebrio molitor* were observed after treatment with methanol extract of *Myrtillocactus geometrizans* (Cespedes et al., 2005).

Imperfectly emerged adults, in the present study may be due to the disturbance of normal ecdysteroid titer which is usually needed for the achievement of perfect metamorphosis program or even the inhibition of neurosecretion (prothoracicotropic hormone) causing inhibition of a number of physiological processes, such as metamorphosis and morphogenesis (Josephrajkumar et al., 1999).

Induced solitarization tendency of *S. gregaria*

The desert locust, *S. gregaria*, usually display a dramatic polyphenism, being able to transform reversibly between two forms or phases that differ considerably in many aspects including behaviour, physiology and morphology (Uvarov, 1977; Roessingh et al., 1993; Tawfik et al., 1999; Rogers et al., 2004; Pener & Simpson, 2009; Gordon et al., 2012; Harano et al., 2012; Rogers et al., 2014). Many studies have been performed searching for the exogenous and endogenous causes of phase changes in *S. gregaria*. They has focused on the changes from gregarious to solitary, since only gregarious locusts form large migratory swarms capable of invading and inflicting serious damage to crops. No striking interpretation was introduced more than the suggestion about the role of ecdysteroids, juvenoids, and possibly also pheromones in initiating and regulating this process (Pener, 1983).

As reported in the available literature, phase shift from gregaria to solitaria in *S. gregaria* was caused by some extracts of *Melia volkensii* (Rembold & Mwangi, 1989; Nasseh et al., 1993). A clear tendency to solitarization was elicited after treatment of *S. gregaria* gregarious phase with neem oil (Nicol and Schmutterer, 1991; Schmutterer et al., 1993; Langewald et al., 1995). Also, treatment of earlier instar nymphs of *Locusta migratoria migratorioides* resulted in behavior toward the solitary phase (Schmutterer & Freres, 1990). Treatment of gregarious penultimate or last instar nymphs of *S. gregaria* with the ethanol extract of *C. rotendus* resulted in a solitary tendency in adult females (Bakr et al., 2008). The n-butanol extract of *F. bruguieri* enhanced the solitarious tendency in adult females of gregarious *S. gregaria*, regardless the time of nymphal treatment (Aly et al., 2010). In connection with the phase transition, in the present study, treatment of penultimate instar nymphs of *S. gregaria* with n-butanol extract of *N. sativa* seeds (at 15.0 %) induced the solitarious tendency of *S. gregaria* because 50 % of the deformed adults appeared with deeply green colour (characteristic of solitary phase).

The phase transition can be explained on the hormone basis. Allatectomy (surgical removal of corpora allata, CA, responsible for the production of juvenile hormone, JH) resulted in no gregarious behavior in locusts (Richard et al., 2001). Such observation rationally explains the higher activity of CA in solitary *S.*

gregaria causing higher titers of JH in haemolymph and a green colouration of the cuticle (Uvarov, 1966). On the pheromone basis, the existence of 'gregarization pheromone' was postulated (Nolte, 1963; Gillett & Phillips, 1977). The solitarization effect of *N. sativa* n-butanol extract, in the present study, may be due to their influence on this pheromone or to its influence on the hormonal system of the insect (Langewald et al., 1995). For some detail, JH influences the response of olfactory interneurons in the antennal lobe to aggregation pheromone, whereas the responsiveness of antennal receptors neurons is not changed (Richard et al., 2001). In conclusion, it is reasonable to suggest the existence of a juvenilizing, and subsequently antigregarizing, substance in *N. sativa* extracts but more deep investigation is needed to disclose some aspects of our suggestion since juvenilizing effects of some other plant species, such as *Ajuga chamaepitys*, were determined (Jacobson, 1989).

Disturbed adult longevity of *S. gregaria*

In Orthoptera, the sexual maturity usually needs a time interval elapsed between 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, while others prohibited the ovarian maturation, and delayed the sexual maturity. An enhancing effect on the ovarian maturation of *S. gregaria* was exhibited by certain concentrations of Neemazal (a neem preparation)(Hamadah et al., 2013) as well as by methanolic and petroleum ether extracts of *F. bruguieri* (Aly et al., 2010). In contrast, some extracts of *C. rotendus* completely retarded the ovarian maturation of the same locust (El-Sokkary, 2003), n-butanol extract of *F. bruguieri* exhibited a delaying effect on the same process (Aly et al., 2010) and some extracts of *P. granatum* peel slightly or remarkably retarded this vital process (Ghoneim et al., 2014b). On the other hand, no effect was exhibited on it in *M. domestica* by Margosan-O (a neem preparation) or Jojoba oil (Hamadah, 2003).

In the present investigation, treatment of penultimate instar nymphs of *S. gregaria* with methanolic extract of *N. sativa* seeds resulted in pronouncedly prohibited ovarian maturation but petroleum ether extract or n-butanol extract exhibited a slight inhibitory effect. Moreover, predominantly retarding effect on this vital process during prolonged duration was recorded after treatment of last instar nymphs, especially at the higher concentrations. An appreciable interpretation of the prolonged pre-oviposition period, indicating delayed sexual maturity and regressed ovarian maturation rate, in *S. gregaria* after treatment with *N. sativa* extracts, in the present study, is still obscure but some active compounds in these extracts may interfere with the hormonal regulation of this physiological event.

As reported in the literature, treatments of some insects with extracts of 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 of *M. volkensii* shortened the reproductive life-time (Nasseh et al., 1993). A similar result was reported after nymphal treatments with *F. bruguieri* (Basiouny, 2008) or *P. granatum* peel extracts (Ghoneim et al., 2014b). In addition, shortened reproductive life-time of some other insects was caused by several botanicals, such as *M. domestica* by an aqueous extract of *Hyoscyamus*

muticus (Abou El-Ela et al., 1995) and Margosan-O or Jojoba oil (Hamadah, 2003) and *Chrysomya chloropyga* by some extracts of certain Nigerian plants (Muse et al., 2003). In agreement with these reported results, the current study revealed an enforcing action of all *N. sativa* seed extracts on the reproducing adult females of *S. gregaria* to quickly lay eggs during significantly or insignificantly shortened period. An exceptional case of prolonged time was recorded after treatment of last instar nymphs only with methanolic extract. Unfortunately, no acceptable interpretation of the general shortening effect of *N. sativa* extracts on the reproductive life-time, or enforcing the adult females of *S. gregaria* to quickly lay eggs, is available right now!! Therefore, further investigation should be carried out to explore the mode of action of certain chemical constituents of these extracts on this crucial physiological criterion.

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*. As reported in the available literature, several neem products pronouncedly affected the total adult longevity of some insect pests, such as *Spodoptera litura* (Steffens & Schmutterer, 1982; Gujar & Mehrotra, 1983a, b; Mehrotra & Gujar, 1984; Di Ilio et al., 1999), *M. stabulans* (Ghoneim & Al-Dali, 2002), *M. domestica* (Amer et al., 2004), *Chrysomya megacephala* (Siriwattanarungsee et al., 2008). Also, the adult longevity of *S. littoralis* was shortened by larval treatments with extracts from *Melia azedarach* (Schmidt et al., 1997; Hassan, 2002). Considering the present experimental locust, *S. gregaria*, Neemazal treatments of penultimate instar nymphs resulted in remarkably shortened adult longevity but a reversal effect was recorded after treatment of last instar nymphs (Hamadah, 2009). Similar results had been reported for the same locust by extracts of *F. bruguieri* (Aly et al., 2010). Also, accelerating or delaying action of *P. granatum* peel extracts was exhibited on the adult females of the same locust, depending on the extract and time of nymphal treatment (Ghoneim et al., 2014b).

In the present study, treatment of penultimate instar nymphs of *S. gregaria* with methanolic extract or petroleum ether extract of *N. sativa* seeds resulted in a slight prolongation of total adult longevity (delayed aging), irrespective of the concentration. On the contrary, n-butanol extract exhibited a significant shortening effect on the longevity (accelerated aging). After treatment of the last instar nymphs, a shortening effect of both petroleum ether and n-butanol extracts was remarkably exhibited on the longevity, at the higher two concentrations. In contrast, methanolic extract affected the adult life in an insignificantly prolonged longevity or delayed aging. The probable cause of shortened and prolonged adult longevity, as described by Gujar & Mehrotra (1983a, b) and Mehrotra & Gujar (1984), is due to azadirachtin's interference with the neuro-endocrine system of the insects. Delaying of adult aging (or prolonged longevity) in *S. gregaria*, in the current study, may be attributed to the antioxidant properties of some constituents of *N. sativa* seeds as extracted by certain solvents. On the other hand, accelerating of adult aging (or shortened longevity) may be explicated by the action of some chemicals extracted from the tested plant by certain solvents on a hormonal activity because there is a close relation between certain hormones and adult longevity (Clancy et al., 2001; Simon et al., 200**3**; Broughton et al., 2005; Yamamoto et al., 2013).

CONCLUSION

As clearly shown in the present study, *N. sativa* seed extracts exhibited slight or remarkable effects on various parameters of adult performance of *S. gregaria*. In addition, n-butanol extract induced the phase transition from gregaria to solitaria. It prohibited the gregarization tendency of *S. gregaria* and hence the swarm formation necessary for invasion can be avoided. Therefore, *N. sativa* seed extracts can be used as a complementary agent in the integrated control of this destructive locust. However, further investigation should be carried out to ascertain the active ingredient (s) contained in these extracts responsible for these effects.

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A B C

Figure 1. Different adult malformations of *S. gregaria* were produced as a result of the nymphal treatments with *N. sativa* extracts. A) Normal adult. B) Treated adult with curled legs, incompletely developed short antennae and crumbled wings with transparent posterior area. C) Treated adult with crumbled wings of transparent posterior area and coiled antenna.

A B C

Figure 2. Different degrees of adult failure to completely get rid the last nymphal exuvia as a result of the nymphal treatments with *N. sativa* extracts. A) Nymphal exuvium attached to abdomen, wings and legs. B) Nymphal exuvium attached to wings, legs and mouth parts. C) Nymphal exuvium attached to wings.

A B

Figure .3 Phase shift of *S. gregaria* from gregaria to solitaria as a result of the nymphal treatments with some concentrations of *N. sativa* extracts. A) Normal gregarious adult. B) Solitarized adult.

Table 1. Adulticidal activity (%) of *N. sativa* extracts on *S. gregaria*.

Solvent	Conc. (%)	After treatment of 4th instar nymphs	After treatment of 5th instar nymphs
Methanol	30.0	20.0	10.0
	15.0	00.0	00.0
	07.5	00.0	00.0
	03.7	00.0	10.0
	01.8	00.0	00.0
	Controls	00.0	00.0
Petroleum ether	30.0	50.0	50.0
	15.0	12.5	50.0
	07.5	14.2	22.2
	03.7	14.2	22.2
	01.8	00.0	11.1
	Controls	00.0	00.0
n-butanol	30.0	---	22.2
	15.0	50.0	22.2
	07.5	50.0	42.9
	03.7	40.0	22.2
	01.8	33.3	11.1
	Controls	00.0	00.0

Conc.: Concentration level. ---: No adult could metamorphose from the treated nymphs.

Table 2. Affected adult emergence and morphogenesis of *S. gregaria* by nymphal treatments with *N. sativa* extracts.

Solvent	Conc. (%)	After treatment of 4th instar nymphs			After treatment of 5th instar nymphs		
		Emergence (%)	Deformed %	Solitarian %	Emergence (%)	Deformed %	Solitarian %
Methanol	30.0	62.5	20.0	0.0	090.0	0.0	0.0
	15.0	85.7	0.0	0.0	100.0	0.0	0.0
	07.5	88.9	0.0	0.0	100.0	0.0	0.0
	03.7	87.5	0.0	0.0	090.0	0.0	0.0
	01.8	88.9	0.0	0.0	100.0	0.0	0.0
	Controls	88.9	0.0	0.0	100.0	0.0	0.0
Petroleum ether	30.0	20.0	50.0	0.0	60.0	33.3	0.0
	15.0	85.7	12.5	0.0	40.0	25.0	0.0
	07.5	85.7	14.2	0.0	90.0	11.1	0.0
	03.7	85.7	14.2	0.0	90.0	22.2	0.0
	01.8	88.9	0.0	0.0	90.0	11.1	0.0
	Controls	88.9	0.0	0.0	90.0	0.0	0.0
n-butanol	30.0	---	---	0.0	90.0	22.2	0.0
	15.0	50.0	40.0	**50.0**	90.0	11.1	0.0
	07.5	40.0	33.3	0.0	90.0	0.0	0.0
	03.7	60.0	33.3	0.0	90.0	11.1	0.0
	01.8	60.0	25.0	0.0	90.0	11.1	0.0
	Controls	90.0	0.0	0.0	90.0	0.0	0.0

Conc., ---: see footnote of Table (1). Mean ± SD followed by letter (a): not significantly different (P>0.05), (b): Significantly different (P<0.05), (c): Highly significantly different (P<0.01), (d): Very highly significantly different (P<0.001).

Table 3. Influenced ovarian maturation period (Mean days±SD) of *S. gregaria* by nymphal treatments with *N. sativa* extracts.

Solvent	Conc. (%)	After treatment of 4th instar nymphs	After treatment of 5th instar nymphs
Methanol	30.0	31.3 ± 1.5 c	25.7 ± 1.1 b
	15.0	28.7 ± 1.5 c	26.0 ± 1.3 b
	07.5	28.7 ± 0.6 c	26.0 ± 1.0 b
	03.7	24.0 ± 1.7 a	24.3 ± 1.2 a
	01.8	23.7 ± 1.5 a	24.0 ± 1.0 a
	Controls	22.0 ± 1.7	22.0 ± 1.7
Petroleum ether	30.0	---	28.3 ± 1.2 c
	15.0	25.0 ± 1.0 a	26.7 ± 1.3 b
	07.5	23.0 ± 1.7 a	24.7 ± 1.5 a
	03.7	23.0 ± 1.0 a	24.3 ± 0.6 a
	01.8	22.7 ± 1.2 a	24.7 ± 1.2 a
	Controls	22.0 ± 1.7	23.7 ± 1.2
n-butanol	30.0	---	24.0 ± 1.0 c
	15.0	27.7 ± 3.1 a	28.3 ± 1.2 a
	07.5	28.7 ± 4.2 a	27.7 ± 1.2 a
	03.7	28.0 ± 3.6 a	27.7 ± 0.6 a
	01.8	30.7 ± 1.5 a	28.3 ± 0.6 a
	Controls	32.0 ± 3.7	28.0 ± 1.0

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

Table 4. Disturbed reproductive life-time (Mean days±SD) of *S. gregaria* by nymphal treatments with *N. sativa* extracts.

Solvent	Conc. (%)	After treatment of 4th instar nymphs	After treatment of 5th instar nymphs
Methanol	30.0	10.7 ± 1.2 c	17.3 ± 1.2 a
	15.0	14.0 ± 1.7 a	17.0 ± 1.0 a
	07.5	14.0 ± 1.0 a	16.7 ± 1.2 a
	03.7	16.7 ± 1.2 a	17.3 ± 2.1 a
	01.8	16.3 ± 1.5 a	17.3 ± 1.5 a
	Controls	16.7 ± 1.5	16.7 ± 1.5
Petroleum ether	30.0	---	11.7 ± 1.6 c
	15.0	08.7 ± 1.2 c	11.3 ± 1.5 c
	07.5	09.7 ± 1.5 c	17.0 ± 1.0 a
	03.7	15.3 ± 1.5 a	17.7 ± 1.2 a
	01.8	16.3 ± 1.5 a	17.0 ± 2.0 a
	Controls	16.7 ± 1.5	19.0 ± 1.0
n-butanol	30.0	---	09.3 ± 1.2 c
	15.0	07.7 ± 1.5 c	11.3 ± 1.5 b
	07.5	08.7 ± 0.6 c	13.3 ± 1.5 a
	03.7	09.7 ± 1.5 b	14.7 ± 1.2 a
	01.8	11.7 ± 1.2 a	15.3 ± 0.6 a
	Controls	13.7 ± 2.1	15.7 ± 1.5

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

Table 5. Disturbed total adult longevity (Mean days±SD) of *S. gregaria* by nymphal treatments with *N. sativa* extracts.

Solvent	Conc. (%)	After treatment of 4th instar nymphs	After treatment of 5th instar nymphs
Methanol	30.0	46.3 ± 2.5 a	45.7 ± 2.1 a
	15.0	46.3 ± 1.5 a	45.7 ± 2.9 a
	07.5	46.7 ± 1.2 a	45.3 ± 1.5 a
	03.7	44.7 ± 3.5 a	45.0 ± 2.0 a
	01.8	44.3 ± 1.5 a	45.0 ± 1.0 a
	Controls	43.3 ± 2.1	43.3 ± 2.1
Petroleum ether	30.0	---	45.0 ± 1.0 c
	15.0	42.3 ± 2.1 a	45.7 ± 3.1 b
	07.5	41.3 ± 3.5 a	48.3 ± 1.5 a
	03.7	43.3 ± 3.2 a	49.3 ± 1.5 a
	01.8	44.0 ± 1.0 a	49.0 ± 3.5 a
	Controls	43.3 ± 2.1	53.0 ± 2.6
n-butanol	30.0	---	38.7 ± 2.3 d
	15.0	39.7 ± 3.5 c	48.0 ± 2.6 b
	07.5	43.0 ± 3.6 c	49.0 ± 2.6 a
	03.7	43.0 ± 3.5 c	50.7 ± 1.5 a
	01.8	48.7 ± 2.5 b	52.3 ± 1.2 a
	Controls	58.8 ± 4.6	53.0 ± 1.0

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