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Disrupted survival, growth and development of desert locust *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae) by extracts from toothpick weed *Ammi visnaga* Lamarck (Apiaceae)

Karem Ghoneim[1*], Moneir Amer[1], Alaa Al-Daly[1], Abdel-Hamid Mohammad[1], Faisal Khadrawy[2], Muhammad Mahmoud[1]

[1]*Faculty of Science, Al-Azhar University, Madenit Nasr, Cairo, Egypt*

[2]*Faculty of Pharmacy, Al-Azhar University, Madenit Nasr, Cairo, Egypt*

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Abstract

The present study aims to investigate the survival, growth and developmental effects of *Ammi visnaga* fruit extracts on the destructive desert locust *Schistocerca gregaria*. Treatment of penultimate instar nymphs with the highest concentration of ethanol, petroleum ether and n-butanol extracts resulted in 50, 70 and 60% nymphicidal activity, respectively. Also, the successfully moulted last instar nymphs suffered a lethal action of all extracts. LC_{50} values were 21.0, 12.0 and 22.5% of ethanol extract, petroleum ether extract and n-butanol extract, respectively. After treatment of last instar nymph, a dose-dependent course of the nymphicidal activity could not be detected after treatment with ethanol extract but with petroleum ether extract or n-butanol extract. LC_{50} values 22.3, 20.8 and 22.0% of ethanol extract, petroleum ether extract and n-butanol extract, respectively. With regard to the growth and development, treatment of penultimate instar nymphs with ethanol extract and petroleum ether extract led to pronouncedly prohibited growth. All extracts affected the development. No nymphal-adult intermediates had been formed by ethanol extract or n-butanol extract at 40% concentration. In addition, suppressed developmental rate was caused by ethanol extract but not by other extracts. All *A. visnaga* extracts prohibited the adult emergence in no certain trend.

* **Corresponding Author:** Karem Ghoneim ✉ kar_ghoneim@yahoo.com

Introduction

Plagues of the desert locust *Schistocerca gregaria* (Forskal) have threatened agricultural production in Africa, the Middle East and Asia (Showler, 1995; Ceccato *et al.*, 2007). Damage is caused as a consequence of its polyphagous behaviour, high population density, and the nature to aggregate and swarm. Each individual gregarious locust can consume roughly its own weight of foliage daily (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 and 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, 1990 a, b; Krall and Wilps, 1994). The botanical control agents are generally pest-specific and relatively harmless to non-target organisms and they are biodegradable and consequently harmless to the environment (Rembold, 1994). Majority of botanicals are still at the experimental stage as far as locust control is concerned and large scale production is problematic and difficulties with the registration of variable products will limit adoption (Meinzingen and Kooyman, 1997). Otherwise, prior results on the effects of plant extracts on the desert locust were encouraging for implementing an alternative method to chemical control (Abbasi *et al.*, 2003).

Toothpick weed *Ammi visnaga* Lamarck (Apiaceae= Umbelliferaceae) is commonly known as khella or Al-khillah, especially in Egypt. It is native to Europe, Asia and North Africa but can be found throughout the world as an introduced species. Now, the main chemical constituents in *A. visnaga* are khellin, visnagin and some pyranocouramin fractions (Ziment, 1998). The *A. visnaga* extracts have been used in the traditional medicine and their chemical components have been used in the modern medicine (Khan *et al.*, 2001; Kwon, *et al.*, 2010; Vanachayangkul *et al.*, 2011). Also, khellin and visnagin were reported as pharmacological agents (Duarte *et al.*, 1998; Cordero *et al.*, 2004; Whitton *et al.*, 2008; Lee *et al.*, 2010). However, the research work on using this plant, or some of its chemical constituents, in pest control, is unfortunately scarce. The available literature reported an ovicidal activity of the *A. visnaga* extracts against hessian fly *Mayetiola destructor* (Lamiri *et al.*, 2001a) and a larvicidal activity against some mosquito species (Amer and Mehlhorn, 2006 a, b; Pavela, 2008). These extracts had been reported, also, as a grain protectant against granary weevil *Sitophilus granarius* (Abdel-Latif, 2004) and rice weevil *Sitophilus oryzae* (Ahmed and Al-Moajel, 2005). The present study was conducted aiming to investigate the possible effects of some *A. visnaga* extracts on survival, development and metamorphosis of the dangerous desert locust *S. gregaria*.

Materials and methods

Experimental insect

All parts of the present work had been carried out at Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, Cairo, Egypt. The desert locust *S. gregaria* 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 **(**1961) and improved by Ghoneim *et al.* (2009), insects were reared in wood formed cages (60 x 60 x 70 cm). The bottom was furnished with a sandy layer (20 cm depth) and provided with 10-15% humidity suitable for egg laying. An electric bulb (100 watt) was adjusted in each cage to maintain a continuous photoperiod (12 L: 12 D) as well as an ambient temperature (32±2°C). The insects were reared and handled under the crowded conditions. The feces, dead locusts and food remains were removed daily before introducing fresh food. Care was seriously taken to clean these cages at regular intervals and the

sand was sterilized in drying oven (at 140°C for 24 hours) to avoid contamination with any pathogenic microorganisms. Fresh clean leaves of clover *Trifolium alexandrinum* were provided as a food.

Plant extraction

A. Visnaga fruits (1.5 Kg) purchased from an Egyptian market were thoroughly cleaned with tap water for disposing of impurities. The fruits were shade dried and then finely ground by a micromill. Solvents of different polarities were used for the extraction, as follows. The pulverized powder was macerated with ethanol in a closed container for a defined period with frequent agitation until soluble matter was dissolved as adopted from Ncube *et al.* (2008). The ethanol extract was divided into two parts: a part of the ethanol extract was evaporated for obtaining 25 gm dried extract. Another part was concentrated into 300 ml by rotary evaporator, and then diluted with 300 ml distilled water. Using a separating funnel, the dilute was fractionalized by petroleum ether (300 ml X 5) and n-butanol (300 ml X 5) giving 27 and 23 gm, respectively. From each of the crude ethanol extract and the fractionalized petroleum ether and n-butanol extracts, six concentration levels were prepared: 80.0, 40.0, 20.0, 10.0, 5.0 and 2.5%.

Nymphal treatments

The newly moulted 4th (penultimate), or 5th (last) instar nymphs of *S. gregaria* were fed on fresh leaves of *T. alexandrinum* after dipping in 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 leaves. 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 weighed every day using a digital balance and also examined for recording mortality and other observations.

Criteria studied

To determine the nymphicidal and adulticidal activities of the present plant extracts, mortality of treated and control insects was recorded daily after 24 h post-feeding treatment. Corrected mortality was calculated for general mortality using the Abbott's equation (1925):

$$\%\text{ of corrected mortality} = \frac{\%\text{ of test mort.} - \%\text{ of control mort.}}{100 - \%\text{ of control mort}} \times 100$$

The LC_{50} values were calculated for general mortality by Microsoft office Excel, 2007, according to Finny (1971).

Growth of nymphs was indicated by their weight gain. It was calculated as follows:

Initial weight (before the beginning of experiment) - *final weight* (at the end of experiment).

Dempster's equation (1957) was applied for calculating the developmental duration, and Richard's equation (1957) was used for calculating the developmental rate.

The deranged metamorphosis program of the desert locust was observed and calculated in nymphal-adult intermediate % and imperfectly emerged adults %.

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

Lethal effects of *A. visnaga* on *S. gregaria*

The treated penultimate instar nymphs had been subjected to the nymphicidal activity of all extracts as shown in Table (1). Treatment with the highest concentration level (80%) of ethanol, petroleum ether and n-butanol extracts resulted in 50, 70 and 60% nymphal mortality, respectively (in comparison with 10% natural mortality). On the other hand, the least

potent nymphicidal action was exerted at 2.5% concentration level of both ethanol and petroleum ether extracts alongwith 5.0 and 2.5% concentration levels of the n-butanol extract. With few exceptions, a dose-dependent manner of the nymphicidal action could be conceived.

Moreover, the successfully moulted last instar nymphs suffered a lethal action of all extracts. Data arranged in the same table reveal that the ethanol extract was the most potent one, at the highest concentration level (50% compared to 30 and 40% mortality caused by petroleum ether extract and n-butanol extract, respectively). However, no mortality was recorded at 2.5% of ethanol extract, at 5.0 and 2.5% of petroleum ether extract or at 5.0% of n-butanol extract. No adults emerged after treatment of penultimate instar nymphs with the highest concentration level, regardless the extract. The adulticidal activity could be detected in a dose-dependent fashion although treatment with the lower two concentration levels of n-butanol extract resulted in no adult mortality.

Table 1. Mortality (%) of penultimate and last instar nymphs of *S. gregaria* after treatment of penultimate nymphs with different *A. visnaga* extracts.

Solvent	Conc. (%)	Nymphal mort.		Adult mort.	Total mort.	Corrected mort.	LC_{50}
		Penult.	Last				
Ethanol	80.0	50.0	50.0	—	100.0	100.0	21. 0
	40.0	30.0	28.6	40.0	070.0	066.6	
	20.0	20.0	25.0	50.0	070.0	066.6	
	10.0	20.0	12.5	14.3	040.0	044.4	
	05.0	20.0	10.0	22.2	030.0	022.2	
	02.5	10.0	00.0	11.1	020.0	011.1	
	Control	10.0	00.0	00.0	010.0	- - -	
Petroleum ether	80.0	70.0	30.0	—	100,0	100.0	12.0
	40.0	50.0	60.0	—	100.0	100.0	
	20.0	30.0	42.9	66.7	090.0	087.5	
	10.0	30.0	14.3	20.0	060.0	050.0	
	05.0	30.0	00.0	11.7	050.0	037.5	
	02.5	10.0	00.0	22.2	030.0	012.5	
	Control	10.0	00.0	11.1	.20.0	- - -	
n-butanol	80.0	60.0	40.0	—	100.0	100.0	22.5
	40.0	20.0	25.0	25.0	070.0	066.6	
	20.0	20.0	25.0	16.7	060.0	055.5	
	10.0	20.0	22.2	30.0	060.0	055.5	
	05.0	10.0	00.0	00.0	020.0	022.2	
	02.5	10.0	22.2	00.0	010.0	000.0	
	Control	00.0	10.0	11.1	010.0	- - -	

Conc.: Concentration level. mort.: Mortality. Penult.: penultimate instar nymphs, Last: last instar nymphs. — : No transformed adults.

The corrected mortality was calculated in 100% at the highest concentration level of ethanol extract or n-butanol extract but at the higher two concentration levels of petroleum ether extract. In addition, LC_{50} values were estimated in 21.0, 12.0 and 22.5% of ethanol, petroleum ether and n-butanol extracts, respectively.

As shown in Table (2), the strongest nymphicidal action of *A. visnaga* was exhibited at the highest concentration level of all extracts (40, 80 and 60% by ethanol, petroleum ether and n-butanol extracts, respectively). No dose-dependent course of the nymphicidal activity could be appreciated after treatment with ethanol extract while it may be found after treatment with each of other extracts. Data

contained in the same table clearly indicate complete adult mortality by ethanol extract or n-butanol extract, at the highest concentration level but at the higher two concentration levels of petroleum ether extract. On the contrary, no adulticidal activity was exerted after nymphal treatment with the lowest concentration level of ethanol extract or n-butanol extract. However, the calculated corrected mortality was found in a similar trend. LC_{50} values of ethanol, petroleum ether and n-butanol extracts had been measured in 22.3, 20.8 and 22.0%, respectively.

Table 2. Mortality (%) of last instar nymphs of *S. gregaria* by *A. visnaga* extracts.

Solvent	Conc. (%)	Nymphal mort.	Adult mort.	Total mort.	Corrected mort.	LC_{50}
Ethanol	80.0	40.0	100.0	100.0	100.0	22.3
	40.0	10.0	022.2	070.0	070.0	
	20.0	10.0	011.1	060.0	060.0	
	10.0	10.0	011.1	030.0	030.0	
	05..0	10.0	022.2	030.0	030.0	
	02.5	10.0	000.0	010.0	010.0	
	Control	00.0	000.0	00.0	- - -	
Petroleum ether	80.0	80.0	100.0	100.0	100.0	20.8
	40.0	60.0	100.0	100.0	100.0	
	20.0	30.0	028.6	050.0	037.5	
	10.0	20.0	025.0	040.0	025..0	
	05.0	20.0	012.5	030.0	012.5	
	02.5	20.0	012.5	030.0	012.5	
	Control	00.0	011.1	20.0	- - -	
n-butanol	80.0	60.0	100.0	100.0	100.0	22.0
	40.0	20.0	033.3	070.0	066.6	
	20.0	20.0	025.0	070.0	066.6	
	10.0	20.0	025.0	050.0	044.4	
	05.0	10.0	011.1	030.0	022.2	
	02.5	10.0	000.0	010.0	000.0	
	Control	00.0	000.0	010.0	- - -	

Conc., mort.: See footnote of Table (1).

Growth and developmental effects of *A. visnaga* on *S. gregaria*

After treatment of penultimate instar nymphs

Effects on growth

Table (3) depicts the most important growth criteria of *S. gregaria* after treatment of penultimate instar nymphs with *A. visnaga* fruit extracts. With regard to the nymphal growth, pronouncedly inhibitory effects of ethanol and petroleum ether extracts had been exhibited. In contrast, no significantly devastating effects could be exerted by n-butanol extract, especially at the lower two concentration levels (425.0±59.1 and 413.0±42.0 mg vs. 443.1±51.4 mg of control congeners). The most drastically depressed weight gain was recorded at 40% of ethanol extract (198.5±54.3 mg vs. 466.3±66.2 mg of control congeners), at 80% of petroleum ether extract (290.1±31.2 mg vs. 488.1±53.6 mg of control congeners) and at the same highest concentration level of n-butanol extract (301.4±44.2 vs. 443.1±51.4 mg of control congeners). Growth of successfully moulted last instar nymphs was deranged by *A. visnaga* extracts as exiguously seen in Table (3). At the lower two concentration levels (5.0 and 2.5%) of ethanol and at the lower three concentration levels (10.0, 5.0 and 2.5%) of petroleum ether extract, nymphal growth was not significantly influenced. At other concentration levels of both extracts, the growth was remarkably inhibited since the somatic weight gain highly decreased.

Effects on development

In the light of data arranged in the same table, all extracts remarkably affected the developmental duration because all treated nymphs survived for significantly prolonged time intervals, regardless the

concentration level, and consequently developed only in a slow rate. On the other hand, development of those successfully moulted last instar nymphs had been subjected to a significantly or non-significantly prohibiting action of all extracts. The prohibiting action (as appeared in prolonged developmental duration and slow down rate) of that extract derived by ethanol was considerably exhibited at its higher three concentration levels but at the higher two concentration levels of n-butanol extract and only at 10% concentration level of petroleum ether extract.

Table 3. Growth and development of *S. gregaria* after treatment of the newly moulted penultimate instar nymphs with different extracts of *A. visnaga*.

	Conc. (%)	Nymphs							Adult emergence (%)
		Penultimate instar nymphs			Last instar nymphs				
Solvent		Weight gain (Mean mg ± SD)	Duration (Mean days ± SD)	Develop. rate	Weight gain (Mean mg ± SD)	Duration (Mean days ± SD)	Develop. rate	Nymphal-adult inter. (%)	
Ethanol	80.0	201.3 ±0 60.1d	8.2 ± 1.0 b	12.2	—	—	—	—	—
	40.0	198.5 ± 054.3 d	8.7 ± 0.8 b	11.5	326.3 ± 62.0 c	11.2 ± 1.6 b	08.9	0.0	50.0
	20.0	264.1± 061.8 d	8.8 ± 0.9 b	11.4	367.3 ± 64.2 c	11.8 ± 1.2 d	08.5	0.0	60.0
	10.0	340.9 ± 051.8 d	9.1 ± 1.1 c	11.0	420.2 ± 22.6 c	11.0 ± 1.4 b	09.1	0.0	70.0
	05.0	332.1 ± 126.8 d	9.8 ± 0.8 d	10.2	423.7 ± 97.1 a	10.6 ± 1.5 a	09.9	0.0	90.0
	02.5	408.9 ± 069.3 d	9.1 ± 0.8 d	11.0	440.3 ± 43.8 a	10.2 ± 1.1 a	09.8	0.0	90.0
	Control	466.3 ± 066.2	8.0 ± 0.7	12.5	462.8 ± 48.2	10.2 ± 1.1	10.1	0.0	90.0
Petroleum ether	80.0	290.1 ± 31.2 d	9.9 ± 1.1 d	10.1	—	—	—	—	—
	40.0	362.3 ± 42.7 d	10.8 ± 1.3 d	09.2	547.4 ± 91.9b	11.0 ± 2.8 a	09.0	40.0	—
	20.0	416.9 ± 62.9 d	10.7 ± 0.5 d	09.3	525.8 ± 43.1b	10.3 ± 0.5 a	09.7	14.3	30.0
	10.0	374.3 ± 32.5 d	10.0 ± 0.8 d	10.0	611.9 ± 50.8a	11.2 ± 1.7 c	08.9	14.3	50.0
	05.0	399.6 ± 51.2 d	10.3 ± 0.8 d	09.7	587.1 ± 24.1a	10.9 ± 0.7 a	09.1	14.3	60.0
	02.5	394.3 ± 28.5 d	9.8 ± 0.8 d	10.2	585.7 ± 34.1a	10.8 ± 1.2 a	09.2	00.0	90.0
	Control	488.1 ± 53.6	8.5 ± 1.0	11.5	640.9 ± 50.2	10.0 ± 0.7	10.0	00.0	90.0
n-butanol	80.0	301.4 ± 44.2 c	11.2 ± 1.2 d	08.9	—	—	—	—	—
	40.0	340.3 ± 55.8 c	10.5 ± 0.8 d	09.5	463.8 ± 53.2 c	10.8 ± 0.4 b	09.2	25.0	40.0
	20.0	366.6 ± 37.9 b	10.4 ± 0.5d	09.6	578.1 ± 45.6 c	10.8 ± 1.2 b	09.2	00.0	60.0
	10.0	374.0 ± 45.1 b	9.6 ± 0.8 b	10.4	476.9 ± 47.1 c	10.7 ± 1.2 a	09.3	00.0	70.0
	05.0	425.0 ± 59.1 a	10.6 ± 0.9 d	10.1	477.0 ± 49.7 c	10.6 ± 1.1 a	09.4	00.0	80.0
	02.5	413.0 ± 42.0 a	9.9 ± 0.6 b	10.8	570.2 ± 21.2 b	10.0 ± 0.6 a	10.0	00.0	90.0
	Control	443.1 ± 51.4	8.7 ± 1.0	11.5	594.4 ± 79.7	9.7 ± 0.9	10.3	00.0	90.0

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). — : died nymphs and subsequently no adults. Develop.: Developmental .Nymphal-adult inter.: Nymphal-adult intermediates.

Effects on metamorphosis

In connection with the effects of *A. visnaga* extracts on the metamorphosis program, no nymphal-adult intermediates had been formed by ethanol extract or n-butanol extract, except at 40% of the latter. On the contrary, petroleum ether extract exhibited a remarkably impairing action on this program. The most important nymphal-adult intermediates can be seen in Fig. (1). Just a look at data of Table (3) reveals a dose-dependent inhibitory effect of all *A. visnaga* extracts on the adult emergence with few exceptions at the lowest concentration level of n-butanol extract or the lower two concentration levels of both ethanol and petroleum ether extracts.

After treatment of last instar nymphs

Effects on growth

As summarized in Table (4), *A. visnaga* disruptively affected the growth and development of *S. gregaria* after treatment of last instar nymphs with different fruit extracts. Based on these data, the most potent extract for suppressing the somatic weight gain of nymphs was that derived by ethanol because significantly decreased weights had been gained. Furthermore, such suppressing action was detected in a dose-dependent course (322.2±72.1 mg and 573.9±80.4 mg at the highest and lowest concentration levels, respectively, compared to 677.4±67.7 mg of control congeners). The inhibitory

effect of this extract on the somatic weight gain was followed by petroleum ether extract and then n-butanol extract (for detail, see Table 4).

Effects on development

In addition, an evidently prolonged developmental duration was recorded by ethanol extract since the most lengthened duration was 11.4±0.9 days and the least prolonged one was 10.1±1.1 days (vs. 9.9±1.1 days of control congeners). In contrast, neither petroleum ether extract nor n-butanol extract remarkably affected the developmental duration, and consequently did not affect the developmental rate of last instar nymphs.

Table 4. Growth and development of *S. gregaria* after treatment of the newly moulted last instar nymphs with different extracts of *A. visnaga*.

Solvent	Conc. (%)	Nymphs				Adult emergence (%)
		Weight gain (Mean mg ± SD)	Duration (Mean days ± SD)	Develop. rate	Nymphal-adult inter. (%)	
Ethanol	80.0	322.2 ± 072.1 d	11.4 ± 0.9 b	08.8	0.0	030.0
	40.0	347.8 ±0 93.3 d	11.7 ± 1.0 c	08.6	0.0	090.0
	20.0	413.8 ± 108.9 d	11.6 ± 0.9 c	08.7	0.0	090.0
	10.0	435.6 ± 088.6 d	11.2 ± 0.8 c	08.9	0.0	090.0
	05.0	503.1 ± 080.4 d	11.0 ± 0.9 b	09.1	0.0	090.0
	02.5	573.9 ± 080.4 b	10.1 ± 1.1 b	09.8	0.0	090.0
	Control	677.4 ± 076.7	09.9 ± 1.1	10.1	0.0	100.0
Petroleum ether	80.0	473.4 ± 66.2 d	10.8 ± 1.4 a	09.3	0.0	20.0
	40.0	539.1 ± 79.4 b	11.0 ± 2.0a	09.1	0.0	40.0
	20.0	461.0 ± 81.0d	10.4 ± 1.3a	11.1	0.0	70.0
	10.0	511.6 ± 50.9 b	10.0 ± 2.1a	10.0	0.0	80.0
	05.0	531.1 ± 78.6b	10.3 ± 1.2a	09.7	0.0	80.0
	02.5	625.4 ± 64.4a	11.0 ± 1.4a	09.1	0.0	80.0
	Control	659.2 ± 42.9	10.0 ± 0.9	10.0	0.0	90.0
n-butanol	80.0	442.1 ±0 80.1 c	11.0 ± 1.2 a	09.1	0.0	40.0
	40.0	513.7 ± 103.8 b	10.7 ± 1.2a	09.3	0.0	90.0
	20.0	570.0 ± 096.5 a	10.9 ± 1.1 a	09.2	0.0	80.0
	10.0	586.7 ± 079.7 a	10.1 ± 1.1a	09.9	0.0	80.0
	05.0	575.9 ± 098.3 a	10.6 ± 1.1a	09.4	0.0	90.0
	02.5	597.8 ± 077.1 a	10.0 ± 1.3a	10.0	0.0	80.0
	Control	631.3 ± 084.6	09.8 ± 0.7	10.2	0.0	90.0

Conc.: See footnote of Table (1). Develop., Nymphal-adult inter., a, b, c, d: See footnote of Table (3).

Effects on metamorphosis

It is of interest to know that all fruit extracts of *A. visnaga* failed to affect the metamorphosis program since no nymphal-adult intermediates had been observed. On the contrary, all extracts prevented the adult emergence but in no certain trend albeit the most preventive action was exerted by all extracts at the highest concentration level (30, 20 and 40% adult emergence after treatment with ethanol, petroleum ether and n-butanol extracts, respectively, vs. 100 or 90% adult emergence of control congeners). Also, different degrees of adult failure to completely get rid the last nymphal exuvia, as a result of the nymphal treatments with different extracts of *A. visnaga*, was noticed (for detail, see Fig. 2).

Discussion

Influenced survival potential of S. gregaria by A. visnaga extracts

Plant sources contain natural compounds which may have toxic, repellent, and antifeedant or antihormonal characteristics (Thomas and Callaghan, 1999). The most famous plant for several decades ago is the neem tree *Azadirachta indica* (Meliaceae) from which hundreds of products and formulations were assessed on different insects and pests. Azadirachtin

(a neem seed kernel extract) has a lethal activity against various insects (e.g. Meisner *et al.*, 1991; Meisner and Nemny, 1992; Osman, 1993; Schmutterer *et al.*, 1993; Yoshida and Toscano, 1994; AliNiazee *et al.*, 1997; Ghoneim *et al.*, 2000; Ghoneim and Al-Dali, 2002; Athanassiou *et al.*, 2005; Senthil Nathan *et al.*, 2006a; Senthil Nathan *et al.*, 2007; Abdel-Ghaffar *et al.*, 2008; Hamadah *et al.*, 2013). Outside the neem extracts and preparations, strong lethal effects of extracts derived from so many plant species have been reported on several pests (e.g. El-Sokkary, 2003; Athanassiou *et al.*, 2005; Zouiten *et al.*, 2006; Senthil Nathan *et al.*, 2006 b; Abdel-Ghaffar *et al.*, 2008; Begum *et al.*, 2010; Ghoneim *et al.*, 2009; Seffrin *et al.*, 2010; Martins *et al.*, 2012; Hamadah *et al.*, 2013).

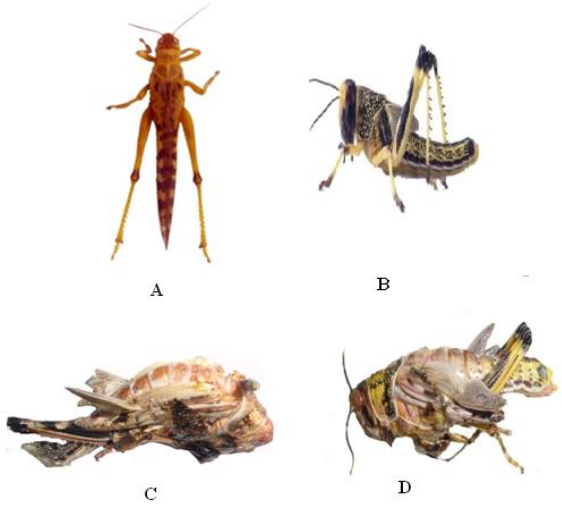

Fig. 1. Nymphal-adult intermediates as disturbed metamorphosis program features after nymphal treatments with different extract of *Ammi visnaga*. (A): Normal adult. (B): Normal last instar nymph. (C, D): Nymphal-adult intermediates after treatment of penultimate instar nymphs with 40%, 20%, 10.0% or 5.0% of petroleum ether extract as well as with 40.0% of n- butanol extract.

In the present study, treatment of penultimate instar nymphs of *S. gregaria* with the highest concentration level (80%) of ethanol, petroleum ether and n-butanol extracts of *A. visnaga* fruits resulted in 50, 70 and 60% nymphicidal activity, respectively (in comparison with 10% natural mortality). With few exceptions, a dose-dependent course of this toxic effect could be conceived. Moreover, the successfully moulted last instar nymphs suffered a lethal action of all extracts. LC_{50} values were 21.0, 12.0 and 22.5% of ethanol, petroleum ether and n-butanol extracts, respectively. After treatment of last instar nymphs, the strongest nymphicidal activity was exhibited in 40, 80 and 60% at the highest concentration level of ethanol, petroleum ether and n-butanol extracts, respectively. The current results agree, to some extent, with the nymphal mortality of *S. gregaria* after treatment with curcuminoids or turmeric oil (Chowdhury *et al.*, 2000), seed oil of *Jatropha curcas* (Euphorbiaceae) (Bashir and El-Safie, 2013) and extracts of *Nerium oleander* (Apocynaceae) (Bagari *et al.*, 2013). The present results are, also, in accordance with the larvicidal activity of methanol extract of *A. visnaga* on the mosquito *Culex quinquefasciatus* (Pavela, 2008) while exposure of *S. granaries* to linalool, 3-pentylmethylbutanoate (main component of essential oils in *A. visnaga*) resulted in low or null toxicity (Lamiri *et al.*, 2001 b). On another weevil, *S. oryzae*, chloroform extract of *A. visnaga* was the most toxic (Ahmed and Al-Moajel, 2005). As reported in the literature, the most important chemical constituents of *A. visnaga* are khellin and visnagin (Ziment, 1998; Lee *et al.*, 2010) as well as pyranocouramin fractions (Gunaydin and Beyazit, 2004). Topical treatment of the milkbug *Oncopeltus fasciatus* with khellin resulted in 40-50% nymphal mortality at 10 and 100 µg/nymph (Maleck *et al.*, 2013). Khellin was, also, tested against the dengue vector *Aedes aegypti* and caused 50% larval toxicity at 50 µg/nymph (Maleck *et al.*, 2013).

With special reference to the adulticidal activity of *A. visnaga* fruit extracts on *S. gregaria*, in the present study, no adults survived after treatment of penultimate instar nymphs with the highest concentration level (80%), regardless the extract. After treatment of last instar nymphs, complete mortality was caused by ethanol extract or n-butanol extract, at the highest concentration level but at the higher two concentration levels of petroleum ether extract. On the contrary, no adulticidal activity was exhibited after treatment of last instar nymphs with the lowest concentration level of ethanol extract or n-

butanol extract. More or less, these results are in consistent with the adulticidal activity of extracts derived from the mangrove tree *Rhizophora mucronata* (Rhizophoraceae) on *S. gregaria* (Kabaru and Gichia, 2001) and with the adulticidal activity of *A. visnaga* extracts, or its khellin, on some other pests (Pavela, 2008; Maleck *et al.*, 2013).

Fig. 2. Different degrees of adult failure to completely get rid the last nymphal exuvia as a result of nymphal treatments with different extracts of *Ammi visnaga*. (A): Normal adult. (B): Nymphal exuvia attached to abdomen, legs and mouth parts after treatment of newly moulted penultimate instar nymphs with 40%, 20.0% or 10.0% of ethanol extract as well as with 40%, 20.0% or 5.0% of n- butanol extract. (C, D): Nymphal exuvia attached to the legs after treatment of last instar nymphs with 40%, 20.0% or 5.0% of ethanol extract as well as with 40%, 20.0% or 10.0% of n- butanol extract.

However, the nymphicidal, or adulticidal, activity of *A. visnaga* fruit extracts, as exerted on *S. gregaria* in the present study, may be interpreted by the occurrence of certain secondary metabolites. The lethal effects of *A. visnaga* extracts on the nymphs of *S. gregaria* may be attributed to the feeding inhibition which usually leads to continuous starvation and subsequently death (Ghoneim *et al.*, 2000) or to the inability of the moulting nymphs to swallow sufficient volumes of air to split the old cuticle and expand the new one during ecdysis (Linton *et al.*, 1997). Disturbance of nymphal ecdysis and prevention of exuviation in the integument had been suggested for the toxic effects of *Cestrum parquii* (Solanaceae) on *S. gregaria* (Ammar and N'cir, 2008) or due to the destruction of extracellular microorganisms by steroid saponins from this plant (Chaieb *et al.*, 2007). In addition, deaths of last instar nymphs of *S. gregaria* may be due to a metamorphosis inhibiting effect of the *A. visnaga* extracts, which is possibly based on the disturbance of the hormonal regulation (Al-Sharook *et al.*, 1991) because the prevention of metamorphosing ecdysis, and subsequently death, could be attributed to the reduction in ecdysteroid peak or interference with the release of eclosion hormone (Sieber and Rembold, 1983).

Affected growth, development and metamorphosis of S. gregaria by A. visnaga extracts

Prohibited growth of S. gregaria

Because the body weight, and subsequently the somatic weight gain, is one of the important indicators for evaluating growth (Armbruster and Hutchinson, 2002), the weight gain of *S. gregaria* nymphs was determined in the present study. After treatment of penultimate instar nymphs with *A. visnaga*, ethanol and petroleum ether extracts exhibited pronouncedly prohibiting effects on growth but no considerable effect could be exerted by n-butanol extract. Also, growth of the successfully moulted last instar nymphs had been subjected to some prohibiting effects of these extracts, with exceptions of the lower two concentration levels. Also, after treatment of last instar nymphs, the *A. visnaga* extracts disruptively affected their growth. The most potent extract was that derived by ethanol followed by petroleum ether extract and then n-butanol extract. The current results, however, agree with those reported results for Azadirachtin or some other neem preparations against various insect pests (Wilps, 1986; Jagannadh and Nair, 1992; Ghoneim *et al.*, 2000; Weathersbee III and Tang, 2002; Al-Dali *et al.*,

2003; Amer *et al.*, 2004; Senthil Nathan *et al.*, 2007; Abdel-Ghaffar *et al.*, 2008; Hamadah *et al.*, 2013). Also, extracts from some other plant species inhibited the growth of different insect pests (Huang *et al.*, 2000; Nascimento *et al.*, 2004; Akhtar and Isman, 2004; Sayed *et al.*, 2004; Cespedes *et al.*, 2005; Senthil Nathan, 2006a; Abdel-Ghaffar *et al.*, 2008; Ghoneim *et al.*, 2009; Hamadah *et al.*, 2013). However, the growth inhibition in *S. gregaria* by the action of *A. visnaga* extracts, in the present study, may be a result from the blocked release of morphogenic peptides, causing alteration in the ecdysteroid and juvenoid titers (Sieber and Rembold, 1983; Barnby and Klocke, 1990; Linton *et al.*, 1997). Also, some possible direct effects of *A. visnaga* extracts may affect the tissue and cells undergoing mitosis (Nasiruddin and Mordue, 1994).

Deranged development of S. gregaria

In the present study, treatment of penultimate instar nymphs of *S. gregaria* with the *A. visnaga* extracts remarkably affected the development of the same nymphs (as shown in significantly prolonged nymphal duration and suppressed developmental rate), regardless the extract. After treatment of last instar nymphs, an evidently prolonged developmental duration, and suppressed rate, was caused by ethanol extract but not by petroleum ether extract or n-butanol extract. These results are, however, consistent with those reported results for some neem extracts and extracts derived from other plants against several insect pests (Nicol and Schmutterer, 1991; Jagannadh and Nair, 1992; Wilps *et al.*, 1993; Yoshida and Toscano, 1994; El-Shazly *et al.*, 1996; El-Sherif, 1998; Mohamed, 1998; Mohamed *et al.*, 2000; Ghoneim *et al.*, 2000; Sharda *et al.*, 2000; Zhong *et al.*, 2001; von Elling *et al.*, 2002; Al-Dali *et al.*, 2003; Jeyabalan *et al.*. 2003; Zouiten *et al.*, 2006; Abdel-Ghaffar *et al.*, 2008; Jbilou *et at.*, 2008; Hamadah *et al.*, 2013). On the other hand, the current results of prohibited development of *S. gregaria* by *A. visnaga* extracts disagree with some other results of enhanced development of some insects by extracts of various plant species (Al-Sharook *et al.*, 1991; Saxena *et al.*, 1993; Darvas *et al.*, 1996; Amer *et al.*, 2004; Maleck *et al.*, 2013).

However, the delayed development of *S. gregaria*, in the present study, by the action of *A. visnaga* extracts may be explicated by a retarding effect on ecdysis and transformation (Linton *et al.*, 1997). The chemical component, khellin, visnagin or pyranocouramin (Ziment, 1998; Gunaydin and Beyazit, 2004; Lee *et al.*, 2010), may be responsible for exerting such retarding action. The matter is still obscure and needs further investigation.

Disrupted metamorphosis of S. gregaria

The available literature reported inhibitory action of azadirachtin, some other neem products, and extracts of different plant species on the immature-adult transformation program of several insects. No effects or even contradictory effects had been reported, depending on the plant species and the susceptibility of the insect species (Jagannadh and Nair, 1992; Khalaf and Hussein, 1997; Shaurab *et al.*, 1998; Ghoneim *et al.*, 2000; Hassan, 2002; Al-Dali *et al.*, 2003). In the present study, treatment of newly moulted penultimate instar nymphs of *S. gregaria* with ethanol extract of *A. visnaga* fruits resulted in no nymphal-adult intermediates but treatment with n-butanol extract (at 40% concentration level) led to 25% intermediates. Furthermore, petroleum ether extract remarkably intervened in such program, regardless the concentration level. Treatment of newly moulted last instar nymphs with all extracts failed to cause nymphal-adult intermediates but impaired the program of adult morphogenesis since nymphal exuvia were observed in attachment with abdomen, legs and mouth parts after of nymphal treatments with certain concentration levels of ethanol extract or n- butanol extract. Our results are consistent with some results of deranged nymphal-adult transformation program of *S. gregaria* by extracts of *A. conyzoides* (Pari *et al.*, 2000), *C. rotendus* (El-Sokkary, 2003), *F. bruguieri* (Ghoneim *et al.*, 2000) and *N. sativa* (Hamadah *et al.*, 2013). In addition, treatment of penultimate instar nymphs of *S. gregaria* with *A. visnaga* extracts, in the present study, resulted in a dose-dependent inhibition of

adult emergence with few exceptions. Treatment of last instar nymphs with all extracts detained the emerging adults in no certain trend. The formation of nymphal-adult intermediates or 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). Hence, the *A. visnaga* extracts contain certain active ingredients affecting the hormonal regulation essential to the metamorphosis or morphogenesis of *S. gregaria*, in the present study (ecdysone, juvenile hormone and eclosion, in particular).

Conclusion

The active ingredient (s) in the *A. visnaga* extracts which is responsible for the reduction of survival, prohibition of growth, derangement of development and disruption of metamorphosis of S. gregaria is still questionable right now. A further investigation should be carried out in future to explore the specific active agent (khellin, vishagin, or pyranocouramin) causing the disturbance or imbalance of the enzymatic patter or hormonal hierarchy in life of *S. gregaria*.

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