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RESEARCH ARTICLE

Disturbed acid and alkaline phosphatase activities in desert locust *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae) by extracts from the khella plant *Ammi visnaga* L. (Apiaceae).

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Abstract

The present study aimed to investigate the effects of fruit extracts from toothpick weed *Ammi visnaga* on the acid (ACP) and alkaline (ALP) phosphatases in haemolymph and fat bodies of *Schistocerca gregaria*. LC_{50}s of ethanol, petroleum ether and n-butanol extracts (21.0, 12.0 and 22.5%, respectively) were applied for penultimate instar nymphs. The enzyme activity was determined in the last instar nymphs (of three ages) and newly emerged adult females. Both ethanol and petroleum ether extracts promoted the ACP activity in haemolymph throughout nymphal life and newly emerged adults. In contrast, n-butanol extract caused a drastic reduction in the enzyme activity in haemolymph of nymphs and adults. With regard to ACP activity in fat bodies, a considerable induction in both early- and late-aged nymphs was achieved by each of ethanol and petroleum ether extracts and *vice versa* for n-butanol extract. Enhanced enzyme activity was estimated in newly emerged adults, regardless the extract. ALP activity in haemolymph was significantly reduced throughout the nymphal life after treatment with petroleum ether extract or n-butanol extract. The n-butanol extract exhibited an inhibitory effect on the enzyme activity in only early-aged nymphs. Increasing ALP was measured in the newly emerged adults, irrespective of the extract. Ethanol extract suppressed ALP in fat bodies of early- and mid-aged nymphs and n-butanol extract prohibited it only in early-aged nymphs. The newly emerged adults had significantly stimulated ALP activity by all extracts.

INTRODUCTION:

The desert locust *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae) is a destructive pest to many crops in several African and Asian countries due to its voracious feeding and invasions in swarms. Most recent large-scale outbreaks of it occurred in 1986-1989 and in 2003-2005, mostly on the African continent (Latchininsky, 2013). In 2003-2005, to curtail the outbreak of this locust, 13 million ha were treated with broad-spectrum insecticides in 26 countries on three continents. Such transcontinental operations, together with food aid for affected populations, cost over half a billion USD to the world community, not to mention human and environmental health costs (Belaynech, 2005).

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). Although, hundreds of plant natural products have demonstrated deleterious effects on insects only a handful of botanical insecticides are currently approved for use in industrialized countries (Isman, 1994) for several reasons (out-lined in Isman, 2006). At present there are four major botanical products (pyrethrum, rotenone, neem, and essential oils) used for insect control along with three others (ryania, nicotine, and sabadilla) in limited use (Isman, 2006). Neem extracts, as an example, were considered insect growth regulators (Naqvi *et al*., 1987, 1992) and toxicants (Saxena *et al*., 1989). In addition, they disturb the enzyme pattern in the insect body as reported by some researchers (Naqvi *et al*., 1991; Hosseini-Naveh *et al*., 2007).

Ammi visnaga Lamarck (Apiaceae = Umbelliferaceae) is native to Europe, Asia and North Africa but can be found throughout the world as an introduced species. Among Egyptian people, it is called "Khella", "Chellah" or "Kella", while in Europe the plant has often been referred to "Toothpick herb" or "Bishop's weed" (El-Fiky et al., 1989). Turkish people referred to this plant as "Disotu", "Kilir" and "Hittan" (Gunaydin and Beyazit, 2004). It is of interest to know the main constituents of A. visnaga as couramins and furocumarins (2-4%), the nost important of which are khellin (0.3-1.2%), visnagin (0.05-0.3%), khellol, khellinol, and angular pyranocouramins (0.2-0.5%) including visnadin, samidin and dihydrosamidin (Ortel et al., 1988). In fruits, khellin represents 1% and visnagin represents 0.3% (Martelli *et al*., 1984). Flavonoids have been, also, reported from this herb (Cisowski, 1986; Bencheraiet *et al*., 2011). The essential oil of *A. visnaga*, growing in Morocco, has been reported to contain linalool and aliphatic esters as the main components (Lamiri *et al*., 2001a). Gas-liquid chromatographic analysis revealed the following major components in its essential oil: isobutyl isobutyrate, linalool, thymol, fenchyl acetate, bornyl acetate, α-isophorone, 2,2-dimethyl butanoic acid and croweacin (Khalfallah *et al*., 2011; Talaat *et al*., 2014). Furthermore, the unused parts of *A. visnaga* were found to contain several compounds, such as tetracosanoic acid, β-sitosterol, visnadine, khellin, β-sitosterol glucoside, norkhellol, khellol, rhamnazin, cimifugin and *cis*-khellactone-3-β-glucopyranoside (Ashour *et al*., 2013).

As pointed out by several authors (Duarte *et al*., 1995, 1998; Khan *et al*., 2001; Jouad *et al*., 2002; Cordero et al., 2004; Whitton et al., 2008; Vanachayangkul, 2008; Lee *et al*., 2010; Kwon *et al*., 2010; Vanachayangkul *et al*., 2010; Jan, 2014; Mahmood, 2014; Sabry et al., 2014), *A. visnaga* extracts or some of their components have been used in traditional medicine and modern therapeutics. These extracts or components are reported as anti-inflammatory, antimicrobial, antihyperglycemic, vasodilator and antihyperacidic agents, as well as for inhibition of oxalate nephrlithiasis. The research work on using this plant, or some of its chemical constituents, in the 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*., 2001b) 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).

Four types of detoxifying enzymes have been found to react against botanical insecticides including general esterases (EST), glutathione S-transferase (GST) and phosphatases (Zibaee, 2011). Acid phosphatase (ACP, E.C.3.1.3.2) and alkaline phosphatase (ALP, E.C.3.1.3.1) are the hydrolytic enzymes, which hydrolyze phosphate monoesters under acid or alkaline milieus, respectively (Janda and Benesova, 1991; Bai *et al*., 1993). In insects, ACP and ALP are responsible for cytolysis of tissues during the insect development (Dadd, 1970). Also, these two enzymes may act as hydrolases during the final stages of digestion (Cheug and Low, 1975), gonad maturation and metamorphic moults (Rhadha and Priti, 1969). Their activities are low during the larval moulting stage and increased gradually after moulting (Miao, 2002).

ACP, known as a lysosomal marker enzyme (Csikos and Sass, 1997), is active in guts (Ferreira and Terra, 1980), Malpighian tubules (Srivastava and Saxena, 1967) and is also abundant in the disintegrating tissues and organs subjected to cytolysis (Aidellis *et al*., 1971; Sahota, 1975). This enzyme hydrolyzes a variety of orthophosphate esters and is capable of transphosphorylation reactions to increase the phosphate pool for synthesizing higher energy compounds as adenosine triphosphate (ATP), ATP ase, and genetic materials (DNA or RNA) (Hollander, 1971). ALP is primarily found in the intestinal epithelium of animals and its major function is to provide phosphate ions from mononucleotide and ribonucleo-proteins for a variety of metabolic processes. In insects, ALP is a brush border membrane marker enzyme (Wolfersberger, 1984) and is especially active in tissues with active membrane transport, such as intestinal epithelial cells (Sakharov *et al*., 1989; Caglayan, 1990), Malpighian tubules (Srivastava and Saxena, 1967; Khoja, 1991; Khoja, 1991; Etebari and Matindoost, 2004 a,b) and haemolymph (Etebari *et al*., 2007). It is responsible for cytolysis of tissues during the insect development (Dadd, 1970). In addition to ACP, ALP may act as hydrolase during the final stages of digestion (Cheug and Low, 1975), gonad maturation and metamorphic moults (Rhadha and Priti, 1969). Its activity is low during the larval moulting stage and increased gradually after moulting (Miao, 1988). The available literature contains no report for the effects of *A. visnaga* extracts, or its constituents, on the physiological processes of insects. The present study is a part of an extended

research work on the effects of *A. visnaga* fruit extracts on the destructive locust *S. gregaria*. It was designed to explore the effects of ethanol, petroleum ether and n-butanol extracts on ACP and ALP activities in two tissues of *S. gregaria*.

Materials and Methods

1. Experimental insect

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 **(**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 with10-15% humidity to be suitable for egg laying. An electric bulb (100 watt) was adjusted to maintain a continuous photoperiod (12 L: 12 D) in each cage as well as in order to maintain an ambient temperature (32±2^{o}C). The insects were reared and handled under the crowded conditions. The feces, dead locusts and food remains were removed daily before introducing the freshly food. Care was seriously taken to clean these cages at regular intervals and the sand was sterilized in drying oven (at 140^{o}C for 24 hours) to avoid contamination with any pathogenic microorganisms. Fresh clean leaves of clover *Trifolium alexandrinum* were provided, as a food for insects, every day.

2. Plant extraction:

A weight of 1.5 Kg *Ammi visnaga* fruits, which purchased from an Egyptian market, was 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 is 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%.

3. Nymphal treatments

In a preliminary experiment, different concentration levels of ethanol, petroleum ether, and n-butanol extracts derived from *A. visnaga* fruits had been applied on the newly moulted penultimate (4th) instar nymphs of *S. gregaria* through the fresh food leaves of *Trifolium alexandrinum* dipped once in the extract for 3 minutes. A day after treatment, all nymphs (treated and control) were provided, individually, with untreated fresh food leaves. 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. LC_{50} values were estimated for ethanol, petroleum ether, and n-butanol extracts in 21.0, 12.0 and 22.5%, respectively. After treatment of penultimate instar nymphs with LC_{50}s, the successfully moulted last instar nymphs and emerged adult females were undergone to determine the influenced acid phosphatase (ACP) and alkaline phosphatase (ALP) activities in two tissues: haemolymph and fat body. Three ages of last instar nymphs were only used: early- (1-day old), mid- (4-day old) and late-aged (7-day old) nymphs.

4. Determination of phosphatases

For the determination of phosphatase activity in the haemolymph, it was collected from early-, mid- and late-aged last instar nymphs, as well as newly emerged adult females. Haemolymph was drawn into Eppendorff Pipetman containing few milligrams of phenoloxidase inhibitor (phenylthiourea) to prevent tanning or darkening and then diluted 5× with saline solution 0.7%. For whole blood assays, the diluted haemolymph was frozen for 20s to rupture the haemocytes. The haemolymph samples were then centrifuged at 2000 r.p.m. for 5 min, and only the supernatant fractions were used for assay directly or frozen until use. Three replicates were used and the haemolymph of two individuals were never mixed.

For the determination of phosphatase activity in the fat body, samples were collected from last instar nymphs (of the same ages) and newly emerged adults. The fat body samples were weighed and then homogenized in a saline solution (the fat body of one insect / 1 ml saline solution 0.7 %) using a fine electric homogenizer, tissue grinder for 2 min. Homogenates were centrifuged at 4000 r.p.m. for 15 min. The supernatant was used directly or frozen until the use for the enzymatic determination. Three replicates were used and the fat bodies from two individuals were avoided to be mixed.

ACP activity was determined according to the method of Tietz **(**1986) using a kit of Bioadwic. The enzyme was measured at wave length 405 nm by spectrophotometer. ALP activity was determined according to the method

of Klein *et al*. **(**1960) using a kit of Quimica clinical aplicada S.A. the measured at wave length 550 nm by spectrophotometer.

5. Statistical analysis

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

1. Effects of *A. visnaga* extracts on ACP activity in *S. gregaria*

The data presented in Table (1) clearly reveal different effects on ACP activity in haemolymph of both nymphs and adults, depending on the extract. Both ethanol and petroleum ether extracts remarkably promoted the enzyme activity throughout the nymphal stage and newly emerged adults. The strongest enhancing effect was exhibited by petroleum ether extract (Change%s: +86.8, +95.0, +66.0 and +34.9 in early-, mid- and late-aged nymphs, as well as newly emerged adults, respectively). In contrast, n-butanol extract caused a drastic reduction in the enzyme activity in haemolymph of nymphs and adults (Change%s: -12.4, -34.2 in early- and mid-aged nymphs, as well as -34.3 in adults) with few exception of the late-aged nymphs which appeared with haemolymph containing significantly increased ACP.

With regard to ACP activity in fat bodies of nymphs and adults, as disturbed by *A. visnaga* extracts, data arranged in Table (2) obviously indicate the pronouncedly induced enzyme activity in early- and late-aged nymphs by ethanol extract or petroleum ether extract (Change%s: +96.6 and +34.3 by ethanol extract, as well as +20.9 and +56.5 by petroleum ether extract, respectively). On the other hand, the mid-aged nymphs failed to maintain normal enzyme activity owing to remarkably prohibiting action of these two extracts. Considering the effect of n-butanol extract on ACP in fat bodies of early- and late-aged nymphs, the enzyme activity was profoundly inhibited (Change%s: -12.6 and -14.1, respectively). On the contrary, the mid-aged nymphs appeared with fostered enzyme activity (154.6±4.6 U/L vis. 115.3±3.1 U/L of control congeners). In the light of data assorted in the same table, elaborately enhanced enzyme activity was estimated in fat bodies of newly emerged adults, regardless the extract (144.1±3.8, 149.4±3.2 and 127.4±4.0 U/L vis 110.3±2.6 U/L of control adults).

2. Effects of *A. visnaga* extracts on ALP activity in *S. gregaria*

Another part of the present work was carried out to explore the possible effects of *A. visnaga* extracts on ALP activity in the two tissues of last instar nymphs and newly emerged adults. As shown in Table (3), ALP activity was considerably reduced in haemolymph throughout the nymphal stage after treatment with petroleum ether extract or n-butanol extract. On the other hand, ethanol extract exhibited an inhibitory effect on the enzyme activity in only early-aged nymphs but inducing effect through the remainder of their life (Change%s: -28.3, +17.0 and +40.1 in early-, mid- and late-aged nymphs, respectively). In respect to the newly emerged adults, data of the same table exiguously show increasing ALP activity as a result of treatment with all extracts. The most powerful enhancing action was achieved by ethanol extract (14.4±1.9, 12.5±1.9 and 12.2±2.0 U/L after treatment with ethanol, petroleum ether and n-butanol extracts, respectively, compared to 10.5±3.2 U/L in control adults).

Depending on the data arranged in Table (4), a general promoting action on ALP activity in fat bodies can be easily seen for nymphs, with few exceptions because ethanol extract suppressed the enzyme activity in fat bodies of early- and mid-aged nymphs (Change%s: -56.0 and -14.1, respectively). Another case of the enzyme reduction was detected in only early-aged nymphs after treatment with n-butanol extract (8.3±1.8 U/L vis 12.5±3.2 U/L in control nymphs). The newly emerged adults had been stimulated to produce high level of ALP, regardless the extract.

Table (1): Acid phosphatase activity (U/L) in haemolymph of desert locust *Schistocerca gregaria* after treatment of newly moulted penultimate instar nymphs with LC_{50} of different extracts from ***Ammi visnaga*.**

Solvents		Last instar nymphs			Newly emerged adults
		Early-aged	Mid-aged	Late-aged	
Ethanol	**Mean±SD**	1813.8 ± 4.4 d	1902.3 ± 4.6 d	1968.8 ± 2.4 d	1440.5 ± 4.5 d

	Change (%)	+36.6	+55.8	+41.4	+6.9
Petroleum ether	**Mean±SD**	2071.3 ± 4.1 d	2380.5 ± 4.1 d	2311.7 ± 3.4 d	1817.3 ± 2.8 d
	Change (%)	+86.8	+95.0	+66.0	+34.9
n-butanol	**Mean±SD**	0971.6 ± 3.5 d	0803.4 ± 2.9 d	1467.2 ± 3.4 d	0883.5 ± 4.2 d
	Change (%)	-12.4	-34.2	+05.4	-34.3
Control		1108.6 ± 7.5	1220.9 ± 4.1	1392.4 ± 5.6	1346.9 ± 3.4

Early-aged: 1- day old nymphs, Mid-aged: 4- day old nymphs, Late-aged: 7- day old nymphs. 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 (2): Acid phosphatase activity (U/L) in fat bodies of desert locust *Schistocerca gregaria* after treatment of newly moulted penultimate instar nymphs with LC_{50} of different extracts from *Ammi visnaga*.

Solvents		**Last instar nymphs**			**Newly emerged adults**
		Early-aged	**Mid-aged**	**Late-aged**	
Ethanol	**Mean±SD**	198.1 ± 3.8 d	101.8 ± 4.5 b	161.4 ± 4.2 d	144.1 ± 3.8 c
	Change (%)	+96.6	-11.7	+34.3	+30.6
Petroleum ether	**Mean±SD**	311.6 ± 4.6 d	091.5 ± 4.1 c	188.2 ± 3.7 d	149.4 ± 3.2 d
	Change (%)	+20.9	-20.7	+56.5	+35.4
n-butanol	**Mean±SD**	087.9 ± 3.5 c	154.6 ± 4.6 d	103.3 ± 3.8 c	127.4 ± 4.0 d
	Change (%)	-12.6	+34.1	-14.1	+15.5
Control		100.6 ± 2.6	115.3 ± 3.1	120.2 ± 2.8	110.3 ± 2.6

Early-aged , Mid-aged , Late-aged , b, c, d : See footnote of Table (1).

Table (3): Alkaline phosphatase activity (U/L) in haemolymph of desert locust *Schistocerca gregaria* after treatment of newly moulted penultimate instar nymphs with LC_{50} of different extracts from *Ammi visnaga*.

Solvents		Last instar nymphs			Newly emerged adults
		Early-aged	Mid-aged	Late-aged	
Ethanol	Mean±SD	20.4 ± 2.7 b	26.2 ± 2.1 b	20.6 ± 2.3 b	14.4 ± 1.9 b
	Change (%)	-28.3	+17.0	+40.1	+36.8
Petroleum ether	Mean±SD	23.3 ± 2.3 c	19.4 ± 2.2 b	09.5 ± 1.7 c	12.5 ± 1.9 a
	Change (%)	-18.2	-13.4	-35.1	+18.7
n-butanol	Mean±SD	19.4 ± 2.0 d	15.3 ± 2.1 c	10.2 ± 2.1 c	12.2 ± 2.0 b
	Change (%)	-31.9	-31.5	-30.6	+16.2
Control		28.5 ± 3.2	22.4 ± 2.9	14.7 ± 3.3	10.5 ± 3.2

Early-aged , Mid-aged , Late-aged , a ,b , c , b : See footnote of Table (1).

Table (4): Alkaline phosphatase activity (U/L) in fat bodies of desert locust *Schistocerca gregaria* after treatment of newly moulted penultimate instar nymphs with LC_{50} of different extracts from *Ammi visnaga* .

Solvents		Last instar nymphs			Newly emerged adults
		Early-aged	Mid-aged	Late-aged	
Ethanol	Mean±SD	05.5 ± 1.6 c	07.3 ± 1.8 a	09.4 ± 1.7 c	08.2 ± 2.0 d
	Change (%)	-056.0	-014.1	+064.3	+127.8
Petroleum ether	Mean±SD	13.9 ± 1.5 a	17.3 ± 2.1 c	13.2 ± 2.1 c	07.5 ± 1.7 d
	Change (%)	+010.9	+103.9	+132.3	+107.3

n-butanol	**Mean±SD**	08.3 ± 1.8 b	13.5 ± 2.1 b	07.5 ± 1.7 b	08.1 ± 1.8 d
	Change (%)	-033.6	+058.8	+032.2	+126.0
Control		12.5 ± 3.2	08.5 ±2.1	05.7 ± 1.9	03.6 ± 1.7

Early-aged , Mid-aged , Late-aged , a ,b , c , b : See footnote of Table (1).

Discussion

1. Disturbed ACP activity in *S. gregaria* by *A. visnaga* extracts

Different effects of several botanicals on ACP activity in various insects had been reported in the literature. In *Culex fatigans*, the neem extract RB-a was less effective than other extracts RBU-a and Margosan-O (Naqvi *et al*., 1995). The neem extract RB-b was more effective than RB-a but less than the insecticide parathion in *Anopheles stephensi* (Rajput, 2003). Ghoneim *et al*. (2008) recorded various inducing and reducing effects of Margosan-O and Jojoba oil on activity level of the same enzyme in the pupal stage of *Musca domestica*. Another neem preparation, Neemazal, promoted the enzyme in haemolymph, but gradually prohibited it in fat bodies of last instar nymphs of *S. gregaria* (Hamadah, 2009). To a great extent, similar disruptive effects had been reported for the *Nigella sativa* extracts (Hamadah, 2009) and the *Fagonia bruguieri* extracts (Basiouny *et al*., 2010) on the enzyme activity in the same locust. In addition, treatments of last instar larvae of *M. domestica* with juvenoids pyriproxyfen and buprofezin, or chitin inhibitors hexaflumuron and lufenuron, induced ACP activity but ecdysone analogue tebufenozide reduced it (Assar *et al*., 2010).

In the light of these contradictory effects of botanicals and insect growth regulators (IGRs) on ACP activity, the present study revealed a remarkably promoting action of ethanol and petroleum ether extracts from *A. visnaga* on the enzyme activity in haemolymph throughout the nymphal life and newly emerged adults of *S. gregaria*. On the contrary, n-butanol extract caused a drastic reduction in the enzyme in haemolymph of nymphs and adults. With regard to ACP activity in fat bodies, a significant induction in both early- and late-aged nymphs was achieved by ethanol and petroleum ether extracts. At these two life limits of nymphs, n-butanol extract exhibited a considerable prohibitory effect on the enzyme activity. On the other hand, all extracts induced it in newly emerged adults. The inducing effect of the *A. visnaga* extracts, especially ethanol and petroleum ether extracts, on ACP activity in nymphs and adults of *S. gregaria* are in agreement with those reported enhancing effects of some other botanicals on the same enzyme in various insects, such as *Culex pipiens* (El-Bassal, 1993), *Pectinophora gossypiella* and *Earias insulana* (Anan *et al*., 1993), *Helicoverpa armigra* (Babu *et al*., 1996), *Spodoptera littoralis* (Hassan, 2002; Abdel-Al, 2002), *Agrotis ipsilon* (El-Sheikh, 2002), *Rhynchophorus ferrugineus* (Bream, 2003) and *S. gregaria* (Hamadah, 2009; Basiouny *et al*., 2010). The induced ACP activity, in the present study on *S. gregaria* by the action of *A. visnaga* extracts, may be attributed to increasing number of lysosomes since ecdysone (moulting hormone) is responsible for increase of lysosome number as a lysosomal ACP enzyme (Redford and Misch, 1971; van Pelt-Verkuil, 1979; Bassal and Ismail, 1985). It can be, also, understood because ACP activity, directly or indirectly, interferes with the digestion, absorption and positive transport of nutrient in the midgut (Smirle *et al*., 1996; Senthil Nathan *et al*., 2004).

On the other hand, the inhibitory effects of *A. visnaga* extracts, especially n-butanol extract, on ACP activity, agree, to some extent, with the reported results of similar effects of other plant extracts, such as azadirachtin (Azt.) against *M. domestica* (Saeed *et al*., 1987) and *S. littoralis* (Ayyangar and Rao, 1990); NfD (a fraction of winter neem leaves) against *Sitophilus oryzae* (Naqvi *et al*., 1991); some neem limonoids against *Euprepocnemis plorans* (Al-Dali, 2007); Margosan-O and Jojoba oil against *M. domestica* (Ghoneim *et al*., 2008); Neemazal against *S. gregaria* (Hamadah, 2009); as well as extracts from Ammi *majus*, *Apium graveolens*, *Melia azedarach* and *Vinea rosea* against *Agrotis ipsilon* (Abo El-Ghar *et al*., 1995). In addition, the inhibitory effects of some *A. visnaga* extracts, in the present study, are in conformity with the reducing effects of several IGRs when applied against different pests, such as *S. littoralis* by pyriproxyfen (Mostafa, 1993) and hexaflumuron (Sokar, 1995), as well as *M. domestica* by Triflumuron (BAY SIR)(El-Bermawy, 1994) and tebufenozide (Assar *et al*., 2010). However, the declined ACP in *S. gregaria*, as a response to some extracts from *A. visnaga*, in the current investigation, may be due to strong inhibition of ecdysone which is followed by subsequent decrease in number of lysosomes and in turn decreased levels of ACP (Hassan, 2002). Also, Senthil Nathan *et al*. (2005) suggested that a prohibited ACP activity

reduced phosphorus libration for energy metabolism, decreased rate of metabolism as well as decreased rate of transport of enzyme regulation.

2. Disturbed ALP activity in *S. gregaria* by *A. visnaga* extracts

So many controversial results on ALP activity by several botanicals, IGRs or insecticides can be reviewed in the available literature (e.g. Mostafa, 1993; Abdel-Al, 2002; Senthil Nathan *et al*., 2005, 2006; Etebari *et al*., 2007; Assar *et al*., 2010; Teleb *et al*., 2012). For some detail, Neemazal and *N. sativa* enhanced or suppressed the ALP activity in *S. gregaria*, depending on the developmental stage, tissue, extract and concentration level (Hamadah, 2009). Similarly, various, or even confusing, effects of the *F. bruguieri* extracts on ALP activity in the same locust were reported (Basiouny *et al*., 2010). Outside the botanicals, stimulating action of pyriproxyfen on ALP activity was detected in *P. gossypiella* (Mostafa, 1993), *S. littoralis* (Abdel-Al, 2002) and *M. domestica* (Assar *et al*., 2010). Also, each of buprofezin, hexaflumuron, lufenuron and tebufenozide promoted the ALP activity in last instar larvae of *M. domestica* (Assar *et al*., 2010). As well as, the insecticide pyridalyl exhibited some inducing or reducing effects on activity of the same enzyme in haemolymph and fat bodies of *S. gregaria*, depending on the nymphal age and developmental stage (Teleb *et al*., 2012).

In the present study on *S. gregaria*, ALP activity in haemolymph was evidently reduced as a result of nymphal treatments with ethanol and petroleum ether extracts from *A. visnaga* throughout the last nymphal instar. The n-butanol extract, contrarily, stimulated the enzyme during the majority of nymphal life. Furthermore, increasing ALP level was measured in haemolymph of newly emerged adults, regardless the extract. In fat bodies, a general promoting effect of *A. visnaga* on ALP activity could be detected, with few exceptions. However, the reduced ALP activity in the present study are in accordance with similar reducing effects of some other plant extracts on ALP activity in *C. pipiens* (El-Bokl *et al*., 1998), *Cnaphalocrocis medinalis* (Senthil Nathan *et al*., 2005, 2006), *E. plorans* (Al-Dali, 2007). Also, some IGRs suppressed the ALP activity in *Bombyx mori* (Etebari *et al*., 2007) and *A. ipsilon* (El-Sheikh, 2002). The depressed ALP activity in some tissues at different developmental events in *S. gregaria*, in the present study, may be explicated by some developmental disturbance as an appreciated suggestion of Wu (1990) for the mosquito larvae of *C. pipiens fatigans* after treatment with IGR diflubenzuron. In addition, the A. visnaga extracts contain some components (such as couramins, furocumarins and flavonoids)(Martelli *et al*., 1984; Cisowski, 1986; Ortel *et al*., 1988; Bencheraiet *et al*., 2010) which one or more of their chemicals may affect the gut physiological events (i.e. transport) causing a prohibition of ALP activity, as well as may influence both juvenile hormone and ecdysone regulation, directly or indirectly, as suggested by Sridhara and Bhat (1963) for *B. mori*. On the other hand, increasing ALP activity in some tissues of nymphs or adults of *S. gregaria*, in the present study, may indicate the involvement of this enzyme in detoxification process against the toxicants contained in the *A. visnaga* extracts, as suggested by Shekari *et al*. (2008) for other plant extracts against another insect.

Conclusion

In the present study, no isolated or identified compound was assessed on the phosphatase activities in *S. gregaria*, but crude fruit extracts from *A. visnaga* had been applied on the newly moulted penultimate instar nymphs. Thus, the active ingredient (s) which may be responsible for disturbance of the enzyme activity necessarily needs to be explored by further investigation in future. Nevertheless, and in light of the available literature, the current study represents the first report dealing with the disruptive effects of *A. visnaga* on the enzyme activities in the desert locust *S. gregaria* or even all insects !!

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