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RESEARCH ARTICLE

Lactate dehydrogenase disturbance in the desert locust *Schistocerca gregaria* (Forsk.) (Orthoptera: Acrididae) by extracts of *Punica granatum* Linn. and *Ammi visnaga* L.

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Abstract

The current work was planned and conducted to investigate the effects of *Punica granatum* Linn. (Lythraceae) peel extracts and *Ammi visnaga* L. (Apiaceae) fruit extracts on lactate dehydrogenase (LDH) activity in haemolymph and fat bodies of last instar nymphs and newly emerged adult females of *Schistocerca gregaria*. A prevalent enhancing effect was exhibited by *P. granatum* peel extracts on last instar nymphs of all ages to gain considerably increasing LDH activity in haemolymph, with an exceptional case of inhibited activity in the mid-aged nymphs by n-butanol extract. A similar promoting action was exerted by *A. visnaga* fruit extracts with an exceptional case of an inhibited activity in the late-aged nymphs by petroleum ether extract. LDH activity in haemolymph of the newly emerged adult females was drastically suppressed by all *P. granatum* peel extracts. The ethanol extract of *A. visnaga* fruits prohibited the enzyme activity but both petroleum ether extract and n-butanol extract induced it. All *P. granatum* peel extracts caused remarkable inhibition of LDH in fat bodies of early-aged nymphs. In fat bodies of the mid- and late-aged nymphs, petroleum ether extract prohibited the enzyme activity but both ethanol extract and n-butanol extract stimulated it. Treatment with *A. visnaga* fruit extracts resulted in dramatically declined enzyme level in both early- and late-aged nymphs but enhanced in mid-aged ones. With regard to adults, LDH activity was unexceptionally induced in fat bodies by all plant extracts.

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INTRODUCTION

As pointed out by some authors (Uvarov, 1977; Showler and Potter, 1991; Jahn, 1993; Showler, 1995; Ullman, 2006; Ceccato et al., 2007; Tawfik, 2012), the desert locust, *Schistocerca gregaria* is potentially the most dangerous among the locust pests because of its ability of swarms to fly rapidly across great distances affecting some 58 countries. It is a dangerous pest in a vast area of the old world stretching from Mauritania in the west to India in the east, and from Turkey to as far south as Tanzania. Plagues of the desert locust have threatened agricultural production in Africa, the Middle East, and Asia for centuries.

The widespread use of 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, alternatives to conventional pesticides are required to be developed from the active ingredients of plant origin (Copping and Menn, 2001). The plant extracts and plant products are reported to be more effective, less expensive, rapidly biodegradable, have low toxicity to natural enemies and mammals, safe for mankind (Singh et al., 1996) and more selective in action than synthetic pesticides (Keita et al., 2001; Rahman and Siddiqui, 2004). Along the late decades, so many plant species in the world have been assessed for their insecticidal activities, antifeedant, growth retarding, morphogenic impairing or reproductive disturbing effects on various insect pests. *Azadirachta indica* and *Melia azedarach* are two species among the most well investigated plants and trees in this context affecting survival, feeding, growth,

fecundity, fertility and several anatomical and physiological processes of insects (Mordue et al., 1998; Breuer and De Loof, 2000; Sayah, 2002; Breuer et al., 2003; Huang et al., 2007; Senthil Nathan et al., 2008; Correia et al., 2009).

Among the methods used to fight against desert locust, chemical control remains the most accessible and most effective (Abou et al., 2005). Nevertheless, various researches on the effect of botanical biopesticides on desert locust had been carried out as alternatives to the currently used harmful pesticides (Krall and Wilps, 1994). Several plant species affect differentially the fertility, development, behaviour, and survival of this pest (Idrissi Hassani, 2000; Abbassi et al., 2004). As for examples, *Fagonia bruguieri* (Tanani et al., 2009; Hamadah et al., 2010; Basiouny et al., 2010; Aly et al., 2010), *Nigella sativa* (Hamadah et al., 2013), pomegranate (Ghoneim et al., 2014a,b,c; Mahmoud, 2014) and toothpick weed (Ghoneim et al., 2014d,e,f; Mahmoud, 2014) had been assessed against this locust. These botanicals are still at the experimental stage and the large scale production is problematic and difficulties with the registration of variable products will limit adoption (Meinzingen and Kooyman, 1997). However, prior results on the effects of plant extracts on the desert locust have encouraging for implementing an alternative measure to synthetic pesticides (Idrissi Hassani, 2000; Abbassi et al., 2003).

Pomegranate (*Punica granatum* Linn., Lythraceae) is one of the oldest cultivated plants in the world (Lye, 2008). It is cultivated in Central Asia and the drier parts of Southern Asia (Holland et al., 2009), as well as in the Mediterranean, tropical and subtropical areas (Mars, 2000). It was introduced into Latin America, California and Arizona (Khan and Hane, 2011). From the medical point of view, pomegranate is of a great interest to research in pharmaceutical and new drug development fields because of its distinctive bioactivities (Singh et al., 2002; Negi and Jayaprakasha, 2003; Vasconcelos et al., 2006; Reddy et al., 2007; Lansky and Newman, 2007; Jurenka, 2008; Tayel et al., 2009; Augusta et al., 2010; Abdollahzadeh et al., 2011; Das and Barman, 2012; Dkhil, 2013; Eldiasty et al., 2014). For the pest control, extract of *P. granatum* fruit rind was toxic against tape-worms, earthworms and round-worms (Hukkeri et al., 1993). Extracts of the bark exhibit molluscicidal activity (Tripathi et al., 2004). With regard to the insect pests, available literature reported insecticidal effects of *P. granatum* extracts and their disruptive effects on growth and development (Alanis et al., 2005; Melendez and Capriles, 2006; Liu et al., 2007; Sharma and Rajguru, 2009; Mansour et al., 2010; Ghandi et al., 2010; Mahmood, 2010; Ghandi and Pillai, 2011; Mansour et al., 2012; Mohammad, 2012; Eldiasty et al., 2014; Ghoneim et al., 2014a). Also, *P. granatum* peel extracts affected the adult performance and transaminase activity in *S. gregaria* (Ghoneim et al., 2014b, c). Toothpick weed (*Ammi visnaga* Lamarck, Apiaceae= Umbelliferaeae) 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. The *A. visnaga* extracts have been used in the traditional medicine and their chemical components have been used in the modern medicine (Duarte et al., 1998; Khan et al., 2001; Cordero et al., 2004; Whitton et al., 2008; Kwon, et al., 2010; Lee et al., 2010; Vanachayangkul et al., 2011). However, the research work on using this plant, or some of its chemical constituents, in the pest control, is unfortunately scarce. The available literature reported its ovicidal activity against *Mayetiola destructor* (Lamiri et al., 2001), larvicidal activity against some mosquito species (Amer and Mehlhorn, 2006 a, b; Pavela, 2008) and its use as a grain protectant against weevils (Abdel-Latif, 2004; Ahmed and Al-Moajel, 2005). Also, fruit extracts disrupted the growth, development, general metabolism and phosphatase activity in *S. gregaria* (Ghoneim et al., 2014 d, e, f).

Dehydrogenases are very important tools for the investigation of insect metabolic activities during the course of development. The relative activities of the insect dehydrogenases may be related to the function and energy yielding demands of the tissues (Dickinson and Sullivan, 1975). Lactate dehydrogenase (LDH) (EC 1.1.1.28) is an important glycolytic enzyme present in virtually all animal tissues (Kaplan and Pesce, 1996). It is involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress and toxicology (Wu and Lam, 1997; Ribeiro et al., 1999; Diamantino et al., 2001; Senthil Nathan et al., 2006a). This enzyme may be a sensitive criterion in laboratory (Senthil Nathan et al., 2005). The aim of the present study was to assess the disturbing efficacy of *P. granatum* peel extracts and *A. visnaga* fruit extracts on the LDH activity in haemolymph and fat body of *S. gregaria*.

MATERIALS AND METHODS:

1) The insect.

The desert locust *Schistocerca gregaria* (Forsk.) (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 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.

2) Plant extraction.

A weight of 1.5 Kg *P. granatum* peel was purchased from the Egyptian market and thoroughly cleaned with tap water for disposing of impurities. The peel was shade dried and then finely grinded by a micromill. The pulverized powder was macerated with ethanol in a stoppered container for a defined period with frequent agitation until soluble matter is dissolved as adopted from Ncube et al. (2008). The ethanol extract was divided into two parts, a part of the ethanol extract was evaporated for obtaining 37 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 29 and 34 gm, respectively. From each of the crude ethanol extract and the fractionalized petroleum ether and n-butanol extracts, a series of concentrations were prepared: 80, 40, 20, 10, 5 and 2.5%. A weight of 1.5 Kg *A. visnaga* fruits was purchased from an Egyptian market and 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) and treated as previously mentioned for *P. granatum*.

3) Nymphal treatments.

In a preliminary experiment, different concentration levels of ethanol, petroleum ether, and n-butanol extracts derived from *P. granatum* peel or *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 each extract for 3 minutes. LC₅₀ values were calculated in 36.7, 22.2 and 40.7% of ethanol, petroleum ether and n-butanol extracts of *P. granatum* peel, respectively. LC₅₀ values were calculated in 21.0, 12.0 and 22.5% of ethanol, petroleum ether, and n-butanol extracts of *A. visnaga* fruits, respectively. After treatment with these LC₅₀s, the successfully moulted last instar nymphs and newly emerged adult females were used to determine the influenced LDH activity in two tissues: haemolymph and fat body. Three ages of last instar nymphs were used: early- (1-day old), mid- (4-day old) and late-aged (7-day old) nymphs.

4) Tissue preparation and enzyme assay.

For the determination of LDH activity, samples of haemolymph had been collected from the last instar nymphs and newly emerged adult females. Haemolymph was obtained by amputation of one or two hind legs of the nymph or adult with fine scissors. Gentle pressure was done on the thorax until a drop appeared at the point of amputation. Haemolymph was drawn into Eppendorff Pipetman containing few milligrams of phenoloxidase inhibitor (phenylthiourea) to prevent tanning or darkening and then diluted 5x with saline solution 0.7%. For whole 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 directly or frozen until the use. Three replicates were used and the haemolymph of two individuals were never mixed. Also, samples of fat bodies (parietal and visceral) were collected from nymphs (of the same ages) and newly emerged adult females. The fat body was weighed and then homogenized in a saline solution (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. Three replicates were used and the fat bodies from two individuals were avoided to be mixed. LDH activity was determined according to the method of Tietz (1999) using a kit of Biolap reagents. The enzyme was measured at wave length 340 nm by spectrophotometer.

5) 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:

1) Effects on LDH activity in haemolymph.

After treatment of the penultimate instar nymphs of *S. gregaria* with LC₅₀ concentrations of *P. granatum* peel, data assorted in Table (1) obviously reveal a prevalent enhancing effect on last instar nymphs of all ages to gain considerably increasing LDH activity in haemolymph with an exceptional case of seriously inhibited activity in the mid-aged nymphs by n-butanol extract (46640.6±164.4 U/L compared to 48340.9±200.1 U/L of control nymphs).

The strongest enhancing effect was exhibited by n-butanol extract on the enzyme activity in haemolymph of the early-aged nymphs (30.0% increment).

As clearly shown in Table (2), *A. visnaga* fruit extracts exerted a similar promoting actions on the nymphs to gain pronouncedly increasing enzyme activity with an exceptional case of a slightly inhibited activity in haemolymph of late-aged nymphs by petroleum ether extract (60188.2±157.1 U/L compared to 61346.7±102.2 U/L of control nymphs). The most potent inducing effect was exhibited by ethanol extract on the enzyme activity in haemolymph of early-aged nymphs (50.7% increment).

With regard to the newly emerged adult females, LDH activity in haemolymph was drastically suppressed by all *P. granatum* peel extracts (Table 1). Ethanol extract of *A. visnaga* suppressed the enzyme activity in haemolymph of adults (3.1% reduction). In contrast, petroleum ether extract and n-butanol extract significantly or slightly induced the enzyme activity (1.3 and 0.5% increments by petroleum ether extract and n-butanol extract, respectively, Table 2).

2) Effects on LDH activity in fat bodies.

According to data of Table (3), LDH activity in fat bodies of nymphs was remarkably **disturbed** by *P. granatum* peel extracts. The enzyme activity was significantly inhibited in fat bodies of early-aged nymphs, regardless the extract. The most potent inhibitory effect was exhibited by petroleum ether extract (38.1% reduction). The same extract caused continuously inhibited activity throughout the nymphal life (12.4 and 28.5% reductions in fat bodies of mid- and late-aged nymphs, respectively). On the contrary, ethanol extract caused largely stimulated LDH activity in mid- and late-aged nymphs (12.9 and 26.6% increments, respectively) and n-butanol extract enhanced the enzyme in the nymph congeners (47.9 and 12.7% increments, respectively).

In respect of the effects of *A. visnaga* fruit extracts on LDH activity in fat bodies of nymphs, data of Table (4) clearly show dramatically declined enzyme level in both early- and late-aged nymphs. The strongest inhibitory action was exerted by ethanol extract in fat bodies of late-aged nymphs (34.4% reduction). LDH activity was reversely affected in fat bodies of mid-aged nymphs since increasing activity was determined (23741.4±184.9, 21724.2±188.1 and 23442.6±92.9 U/L by ethanol extract, petroleum ether extract and n-butanol extract, respectively, vs. 15361.3±127.5 U/L of control nymphs).

In fat bodies of the newly emerged adults, unexceptionally increasing LDH activity was pronouncedly induced by both *P. granatum* and *A. visnaga* extracts (see tables 3 and 4). The most enhancing potency was exerted by ethanol extract of *P. granatum* peel (78.5% increment).

Table 1: Lactate dehydrogenase activity (U/L) in haemolymph of *S. gregaria* nymphs and adults as affected by LC₅₀ of *P. granatum* extracts.

Solvent		Last instar nymphs			Newly emerged adults
		Early-aged	Mid-aged	Late-aged	
Ethanol	Mean±SD	62236.6 ± 159.5 d	58319.6 ± 119.0 d	75182.6 ± 119.0 d	63334.1 ± 165.8 c
	Change (%)	+21.4	+20.6	+22.6	-1.8
Petroleum ether	Mean±SD	58336.3 ± 176.6 d	52347.4 ± 190.5 d	66643.9 ± 156.8 d	63164.4 ± 124.5 c
	Change (%)	+13.8	+08.3	+08.6	-2.1
n-butanol	Mean±SD	66744.4 ± 146.3 d	46640.6 ± 164.4 d	62097.9 ± 98.0 c	63331.2 ± 164.3 c
	Change (%)	+30.2	-3.5	+01.2	-02.0
Control		51269.4 ± 179.3	48340.9 ± 200.1	61346.7 ± 102.2	64503.6 ± 169.4

Mean ± SD followed by letter (c): Highly significantly different (P<0.01), (d): Very highly significantly different (P<0.001).

Table 2: Lactate dehydrogenase activity (U/L) in haemolymph of *S. gregaria* nymphs and adults as affected by LC₅₀ of *A. visnaga* extracts.

Solvent		Last instar nymphs			Newly emerged adults
		Early-aged	Mid-aged	Late-aged	
Ethanol	Mean±SD	77268.0 ± 184.0 d	51347.6.6 ± 169.3 d	66263.6 ± 171.8 d	62504.8 ± 213.6 d
	Change (%)	+50.7	+06.2	+08.0	-03.1
Petroleum ether	Mean±SD	61320.6 ± 180.2 d	50334.0 ± 173.8 d	60188.2 ± 157.1 a	65332.0 ± 176.5 c
	Change (%)	+19.6	+04.1	-01.9	+01.3
n-butanol	Mean±SD	65782.0 ± 149.3 d	61261.5 ± 158.0 d	69537.3 ± 176.0 d	64838.5 ± 169.1 a
	Change (%)	+28.3	+26.7	+13.4	+00.5
Control		51269.4 ± 179.3	48340.9 ± 200.1	61346.7 ± 102.2	64503.6 ± 169.4

Mean ± SD followed by letter (a): is not significantly different (P>0.05), c, d: See footnote of Table 1.

Table 3: Lactate dehydrogenase activity (U/L) in fat bodies of *S. gregaria* nymphs and adults as affected by LC₅₀ of *P. granatum* extracts.

Solvent		Last instar nymphs			Newly emerged adults
		Early-aged	Mid-aged	Late-aged	
Ethanol	Mean±SD	19328.3 ± 180.9 d	17349.6 ± 171.3 d	25530.2 ± 168.5 d	20250.8 ± 159.5 d
	Change (%)	-32.8	+12.9	+26.6	+78.5

Petroleum ether	Mean±SD	17807.1 ± 175.4 d	13450.2 ± 187.2 d	14429.3 ± 177.1 d	16365.1 ± 159.7 d
	Change (%)	-38.1	-12.4	-28.5	+44.2
n-butanol	Mean±SD	20178.9 ± 119.7 d	22727.0 ± 167.2 d	22725.0 ± 171.7 c	19349.0 ± 184.6 d
	Change (%)	-29.8	+47.9	+12.7	+70.5
Control		28747.8 ± 139.8	15361.3 ± 127.5	20167.3 ± 116.6	11347.5 ± 152.9

c, d: See footnote of Table 1.

Table 4: Lactate dehydrogenase activity (U/L) in fat bodies of *S. gregaria* nymphs and adults as affected by LC₅₀ of *A. visnaga* extracts.

Solvent		Last instar nymphs			Newly emerged adults
		Early-aged	Mid-aged	Late-aged	
Ethanol	Mean±SD	25330.0 ± 183.9 d	23741.4 ± 184.9 d	13229.4 ± 168.7 d	16334.2 ± 178.5 d
	Change (%)	-11.2	+54.6	-34.4	+43.9
Petroleum ether	Mean±SD	25728.4 ± 195.7 d	21724.2 ± 188.1 d	15721.5 ± 175.7 d	13730.0 ± 175.7 d
	Change (%)	-10.5	+41.4	-22.0	+21.0
n-butanol	Mean±SD	21748.5 ± 183.2 d	23442.6 ± 92.9 d	19425.6 ± 200.3 c	20235.0 ± 156.7 d
	Change (%)	-24.3	+52.6	-03.7	+78.3
Control		28747.8 ± 139.8	15361.3 ± 127.5	20167.3 ± 116.6	11347.5 ± 152.9

c, d: See footnote of Table 1.

DISCUSSION:

1) Enhancement of LDH activity in *S. gregaria* by plants extracts.

Dehydrogenases are very important tools for the investigation of insect metabolic activities during the course of development. The relative activities of the insect dehydrogenases may be related to the function and energy yielding demands of the tissues (Dickinson and Sullivan, 1975). Because LDH is an important glycolytic enzyme involved in carbohydrate metabolism in many tissues (Shekari et al., 2008), it has been used as an indicative criterion of exposure to chemical stress and toxicology (Wu and Lam, 1997; Diamantino et al., 2001). As reported in the available literature, LDH activity has been disturbed by plant extracts and insecticides (Nath, 2000; Ghoneim et al., 2014f; Mahmoud, 2014). LDH activity was promoted in haemolymph or fat bodies of *S. gregaria* nymphs and adults by the action of neem preparation Neemazal and *N. sativa* extracts (Hamadah, 2009). The *F. bruguieri* extracts enhanced the enzyme activity in haemolymph of nymphs and adults of the same locust (Hamadah et al., 2010). In addition, various insecticides promoted the LDH activity in several pests by, such as *Tribolium castaneum* (Pak. strain) by pyrethroid (Saleem and Shakoori, 1987), *Culex fatigans* (L.Y. strain) by malathion, cyfluthrin, and DDT (Azmi et al., 2002), *Spodoptera littoralis* by chlorantraniliprole and spinetoram (Rashwan, 2013), *Ephesia kuehniella* by pyriproxyfen (Sharifi et al., 2013) and the same lepidopteran by hexaflumuron (Delkash-Roudsari et al., 2014). In the present study on *S. gregaria*, *P. granatum* peel extracts and *A. visnaga* fruit extracts exhibited a prevalent enhancing effect on the last instar nymphs to gain considerably increasing LDH activity in haemolymph, with few exceptions. Both petroleum ether extract and n-butanol extract of *A. visnaga* induced the enzyme in haemolymph of newly emerged adult females. Both ethanol extract and n-butanol extract of *P. granatum* peel induced the enzyme in fat bodies of the mid- and late-aged nymphs. Also, *A. visnaga* fruit extracts enhanced the mid-aged nymphs to gain increasing LDH level. The enzyme activity was unexceptionally promoted in fat bodies of adults by all extracts of *P. granatum* peel and *A. visnaga* fruits. The enhancement of LDH activity in the present study can be understood because it may indicate an effective stimulation of the portion of Cori cycle responsible for the overall recycling lactate, since this would result in concomitant enhanced production of pyruvate and glucose via gluconeogenesis (Harper et al., 1984).

2) Inhibition of LDH activity in *S. gregaria* by plant extracts.

The pyruvate is the key intermediate of glycolysis, whereas lactate is the end product of glycolysis. The interconvertibility of pyruvate and lactate has a great advantage in the operation of carbohydrate metabolism (Nath, 2000). In the present study on *S. gregaria*, LDH activity in haemolymph of the newly emerged adults was drastically suppressed by all *P. granatum* peel extracts. Only the ethanol extract of *A. visnaga* prohibited the enzyme activity in haemolymph of adults. All *P. granatum* peel extracts caused remarkable reduction of LDH in fat bodies of early-aged nymphs but only petroleum ether extract caused a considerable reduction in fat bodies of mid- and late-aged nymphs. Treatment with *A. visnaga* fruit extracts resulted in dramatically declined enzyme level in fat bodies of both early- and late-aged nymphs. These results are, to a great extent, in agreement with reported results of the inhibited LDH activity in several insect species by various botanicals, such as *Spodoptera litura* (Senthil-Nathan and Kalviani, 2005) and *S. littoralis* larvae (Abd El-Aziz, 2007) by azadirachtin (Azt), *Cnaphalocrocis medinalis* by some neem limonoids or *M. azedarach* (Senthil Nathan, 2006; Senthil-Nathan et al., 2006a), *C. medinalis* by Azt and *Vitex negundo* extracts (Senthil Nathan et al., 2006b), *S. gregaria* nymphal fat bodies by Neemazal, *Nigella sativa* and *F. bruguieri* extracts (Hamadah, 2009; Hamadah et al., 2010), *Callosobruchus chinensis* by *Cassia alata* fruit extracts (Upadhyay et al., 2011), *Sitophilus oryzae* by *Capparis deciduas* extracts (Upadhyay, 2013), *Glyphodes pyloalis* by Achook[®](0.03% azadirachtin) (Khosravi and Sendi, 2013), *Xanthogaleruca luteola* by the same neem preparation (Valizadeh et al., 2013), etc. The inhibited LDH activity in haemolymph and fat bodies of some developmental stages of *S. gregaria* by *P. granatum* peel extracts or *A. visnaga* fruit extracts, in the present study, indicate the inhibition of lactate conversion to pyruvate, resulting in a shift from aerobic to anaerobic metabolism for meeting the required energy demands under toxic conditions.

In **conclusion**, the current results of disturbed LDH activity in haemolymph and fat bodies of nymphs and adults of *S. gregaria* by *P. granatum* peel extracts and *A. visnaga* fruit extracts indicate that the toxic compounds contained in these extracts (Martelli et al., 1984; Ortel et al., 1988; Li et al., 2006; Bencheraiet et al., 2011; Bhandary et al., 2012) might be affecting the synthesis or functional levels of LDH, directly or indirectly, by altering the cytomorphology of the cells (Nath, 2000). Also, the induction or inhibition, of the LDH activities, as recorded in the present study on *S. gregaria*, might be, on molecular levels, referred to depression or mutations of the regulating genes responsible for biosynthesis of polypeptide chains building this enzyme (Hassanein et al., 1996). However, further investigations should be carried out to identify the active ingredients in *P. granatum* peel extracts and *A.*

visnaga fruit extracts responsible for the gene promotion, rate of expression and eventually synthesis of nucleic acid and enzymatic peptides in the target tissues.

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