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Impairing Efficacy of *Nigella sativa* Linn. (Ranunculaceae) Seed Extracts on Phosphatase Activity in Haemolymph and Fat Bodies of *Schistocerca gregaria* (Forskal) (Acrididae: Orthoptera)

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Abstract
The present study aimed to investigate the disturbing effects of sublethal concentrations (15.0% and 7.5%) of methanol, petroleum ether and n-butanol extracts of *Nigella sativa* seeds on acid (ACP) and alkaline (ALP) phosphatases in haemolymph and fat bodies of last instar nymphs and newly emerged adult females of the economically dangerous desert locust *Schistocerca gregaria*. Both methanol and petroleum ether extracts of *N. sativa* exhibited remarkably enhancing effects on ACP activity in haemolymph of nymphs and adults, while n-butanol extract exerted an inhibitory action on the enzyme activity in early- and mid-aged nymphs as well as in adults. With regard to ACP activity in fat bodies, all extracts generally promoted it in both nymphs and adults with few exceptions. Various degrees of disturbance of ALP activity in haemolymph of nymphs and adults were exhibited by *N. sativa* extracts. As most prominent findings, prohibited enzyme activity was detected only in haemolymph of early-aged nymphs, regardless to the extract and concentration. On the contrary, all extracts enhanced the enzyme activity, generally in late-aged nymphs and adults. Concerning the mid-aged nymphs, only methanol extract exhibited a promoting effect on ALP activity while both petroleum ether and n-butanol extracts exhibited reducing effects on it. In fat bodies, methanol extract of *N. sativa* prohibited the ALP activity in early- and mid-aged nymphs but induced it in late-aged congeners. Regarding the newly emerged adult females, ALP activity increased, irrespective of the extract and concentration.

Keywords: methanol, petroleum ether, n-butanol, nymph, adult, haemolymph, fat body.

1. Introduction
The desert locust, *Schistocerca gregaria* (Forskål), is known as a very important insect pest in North Africa (Sanchez-Zapata *et al*., 2007; Ammar *et al*., 2009) [1, 2]. It is characterized by a phase polymorphism (Uvarov, 1966) [3] enabling the transition from a solitary phase to an extremely dangerous gregarious one for the agricultural productions and pastures. *S. gregaria* is perhaps the most dramatic and potentially devastating species, and can devastate the cultures of a whole continent (Lecoq and Mestre, 1988) [4].
Current locust control operations are mainly based on organophosphorus pesticides as a result of the banning of organochlorines (Lecoq, 2001) [5]. 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) [6]. 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) [7-9] because they are generally pest-specific, relatively harmless to non-target organisms and they are biodegradable and consequently harmless to the environment (Rembold, 1984; Isman, 2008) [10, 11]. Because of the multiple sites of action through which the plant materials can act, the probability of developing a resistant population is very low (Isman, 2006) [12].

Nigella plants are widely distributed in countries which border the Mediterranean Sea, central Europe and western Asia (Hedrick, 1972) [13]. There are many species classified in the genus *Nigella* (Ranunculaceae) (Bailey, 1978; Atta, 2003) [14, 15]. Among the most important medicinal crops in Egypt is *Nigella sativa* which is commonly called as known as black seed or black cumin (Rayan *et al*., 2011) [16] and "Habbat al-barakah" (the seed of blessing) in Arabic. Several constituents had been identified and isolated from *N. sativa* seeds, such as conjugated linoleic acid, thymoquinone, nigellone (dithymoquinone), melanthin, nigilline, damascenine, tannins, flavonoids, saponins, alkaloids, proteins, lipids, dithymoquinone carvacol and anethole 4-terpinole, carbohydrates, crude fiber, saponins, ash, fixed oils and essential oil (Burits and Bucar, 2000; Al-Ghamdi, 2001; Ali and Blunden, 2003; Sharma *et al*., 2009; Ali *et al*., 2012; Rahmani and Aly, 2015; Hidayati and Habib, 2015) [17-23]. Recently, Shokri, (2016) [24] identified the major components of the essential oil as thymoquinone, p-cymene, trans-anethole, 2-methyl-5(1-methyl ethyl)-Bicyclo [3.1.0]hex-2-en and γ-terpinene.
Seeds of *N. sativa* and their oil have a long history of folklore usage in various systems of medicines. Different medicinal, pharmacological, traditional values and folk remedies of this herb had been reported (Sharma *et al*., 2009; Rahmani and Aly, 2015; Hidayati and Habib, 2015; Shokri, 2016) [20, 22-24]. In pest control, Deshpande *et al*. (1974) [25] reported that oleic and linoleic acid as insecticidal components from *N. sativa* which were found to be toxic to *Callosobruchus chinensis* and

similar results were obtained (Adebowale and Adeire, 2006; Adabie-Gomez *et al.*, 2006) [26, 27]. *N. sativa* extracts exhibited toxic effects on *Spodoptera littoralis* (Abd ELatif *et al*., 2009) **[28]** and *S. gregaria* (Hamadah *et al*., 2013) [29]. They disrupted the growth, development and metamorphosis of the latter insect (Hamadah *et al*., 2013) [29]. Also, Ahmad *et al*. (2013) **[30]** studied the insecticidal activity of extracts from this herb against the larvae of *Trogoderma granarium* under laboratory conditions. Khan *et al.* (2014) [31] reported the disturbing effects of the acetone seed extract of *N. sativa* on biology and invasion of the stored product pest *Tribolium castaneum*. *N. sativa* extracts showed the highest fumigant mortality against *Tribolium castaneum* (Saleem *et al*., 2014) [32]. Considerably toxic effects of *N. sativa* had been recorded against *Spodoptera littoralis* (Osman and Osman, 2014) [33]. Recently, essential oil of *N. sativa* exhibited a moderate toxic effect on the scale insects *Ceroplastes rusci* and *Asterolcanium pustolans* (Ismail *et al.*, 2015) [34]. *N. sativa* extracts exhibited disruptive effects on the adult performance (Ghoneim *et al*., 2015a) [35], reproductive potential (Ghoneim *et al*., 2015b) [36] and haemogram (Ghoneim *et al*., 2015c) [37] of *S. gregaria*

Acid phosphatase (ACP, E.C.3.1.3.2) and Alkaline phosphatase (ALP, E.C.3.1.3.1) are hydrolyzing enzymes, which are responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids in alkaline and acidic conditions, respectively under the name of dephosphorylation (Janda and Benesova, 1991; Zibaee *et al*., 2011) [38, 39]. Also, these enzymes are involved in lipid hydrolysis in several tissues like midgut, hemolymph and fat bodies (Zibaee *et al*., 2011) [39]. In addition to ACP, ALP may act as hydrolases during the final stages of digestion (Cheug and Low, 1975) [40], gonad maturation and metamorphic moults (Rhadha and Priti, 1969) [41].

ACP, known as a lysosomal marker enzyme (Csikos and Sass, 1997) [42], is active in guts (Ferreira and Terra, 1980) [43], Malpighian tubules (Srivastava and Saxena, 1967) [44] and is also abundant in the disintegrating tissues and organs subjected to cytolysis (Sahota, 1975) [45]. 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) [46].

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 (Ferreira and Terra, 1980; Wolfersberger, 1984) [47, 48] and is especially active in tissues with active membrane transport, such as intestinal epithelial cells (Sakharov *et al*., 1989; Caglayan, 1990) [49, 50], Malpighian tubules (Etebari and Matindoost, 2004 a, b) [51, 52] and haemolymph (Etebari *et al.*, 2007) [53]. It is responsible for cytolysis of tissues during the insect development (Dadd, 1970) [54]. Its primary function is to provide phosphate ions from mononucleotide and ribonucleo-proteins for a variety of metabolic processes (Etebari *et al*., 2005) [55]. In insects, ALP is involved in several biological processes and respond to stress, pathogenesis, or infection (Sukhanova *et al*., 1996; Miao, 1988, 2002) [56-58]. It is one important synthesizing enzyme of tyrosine, the precursor of dopamine and octopamine, which are known to take part in the control of levels of juvenile hormone and 20-hydroxyecdysone (Rauschenbach *et al*., 2007a,b) [59, 60].

In insects, the use of haemolymph as a medium for controlling insect pests has been made because the changes occurring in the haemolymph are quickly transferred to other portions of insect's body (Pugazhvendan and Soundararajan, 2009) [61]. The exposure of an organism to xenobiotic product can modify the synthesis of certain metabolite and disturb the functionality of the organisms (Rodriguez-Ortega *et al*., 2003) [62]. On the other hand, the fat body in insect is the main site for protein synthesis as well as the intermediating metabolism of amino acids, which are utilized for the production of hormones and enzymes and the composition of protein in the body as a whole may be greatly modified (Arrese and Soulages, 2010) [63]. The present study was carried out aiming to investigate the effects of different extracts of *N. sativa* seeds on the phosphatase activities in haemolymph and fat bodies of nymphs and adults of *S. gregaria*.

2. Materials and Methods

2.1. 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) [64] and improved by Ghoneim *et al*. (2009) [65], 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±2ºC). The insects were reared and handled under the crowded conditions. Fresh clean leaves of clover *Trifolium alexandrinum* were provided, as a food for insects, every day.

2.2. Plant Extraction

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

2.3. Nymphal Treatments

According to Hamadah (2009) [66] 15.0% and 7.5% were the sublethal concentrations of methanol, petroleum ether, and n-butanol extracts derived from *N. sativa* seeds. After treatment of the newly moulted penultimate (4^{th}) instar nymphs of *S. gregaria* through the fresh food leaves of *T. alexandrinum* dipped once in each extract for 3 minutes, 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.

2.4. Tissue Sampling and Determination of Phosphatase Activities

For the determination of phosphatase activity in the haemolymph, it was collected from last instar nymphs and 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, 1999) [67] 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) [68] using a kit of Quimica clinical aplicada S.A. The enzyme activity was measured at wave length 550 nm by spectrophotometer.

2.5. Statistical Analysis

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) [69] for the test significance of difference between means.

3. Results

3.1. Effects of *N. sativa* Seed Extracts on ACP Activity

In the present study, sublethal concentrations (15.0 and 7.5%) of *N. sativa* seed extracts had been applied onto the newly moulted penultimate instar nymphs of *S. gregaria*. As obviously shown in Table (1), both the methanol and petroleum ether extracts of *N. sativa* exhibited remarkably enhancing effects on ACP activity in haemolymph of last instar nymphs and newly emerged adult females. In contrast, n-butanol extract exerted an inhibitory effect on the enzyme activity in both early- and mid-aged nymphs (Change %s: - 6.0 and - 38.5, respectively, at high concentration as well as 0.0 and - 30.2 at low concentration). Moreover, n-butanol extract exerted a prohibiting action on ACP in adults (912.5 ± 21.7 and 950.0 ± 21.7 U/mL at high and low concentrations, respectively, *vs*. 1337.5 ± 21.7 U/mL of control adults). Referring to data of the same table, the strongest promoting effect of *N. sativa* extracts on ACP activity was exhibited by petroleum ether extract at low concentration (Change %: +115.6) whereas the least promoting effect was exhibited by methaol extract (Change %: as +9.6) at high concentration.

With regard to the influenced ACP activity in fat bodies by *N. sativa* seed extracts, it was generally induced in nymphs and adults, with few exceptions depending on the concentration and age. In some detail, data assorted in Table (2) reveal that the ACP activity was promoted in early-aged nymphs with the highest Change %:+302.9 (at high concentration of petroleum ether extract) and the lowest change %: +11.7 (at low concentration of methanol extract). The inhibited ACP activity was only detected in early-aged nymphs, change%: 19.7 (at high concentration of n-butanol extract). With age, the inhibitory effect obviously appeared, regardless to the extract or concentration, since late-aged nymphs had Change %:- 44.5 (as a maximum) and -6.4 (as a minimum) in ACP activity. Only the high concentration of both methanol and petroleum ether enhanced the ACP activity in late-aged nymphs (Change %s: + 7.6 and + 2.9, respectively). By the locust metamorphosis, ACP activity in adults was pronouncedly induced to the maximum as 121.1 ± 1.5 U/mL at low concentration of methanol extract and to the minimum as 86.7 ± 2.0 U/mL at low concentration of n-butanol extract (in comparison with 86.3 ± 1.3 U/mL of control congeners).

3.2. Effects of *N. sativa* Seed Extracts on ALP Activity

Concerning the possible effects of *N. sativa* seed extracts, Table (3) includes various degrees of disturbed ALP activity in haemolymph of both nymphs and adults as follows. All *N. sativa* extracts prohibited ALP activity only in haemolymph of early-aged nymphs, regardless to concentration. On the contrary, all extracts generally enhanced the enzyme activity in late-aged nymphs and adults. In regard of the mid-aged nymphs, only methanol extract exhibited promoting effect on ALP activity while both petroleum ether and n-butanol extracts exhibited reducing effects on the enzyme activity. For some detail, the most detrimentally reducing effect was exhibited by the low concentration of n-butanol extract (Change %:-77.5) in early-aged nymphs but the least reducing one was detected in mid-aged nymphs by petroleum ether extract (Change %: -37.2, at low concentration). On the other hand, the strongest activating action on ALP activity in haemolymph was estimated for late-aged nymphs (Change % +157.5, at low concentration of petroleum ether extract).

Data arranged in Table (4) obviously show various degrees of disturbance of ALP activity in fat bodies of nymphs and adults. Methanol extract prohibited the enzyme activity in early- and mid-aged nymphs but induced it in late-aged ones (Change %:- 59.2 and - 66.0 but +325.0 at the high concentration, as well as - 48.5 and -11.3 but +36.8 at low concentration, in the three nymphal ages, respectively). In respect of the petroleum ether, ALP activity was enhanced at both beginning and end of the nymphal instar, regardless the concentration. On the contrary, the enzyme activity was inhibited in fat bodies of mid-aged nymphs by all extracts. Regarding the newly emerged adults, ALP activity increased, regardless to the extract or concentration. The highest increment was measured in 9.8 ± 0.9 U/mL at the high concentration of methanol extract and the lowest increment was measured in 5.3 ± 1.1 U/mL at the low concentration of n-butanol extract (*vs*. 4.8 ± 0.4 U/mL of controls).

Table 1: Effects of *N. sativa* extracts on ACP activity (U/ml) in haemolymph of *S. gregaria*.

Solvent	Conc. %		Last instar nymphs			Newly emerged adults
			Early-aged	Mid-aged	Late-aged	
Methanol	15.0	Mean ± SD	1825.0 ± 57.3 d	1962.5 ± 43.3 d	1937.5 ± 57.3 d	1437.5 ± 21.7 c
		Change %	+73.8	+63.5	+36.0	+7.5
	7.5	Mean ± SD	1312.5 ± 37.5 c	1612.5 ± 37.5 d	2037.5 ± 43.3 d	1337.5 ± 21.7 a
		Change %	+25.0	+34.4	+43.0	0.0
Petroleum ether	15.0	Mean ± SD	2037.5 ± 43.3 d	2300.0 ± 57.3 d	2262.5 ± 43.3 d	1750.0 ± 21.7 d
		Change %	+94.0	+91.7	+58.8	+30.8
	7.5	Mean ± SD	1850.0 ± 43.3 d	2587.5 ± 37.5 d	1862.5 ± 57.3 d	1800.0 ± 37.5 d
		Change %	+76.2	+115.6	+30.7	+34.6
n-butanol	15.0	Mean ± SD	987.5 ± 21.7 a	737.5 ± 57.3 d	1500.5 ± 43.3 b	912.5 ± 21.7 d
		Change %	-6.0	-38.5	+9.6	-31.8
	7.5	Mean ± SD	1050.0 ± 37.5 a	837.5 ± 43.3 d	1550.0 ± 43.3 b	950.0 ± 21.7 d
		Change %	0.0	-30.2	+22.8	-29.0
Controls		Mean ± SD	1050.0 ± 37.5	1200.0 ± 37.5	1425.0 ± 37.5	1337.5 ± 21.7

Conc.: Concentration level; mean ± SD followed with 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: Effects of *N. sativa* extracts on ACP activity (U/mL) in fat bodies of *S.*

Solvent	Conc. %		Last instar nymphs			Newly emerged adults
			Early-aged	Mid-aged	Late-aged	
Methanol	15.0	Mean ± SD	145.6 ± 1.8 d	088.2 ± 1.0 c	107.0 ± 0.9 d	116.3 ± 1.0 d
		Change %	+093.6	-06.4	+07.6	+34.8
	7.5	Mean ± SD	084.0 ± 0.5 c	104.3 ± 1.1 d	055.2 ± 1.0 d	121.1 ± 1.5 d
		Change %	+011.7	+10.7	-44.5	+40.3
Petroleum ether	15.0	Mean ± SD	303.0 ± 5.6 d	107.0 ± 1.1 d	102.3 ± 1.1 b	116.8 ± 1.7 d
		Change %	+302.9	+13.6	+02.9	+35.3
	7.5	Mean ± SD	143.4 ± 5.7 d	133.5 ± 1.2 d	086.8 ± 0.6 d	108.4 ± 1.7 d
		Change %	+090.7	+41.7	-12.7	+25.6
n-butanol	15.0	Mean ± SD	060.4 ± 1.6 c	129.3 ± 2.7 d	085.3 ± 1.0 d	114.6 ± 1.4 d
		Change %	-019.7	+37.3	-14.2	+32.8
	7.5	Mean ± SD	150.0 ± 1.4 d	139.0 ± 1.2 d	088.6 ± 1.5 d	086.7 ± 2.0 a
		Change %	+099.5	+47.6	-10.9	+0.5
Controls		Mean ± SD	075.2 ± 2.8	094.2 ± 1.2	099.4 ± 1.1	086.3 ± 1.3

Conc., a, b, c, d: See footnote of Table 1.

Table 3: Effects of *N. sativa* extracts on ALP activity (U/mL) in haemolymph of *S. gregaria*.

Solvent	Conc. %		Last instar nymphs			Newly emerged adults
			Early-aged	Mid-aged	Late-aged	
Methanol	15.0	Mean ± SD	12.1 ± 3.6 b	39.4 ± 5.3 b	15.1 ± 2.7 a	10.6 ± 2.6 a
		Change %	-63.7	+62.8	+42.5	+76.7
	7.5	Mean ± SD	18.2 ± 4.5 b	34.8 ± 5.3 b	10.6 ± 2.6 a	6.0 ± 2.7 a
		Change %	-45.3	+43.8	0.0	0.0
Petroleum ether	15.0	Mean ± SD	9.1 ± 4.5 c	13.6 ± 4.5 b	25.7 ± 4.3 c	10.7 ± 5.3 a
		Change %	-72.7	-43.8	+142.5	+78.3
	7.5	Mean ± SD	15.1 ± 2.7 b	15.2 ± 3.3 b	27.3 ± 4.5 c	9.1 ± 4.5 a
		Change %	-54.7	-37.2	+157.5	+51.7
n-butanol	15.0	Mean ± SD	7.6 ± 2.7 c	6.0 ± 2.7 c	16.6 ± 5.3 a	12.1 ± 5.3 a
		Change %	-77.2	-75.2	+56.6	+101.7
	7.5	Mean ± SD	7.5 ± 5.3 c	7.6 ± 2.7 c	10.6 ± 2.6 a	7.5 ± 5.3 a
		Change %	-77.5	-68.6	0.0	+25.0
Controls		Mean ± SD	33.3 ± 6.9	24.2 ± 2.7	10.6 ± 2.6	6.0 ± 2.7

Conc., a, b, c, d: See footnote of Table 1

Table 4: Effects of *N. sativa* extracts on ALP activity (U/mL) in fat bodies of *S. gregaria*.

Solvent	Conc. %		Last instar nymphs			Newly emerged adults
			Early-aged	Mid-aged	Late-aged	
Methanol	15.0	Mean ± SD	5.2 ± 0.9 c	3.3 ± 0.7 d	28.9 ± 0.8 d	9.8 ± 0.9 d
		Change %	-59.2	-66.0	+325.0	+104.2
	7.5	Mean ± SD	6.7 ± 0.9 c	8.6 ± 0.7 a	9.3 ± 0.6 c	6.8 ± 0.6 c
		Change %	-48.5	-11.3	+36.8	+41.7
Petroleum ether	15.0	Mean ± SD	32.0 ± 2.7 d	5.2 ± 0.7 c	10.2 ± 1.0 c	8.6 ± 0.6 d
		Change %	+146.2	-46.4	+50.0	+79.2
	7.5	Mean ± SD	19.1 ± 2.0 b	9.0 ± 0.6 a	8.9 ± 0.8 b	8.3 ± 0.6 c
		Change %	+46.9	-7.2	+30.9	+72.9
n-butanol	15.0	Mean ± SD	9.1 ± 1.0 b	6.0 ± 0.9 c	10.3 ± 0.6 d	9.7 ± 0.9 d
		Change %	-30.0	-38.1	+51.5	+102.1
	7.5	Mean ± SD	9.6 ± 0.5 b	7.6 ± 0.7 b	8.8 ± 0.9 b	5.3 ± 1.1 a
		Change %	-26.2	-21.6	+29.4	+10.4
Controls		Mean ± SD	13.0 ± 1.6	9.7 ± 1.0	6.8 ± 0.3	4.8 ± 0.4
Conc., a, b, c, d: See footnote of Table 1						

4. Discussion

In insects, Acid phosphatase (ACP) and Alkaline phosphatase (ALP) are responsible for cytolysis of tissues during the insect development (Dadd, 1970) [54] since they may act as hydrolases during the final stages of digestion (Cheug and Low, 1975) [40], gonad maturation and metamorphic moults (Tsumuki and kanehisa, 1984) [70]. Detoxification enzyme in insects is generally demonstrated as the enzymatic defense against foreign compounds and play significant roles in maintaining their normal physiological functions (Li and Liu, 2007) [71]. Induction of detoxification metabolic system plays an important role in the insect's detoxification mechanism (Terriere, 1984) [72]. The detoxifying enzymes react against insecticides, or compounds exhibiting insecticidal activities. They include general esterases, glutathione S-transferase and phosphatases (Zibaee *et al*., 2011) **[39]**. It may be important to mention that the activities of phosphatases were affected by secondary metabolites of some botanicals. However, the detailed mechanism of action was explained in the review of Senthil-Nathan **(**2013) [73].

4.1. Deteriorated ACP Activity in *S. gregaria* by *N. sativa* Seed Extracts

Different, and sometimes contradictory, effects of several botanicals on ACP activity in various insects had been reported in the literature since Ghoneim *et al*. (2008) [74] recorded various inducing and reducing effects of Margosan-O (a neem preparation) and Jojoba oil on of the enzyme activity in pupal stage of *Musca domestica*. Neemazal (a neem preparation) promoted the enzyme activity in haemolymph, but gradually prohibited it in fat bodies of last instar nymphs of *Schistocerca gregaria* (Hamadah, 2009) [66]. To a great extent, similar various disruptive effects had been reported for the *Fagonia bruguieri* extracts on the enzyme activity in the same locust (Basiouny *et al*., 2010) [75]. In the same locust, also, treatments of penultimate instar nymphs with different extracts of *Ammi visnaga* fruits, ACP activity was promoted or inhibited in haemolymph of last instar nymphs and newly emerged adults, depending on the extract but depending on the nymphal age, in case of fat bodies of last instar nymphs (Ghoneim *et al*., 2014) [76].

In the present study, inducing effects of both methanol and petroleum ether extracts of *N. sativa* seeds on ACP activity had been determined in haemolymph of the nymphs and adults of *S. gregaria*. All extracts exhibited similar inducing effects of the enzyme activity in fat bodies of the same developmental stages, with few exceptions. These results were found in agreement with those reported inducing effects of some other botanicals on the same enzyme in various insects, such as *Culex pipiens* (El-Bassal, 1993) [77], *Pectinophora gossypiella* and *Earias insulana* (Anan *et al*., 1993) [78], *Helicoverpa armigra* (Babu *et al*., 1996) [79], *Spodoptera littoralis* (Hassan, 2002; Abdel-Al, 2002) [80, 81], *Agrotis ipsilon* (El-Sheikh, 2002) [82] and *S. gregaria* (Hamadah, 2009; Basiouny *et al*., 2010) [66, 75]. Also, induced ACP activity was estimated in fat bodies of newly emerged adults of *S. gregaria* after treatment of penultimate instar nymphs with different extracts of *A. visnaga* fruits, regardless the extract (Ghoneim *et al*., 2014) [76]. A significant increase level of ACP was measured in larvae and pupae of *Aedes aegypti* upon exposure to NeemAzal (a neem formulation) (Koodalingam *et al*., 2014) [83]. In addition to botanicals, an increase in ACP activity was recorded in the 4th instar larvae of *P. gossypiella* after treatment of neonate larvae with LC_{50} of the fungus *Verticillium lecanii* (Rashad *et al*., 2015) [84]. The chitin inhibitors Novaluron, Cyromazine and Diofenolan, enhanced the ACP activity in haemolymph and fat bodies of last instar larvae of *S. littoralis* (Hamadah *et al*, 2016) [85].

The induced ACP activity in haemolymph and fat bodies *S. gregaria*, in the present study, by the majority of *N. sativa* seed extracts, may be attributed to certain chemical constituents (Burits and Bucar, 2000; Al-Ghamdi, 2001; Ali and Blunden, 2003; Sharma *et al*., 2009; Ali *et al*., 2012; Shokri, 2016; Vatansev *et al*., 2013) [17-21, 24, 86] responsible for the increasing number of lysosomes since ecdysone (moulting hormone) is responsible for increase of lysosome number as a lysosomal ACP enzyme (van Pelt-Verkuil, 1979; Bassal and Ismail, 1985) [87, 88]. It can be, also, understood because ACP activity, directly or indirectly, interferes with the digestion, absorption and positive transport of nutrient in the midgut (Senthil-Nathan *et al*., 2004) [89].

On the other hand, only n-butanol extract of the *N. sativa* seeds exhibited a remarkable inhibitory effect on ACP activity in haemolymph of the early- and mid-aged nymphs as well as in newly emerged adults, in the current work. This inhibitory effect agrees, to some extent, with some reported results for various insect species by other plant extracts, such as *M. domestica* by azadirachtin (Azt.)(Saeed *et al*., 1987) [90] and

Margosan-O (a neem preparation) or Jojoba oil (Ghoneim *et al*., 2008) [74]; *S. littoralis* (Ayyangar and Rao, 1990) [91] by Azt.; *Sitophilus oryzae* by NfD (a fraction of winter neem leaves) (Naqvi *et al*., 1991) [92]; *Euprepocnemis plorans* by some neem limonoids (Al-Dali, 2007) **[93]**; *S. gregaria* by Neemazal (a neem preparation) (Hamadah, 2009) [66]; *Rhizopertha dominica* by hexane extract of *Capparis deciduas* (Upadhyay, 2013) [94]; *Tribolium castaneum* by various doses of different extracts of *Melia azedarach, Nicotiana tabacum, Azadirachta indica* and *Colosynthus citrullus* (Ali *et al*., 2015) **[95]** or by LC_{50} of the garlic oil (Beltagy and Omar, 2016) [96].

In addition, a similar inhibitory effect of n-butanol extract of the *N. sativa* seeds on ACP activity, in the current work, is in conformity with the reducing effects of several IGRs against different insect pests, such as hexaflumuron against *S. littoralis* (Sokar, 1995) [97]; tebufenozide against *M. domestica* (Assar *et al.*, 2010); lufenuron against *C. pipiens* (Shaurub *et al*., 2015) [98] and Diofenolan against *S. littoralis* (Hamadah *et al*., 2016) [85].

However, the reduced ACP activity in *S. gregaria*, as a response to only n-butanol extract of the *N. sativa* seeds, in the current investigation, may be interpreted by a conceivable suggestion of Senthil-Nathan *et al*. (2004, 2005, 2006) [89, 99, 100] who suggested that decreased ACP level at higher concentration of neem extract can be due to reduced phosphorus liberation for energy metabolism, decreased rate of metabolism, as well as decreased rate of transport of metabolites. It is important to point out that inhibition of the detoxifying enzymes, including ACP, indicates that this enzyme play no role in the detoxification of n-butanol extract of the *N. sativa* seeds and may increase the susceptibility of insect pest against the chemical constituents (Abd-Elaziz and El-Sayed, 2009) [101].

4.2. Deteriorated ALP Activity in *S. gregaria* by *N. sativa* Seed Extracts

So many controversial effects of several botanicals, IGRs or insecticides on ALP activity are available in the literature (Etebari *et al*., 2007; Basiouny *et al*., 2010; Abdel-Al, 2002; Senthil-Nathan *et al*., 2005, 2006; Mostafa, 1993; Assar *et al*., 2010; Teleb *et al*., 2012) [53, 75, 81, 99, 100, 102-104]. In the current investigation, treatment of penultimate instar nymphs of *S. gregaria* with *N. sativa* seed extracts resulted in an inhibition of ALP activity in haemolymph of the early-aged last instar nymphs. Also, inhibited ALP activity was estimated in haemolymph of the mid-aged nymphs after treatment with petroleum ether or n-butanol extract. In fat bodies of the early- and mid-aged nymphs, only methanol extract of *N. sativa* seeds exhibited an inhibitory effect on the enzyme activity. However, these inhibitory effects *N. sativa* seed extracts on ALP activity, in the present study, are in agreement with similar inhibitory effects of some plant extracts on various insects, such as hexane extract of *C. deciduas* on *R. dominica* (Upadhyay, 2013) [94]; different extracts of *Curcuma longa* on *T. castaneum* (Uma devi and Sujatha, 2013) [105]; *A. visnaga* seed extracts on last instar nymphs of *S. gregaria* (Ghoneim *et al*., 2014) [76]; different extracts of *M. azedarach, N. tabacum, A. indica* and *C. citrullus* on *T. castaneum* adults (Ali *et al*., 2015) [95]; LC_{50} of *Acorus calamus* (essential oil) extracts or Biosal (a neem preparation) on *Callosobruchus analis* (Arif *et al*., 2015) [106]. In addition, some bioinsecticides exhibited similar reducing effects on ALP activity in other insects, such as avermactin in *S. littoralis* (Dahi *et al*., 2009) [107]; emamectin benzoate, abamectin and spinosad in 4th larval instar larvae of the same species (Megahed *et al*., 2013) [108] and LC_{50} of fungus *V. lecanii* in the 4th instar larvae of *P. gossypiella* (Rashad *et al*., 2015) [84].

The reduced ALP activity in some tissues of different developmental stages in *S. gregaria* by *N. sativa* seeds extracts, in the present study, may be explicated by some developmental disturbance as an appreciated suggestion of Wu (1990) [109] for the larvae of mosquito *C. pipiens* after treatment with IGR diflubenzuron. In addition, the *N. sativa* extracts contain some components, such as thymoquinone, nigellone (dithymoquinone), melanthin, nigilline, damascenine, tannins, flavonoids, saponins, alkaloids and anethole 4-terpinole (Burits and Bucar, 2000; Al-Ghamdi, 2001; Ali and Blunden, 2003; Sharma *et al*., 2009; Ali *et al*., 2012; Vatansev *et al*., 2013) [17-21, 86] which one or more of them may affect the gut physiological events (i.e. transport) causing a prohibition of ALP activity, as well as may affect both juvenile hormone and ecdysone regulation, directly or indirectly, as suggested by Phillips *et al*. (1988) [110] for *C. medinalis*.

In the present work, treatment of penultimate instar nymphs of *S. gregaria* with methanol extract of *N. sativa* seeds resulted in increasing activity of ALP in haemolymph of mid-aged nymphs. Furthermore, all extracts enhanced the enzyme activity in haemolymph of late-aged nymphs and adults. In fat bodies of *S. gregaria*, induced activity of ALP was determined in late-aged nymphs. Regarding the newly emerged adult females, ALP activity increased, irrespective of extract or concentration. These results are, to a great extent, in accordance with those reported results of enhanced ALP activity in different insects by various botanicals, such as *Pieris rapae* larvae by methanolic extract of *Silybium marianum* (Hasheminia *et al*., 2013) [111]; haemolymph of newly emerged adults of *S. gregaria* by different extracts of *A. visnaga* fruits (Ghoneim *et al*., 2014) [76]; *A. aegypti* larvae by NeemAzal (a neem formulation). (Koodalingam *et al*., 2014) [83]; *Anopheles gambiae* larvae by Biostop Moustiques® (Ahadji-Dabla *et al*., 2015) [112] and *T. castaneum* larvae by LC_{50} of the garlic oil (Beltagy and Omar, 2016) [96]. Outside the botanicals, promoting action of some IGRs on ALP activity in different insects were reported, such as pyriproxyfen against *P. gossypiella* (Mostafa, 1993) [102], *S. littoralis* (Abdel-Al, 2002) [81] and *M. domestica* (Assar *et al*., 2010) [103]; buprofezin, hexaflumuron, lufenuron and tebufenozide against the last instar larvae of *M. domestica* (Assar *et al*., 2010) [103] and Novaluron, Cyromazine and Diofenolan last instar larvae of *S. littoralis* (Hamadah *et al*., 2016) [85].

The 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 *N. sativa* seed extracts (Hasheminia *et al*., 2013) **[111]** or denotes an increasing capability of S. gregaria to detoxify chemicals contained in the tested plant extracts (Sharifi *et al*., 2013) [113]. Also, the increase in ALP activity could be due to a juvenoid effect of the *N. sativa* seed extracts since juvenile hormone leads to increase ALP level in *S. gregaria* (Omar, 2010) [114] or indicates a disturbance in the physiological balance of midgut (Ayyangar and Rao, 1990; Kamel *et al*., 2010) [91, 115].

Conclusion

Because the induction of detoxification metabolic system plays an important role in insect's detoxification mechanism, enhanced ACP and ALP activities in certain tissues of *S. gregaria* nymphs and adults by some seed extracts of *N. sativa* denote an increasing capability of the insect to detoxify them. On the other hand, inhibited enzyme activities in certain tissues of nymphs or adults indicate that some extracts may not be detoxified by these enzymes. So extracts of *N. sativa* seeds which exhibited inhibitory effects on the phosphatase activities may be potential agent for controlling *S. gregaria*, especially as a part of Integrated Pest Management. However, further investigation should be carried out in future to ascertain the active ingredient (s) in the *N. sativa* seed extracts responsible for the inhibition of these detoxifying enzymes.

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