Archives of Agriculture Sciences Journal
Print ISSN: 2535-1680
Online ISSN: 2535-1699

ARCHIVES OF AGRICULTURE SCIENCES JOURNAL

Volume 4, Issue 1, 2021, Pages 127–147

Available online at www.agricuta.edu.eg

DOI: https://dx.doi.org/10.21608/aasj.2021.62324.1054

Enzymatic efficacy of Nimbecidine®, a neem extract, against the phosphatases in certain tissues of the desert locust *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae)

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Abstract

The desert locust *Schistocerca gregaria* is a destructive pest for several crops, particularly which are considered as the main food sources for human and animals. Much attention has been paid to use the plant extracts or plant products for controlling this pest. The present study aimed to investigate the disturbing effect of Nimbecidine on the phosphatase activity in *S. gregaria*. The penultimate instar nymphs were treated, through the fresh food, with 1.0 and 0.3% Nimbecidine and the activities of acid phosphatase (ACP) and alkaline phosphatase (ALP) were estimated in the last instar nymphs and adults. The most important results could be summarized as follows. Nimbecidine significantly promoted the ACP activity in haemolymph, irrespective of the stage, age, or concentration. In addition, Nimbecidine exhibited a remarkable inducing effect on the enzyme activity in fat bodies, regardless the stage and age, with an exception of the late-aged nymphs in which the enzyme activity was suppressed. Regarding the effect of Nimbecidine on ALP activity in haemolymph of the nymphs and adults, results clearly displayed a general reducing effect of Nimbecidine on the enzyme activity in haemolymph of the last nymphal instar. An exceptional case of promoting action was detected in the newly emerged adults, at the higher concentration. In respect of the effect on ALP activity in fat bodies, results indicated that the enzyme activity was significantly induced in nymphs but reduced in adults.

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Keywords: acid phosphatase, alkaline phosphatase, azadirachtin, fat body, haemolymph.

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1. Introduction

The desert locust *Schistocerca gregaria* is a destructive pest for several crops, particularly which are considered as the main food sources for human and animals. In some cases, a single swarm contains 80 billions of adult locusts per square kilometer of an area (Steedman, 1988). Plagues of this pest have been recognized as threat to agricultural production in Africa and western Asia for thousands of years (Showler, 1995, 1996; Ceccato *et al*., 2007). Damage is caused as a consequence of its polyphagous behaviour, high population density, and the nature to aggregate and swarm. Each individual gregarious locust can consume roughly its own weight of foliage daily (Lindsey, 2002). Invasions of this locust are the cause of calamity because they can result 100% crop loss (FAO, 2012; Meinzingen, 1993). Therefore, it is necessary to search and develop some effective control strategies for suppressing the population density and/or inhibition of the phase transition into gregaria to avoid the formation of locust swarms. Because of the difficulty to predict locust outbreaks, the concerned countries usually apply pollutant chemical pesticides for control (Gruys, 1993). As reported by Lecoq (2001), the current control operations against the desert locust are mainly based on organophosphorus pesticides. The indiscriminate uses of many synthetic insecticides lead to destruction of the natural enemies (like parasites, predators), allowing an exponential increase of pest populations (Naqqash *et al*., 2016) and serious toxicological hazards to humans (Costa *et al*., 2008; Mosallanejad and Smagghe, 2009). Also, repeated use of a particular insecticide may result in the development of resistance (Bell *et al*., 2001; FAO, 2003). Therefore, the desert locust remains a serious problem despite the usage of these synthetic insecticides (Ouali-N'goran *et al*., 2013). To avoid the previously mentioned hazards of chemically synthetic insecticides, it is important to search for new effective and safer ways with negligible effects on ecosystem (Dubey *et al*., 2010; Korrat *et al.*, 2012). Much attention has been paid to use the plant extracts or plant products that have some insecticidal effects (Schmutterer, 1990a,b; Krall and Wilps, 1994). Plants may provide potential alternatives to currently used synthetic insecticides because they constitute a rich source of bioactive chemicals (Rembold, 1994; Qin *et al*., 2010). Also, crude extracts of plants could be cheaper, nontoxic to beneficial organisms, bio-degradable, and may have different mode of activities and inadequate resistance development in pests (Cantrell *et al*., 2012; Kabir *et al*., 2013; Senthil-Nathan *et al*., 2009). Majority of botanicals are still at the experimental stage. Unfortunately, the large-scale production is problematic and the difficulties that facing the registration of variable products will limit adoption (Meinzingen and Kooyman, 1997). Otherwise, prior results on the effects of plant extracts on the desert locust were encouraging their implementation as an alternative measure to chemical control (Abbassi *et al*., 2003). Many compounds with biological activities have been extracted from various parts of the neem tree, *Azadirachta indica* A. Juss, but seeds are the main source of the highly bioactive

compounds (Copping and Duke, 2007). Various neem products have been found with insecticidal and feeding deterrent properties (Kumar and Poehling, 2007; Mordue *et al*., 2005; Morgan, 2009). However, the primary active ingredient of most neem-based compounds is Azadirachtin (AZT), a steroid-like tetra-nortriterpenoid, which exhibits a wide range of bioactivity to hundreds of phytophagous insect species belonging to different orders. Along with direct toxicity, AZT affects many different physiological events in insects, including regulation of growth, protein synthesis, reproduction, diapause, and behavior (Abdullah and Subramanian, 2008; Garcia *et al*., 2006; Morgan, 2009). Many entomologists proved the efficacy of extracts and essential oils derived from *Aazadirachta indica*. Therefore, Neem plant was taken as a reference plant for different studies (Sultana *et al*., 2016). Nimbecidine® (Nimc) is a neem-based product containing 0.03% AZT as the major active ingredient in addition to other active compounds. This product has a direct anti-feeding role due to its specific odour which directly affects the gonadotropin production and eventually reduces the production of ovarian protein (Amsalem *et al*, 2014; Wegener *et al*, 2013). After treatment of *Sphaerodema rusticum* with Nimc, different metabolites in haemolymph and fat body were significantly affected (Shoba *et al*., 2011; 2014). Nimc inhibited the vitellogenesis of *Odontopus varicornis via* its effect on the neurosecretory cells (Ramya *et al*., 2014). Nimc influences, also, the growth and development of *Helicoverpa armigera* (Wondafrash *et al*., 2012) and caused significant reduction in fecundity, hatchability and adult emergence of *Earias vittella* (Bhardwaj and Ansari, 2015). Yasmin *et al*. (2016) reported that Nimc acted as an insect repellant, antifeedant, growth regulator and mating disruptor. Haemolymph is the only extra cellular fluid in the insect body that is usually kept in circulation by an open heart within the body cavity. It transports food materials to the cells and metabolic waste products away from those same cells. Hormones that regulate larval moulting, growth, metamorphosis, metabolism and other physiological processes of insects are secreted and circulated in the haemolymph (Hietakangas and Cohen, 2009). Exposure of an organism to xenobiotic product can modify the synthesis of certain metabolite and disturb the functionality of the organism (Rodriguez-Ortega *et al*., 2003). 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). On the other hand, fat body of insects carries out a variety of different metabolic activities comparable to mammalian liver. It 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 (Arrese and Soulages, 2010). Thus, the fat body is the important organ that synthesize and stores energy reserve, in addition to regulate the metabolic activities and reproduction (Park *et al*., 2006; Vivekananthan *et al*., 2010). 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). Also, these enzymes are involved in lipid hydrolysis in several tissues like midgut, hemolymph and fat bodies (Zibaee *et al*., 2011). In addition to ACP, ALP may act as hydrolases during the final stages of digestion (Cheug and Low, 1975), gonad maturation and metamorphic moults (Rhadha and Priti, 1969). 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 (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 (Ferreira and Terra, 1980; Wolfersberger, 1984) and is especially active in tissues with active membrane transport, such as intestinal epithelial cells (Caglayan, 1990; Sakharov *et al*., 1989), Malpighian tubules (Etebari and Matindoost, 2004a,b) and haemolymph (Etebari *et al*., 2007). It is responsible for cytolysis of tissues during the insect development (Dadd, 1970). Its primary function is to provide phosphate ions from mononucleotide and ribonucleo-proteins for a variety of metabolic processes (Etebari *et al*., 2005). In insects, ALP is involved in several biological processes and respond to stress, pathogenesis, or infection (Miao, 1988; 2002; Sukhanova *et al*., 1996). ALP 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). The objective of the present study was to investigate the effect of Nimbecidine on the phosphatase activity in haemolymph and fat bodies of the last instar nymphs and newly emerged adults of *S. gregaria*.

2. Materials and methods

2.1 Experimental insect

The desert locust, *Schistocerca gregaria* was used as an experimental insect in the present study. The present culture was originated by a sample of gregarious nymphs from Plant Protection Research Institute, Ministry of Agriculture, Giza. As designed by Hunter-Jones (1961) and improved by Ghoneim *et al*. (2009), insects were reared in wooden cages (60 x 60 x 70 cm). The bottom was furnished with a sandy layer (20 cm depth) provided with 10-15% humidity suitable for egg

laying. An electric bulb (100 watts) was adjusted in each cage to maintain a continuous photoperiod of 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.

2.2 Neem extract and nymphal treatment

The assessed botanical in the present study was Nimbecidine® (Neem preparation with 0.03% EC Azadirachtin). It was purchased from T. Stanes & company Ltd (Coimbatore, India). In a preliminary experiment, sublethal concentrations of Nimbecidine against *S. gregaria* were determined as 2.0, 1.0, 0.3 & 0.1%. Only two concentrations, 1.0 & 0.3%, were applied to investigate the effect of the present neem extract on the phosphatase activity in *S. gregaria*. 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 concentration of Nimbecidine for 3 minutes, the successfully moulted final instar nymphs and emerged adult females were undergone to determine the influenced acid phosphatase and alkaline phosphatase 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.

2.3 Tissue sampling

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.

2.4 Determination of the phosphatase activity

ACP activity was determined according to the method of (Tietz, 1999) 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 enzyme activity was measured at wavelength 550 nm by spectrophotometer.

2.5 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.

3. Results

3.1 Effect of Nimbecidine on the acid phosphatase (ACP) activity in S. gregaria

According to the data arranged in Table (1), ACP activity in the haemolymph gradually increased with age of control last instar nymphs, starting in 1050.0 ±37.5 U/L and ending in 1425.0±37.5 U/L. Otherwise, the enzyme activity declined in haemolymph of the control newly emerged adults (1337.5±21.7 U/L). After treatment of the penultimate instar nymphs of *S. gregaria* with Nimbecidine, data of the same table revealed a significant promoting effect of Nimbecidine on the enzyme activity, irrespective of the stage, age, or concentration.

Table (1): Effects of Nimbecidine on the of acid phosphatase activity (U/L) in haemolymph of the desert locust *Schistocerca gregaria*.

Conc. %		Last instar nymphs			Newly emerged adults
		Early-aged	Mid-aged	Late-aged	
LC_{75} (1.0%)	Mean ± SD	1137.5 ± 21.7 b	1887.5 ± 57.3 d	1737.5 ± 57.3 c	1850.0 ± 57.3 d
	Change %	+8.3	+57.3	+21.9	+38.3
LC_{50} (0.3%)	Mean ± SD	1087.5 ± 37.5 a	1450.0 ± 57.3 c	1675.0 ± 57.3 c	1362.5 ± 21.7 a
	Change %	+3.6	+20.8	+17.5	+1.9
Controls	Mean ± SD	1050.0 ± 37.5	1200.0 ± 37.5	1425.0 ± 37.5	1337.5 ± 21.7

Conc.: Concentration levels, mean ± SD followed with the same letter (a): is 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).

Its strongest promoting effect was detected on ACP activity in haemolymph of the mid-aged nymphs at higher concentration (57.3% increment) while a slight enhancing effect was found on the enzyme activity in haemolymph of the early-aged nymphs at the lower concentration (3.6% increment). In addition, Nimbecidine exhibited an enhancing effect on ACP activity in

haemolymph of the newly emerged adults in a dose-dependent course (Change % + 38.3 and +1.9 at higher and lower concentrations, respectively). With regard to the effect of Nimbecidine on ACP activity in fat bodies, data assorted in Table (2) exiguously revealed that Nimbecidine exhibited a remarkable inducing effect on the enzyme activity, regardless the stage and age, with an exception of late-aged nymphs in which the enzyme activity was drastically suppressed (32.6% decrement). In some detail, Nimbecidine exerted the strongest enhancing action on ACP activity in fat bodies of the early-aged nymphs at the higher concentration (313.3% increment) but the least enhancing action on the enzyme activity in fat bodies of the mid-aged nymphs, at the same concentration (054.2% increment). In addition, the enzyme activity was pronouncedly promoted in fat bodies of the newly emerged adults (48.1 and 23.3%s increments, at 1.0 and 0.3%, respectively.

3.2 Effect of Nimbecidine on the alkaline phosphatase (ALP) activity in S. gregaria

As exiguously observed in Table (3), ALP activity gradually decreased in the haemolymph of control last nymphal instar with age and in control newly emerged adults (33.3±6.9, 24.2±2.7 and 10.6±2.6 U/L in early-, mid- and late-aged nymphs, respectively, as well as 6.0±2.7 U/L in the newly emerged adults).

Table (2): Effects of Nimbecidine on the acid phosphatase activity (U/L) in fat bodies of the desert locust *Schistocerca gregaria*.

Conc. %		Last instar nymphs			Newly emerged adults
		Early-aged	Mid-aged	Late-aged	
LC_{75} (1.0%)	Mean ± SD	310.8 ± 15.6 d	145.3 ± 0.9 d	112.0 ± 0.8 d	127.8 ± 3.1 d
	Change %	+313.3	+054.2	+12.7	+48.1
LC_{50} (0.3%)	Mean ± SD	163.8 ± 2.0 d	337.3 ± 6.2 d	067.0 ± 0.6 d	106.4 ± 0.8 d
	Change %	+117.8	+258.1	-32.6	+23.3
Controls	Mean ± SD	075.2 ± 2.8	094.2 ± 1.2	099.4 ± 1.1	086.3 ± 1.3

Conc.: Concentration levels, mean ± SD followed with the same letter (a): is not significantly different (P>0.05), (b): significantly different (P<0.05), (c): highly significantly different (P<0.01), (d): very highly significantly different (P<0.001).

Table (3): Effects of Nimbecidine on the alkaline phosphatase (U/L) in haemolymph of the desert locust *Schistocerca gregaria*.

Conc. %		Last instar nymphs			Newly emerged adults
		Early-aged	Mid-aged	Late-aged	
LC_{75} (1.0%)	Mean ± SD	7.5 ± 5.3 c	15.1 ± 2.7 b	6.0 ± 2.7 a	10.6 ± 2.6 a
	Change %	-77.5	-37.6	-43.4	+76.7
LC_{50} (0.3%)	Mean ± SD	13.6 ± 4.6 b	13.6 ± 4.6 b	7.5 ± 5.3 a	6.0 ± 2.7 a
	Change %	-59.2	-43.8	-29.2	0.0
Controls	Mean ± SD	33.3 ± 6.9	24.2 ± 2.7	10.6 ± 2.6	6.0 ± 2.7

Conc.: Concentration levels, mean ± SD followed with the same letter (a): is 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).

To shed some light on the effect of Nimbecidine on ALP activity in haemolymph of the nymphs and adults of *S. gregaria*, the penultimate instar nymphs were treated with two concentrations of this neem preparation. Data of the same table clearly displayed a general reducing effect of Nimbecidine on ALP activity in haemolymph of the last nymphal instar but not in the newly emerged adults. The exceptional case of promoting action of Nimbecidine was detected at the higher concentration in the newly emerged adults (10.6±2.6 *vs*. 6.0±2.7 U/L of control congeners). In some detail, the most drastic reducing effect of Nimbecidine was exhibited on ALP activity in haemolymph of the early-aged nymphs (77.5% decrement)) while the least prohibiting effect was found in haemolymph of the late-aged nymphs (29.2% decrement). In respect of the effect of Nimbecidine on ALP activity in fat bodies of nymphs and adults, data of Table (4) indicated that the enzyme activity was significantly induced in nymphs but reduced in adults. In some detail, the most potent enhancing effect of Nimbecidine was determined in fat bodies of early-aged nymphs (23.3±4.0 *vs*. 13.0±1.6 U/L in control nymphs) while the least enhancing effect of Nimbecidine was detected in the late-aged nymphs (7.4±0.6 *vs*. 6.8±0.3 U/L in control congeners). In general, ALP activity was suppressed in fat bodies of the newly emerged adults, in a reverse course to the concentrations (33.3 and 35.4% decrements, at 1.0 and 0.3%, respectively). However, ALP activity in fat bodies of control insects gradually decreased with the stage and age (13.0±1.6, 9.7±1.0, 6.8±0.3 & 4.8±0.4 U/L in early-, mid- and late-aged nymphs, and the newly emerged adults, respectively).

Table (4): Effects of Nimbecidine on the alkaline phosphatase activity (U/L) in fat bodies of the desert locust *Schistocerca gregaria*.

Conc. %		Last instar nymphs			Newly emerged adults
		Early-aged	Mid-aged	Late-aged	
LC_{75} (1.0%)	Mean ± SD	23.3 ± 4.0 b	16.4 ± 0.9 d	8.9 ± 0.5 b	3.2 ± 0.6 b
	Change %	+79.2	+69.1	+30.9	-33.3
LC_{50} (0.3%)	Mean ± SD	15.3 ± 1.4 a	16.1 ± 1.0 d	7.4 ± 0.6 a	3.1 ± 0.5 b
	Change %	+17.7	+66.0	+8.8	-35.4
Controls	Mean ± SD	13.0 ± 1.6	9.7 ± 1.0	6.8 ± 0.3	4.8 ± 0.4

Conc.: Concentration levels, mean ± SD followed with the same letter (a): is 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).

4. Discussion

In insects, Acid phosphatase (ACP) and Alkaline phosphatase (ALP) are responsible for cytolysis of tissues during the insect development (Dadd, 1970) since they may act as hydrolases during the final stages of digestion (Cheug and

Low, 1975), gonad maturation and metamorphic moults (Tsumuki and kanehisa, 1984). 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). Induction of detoxification metabolic system plays an important role in the insect's detoxification mechanism (Terriere, 1984). The detoxifying enzymes react against insecticides and other compounds that exhibit insecticidal activities. They include general esterases, glutathione S-transferase and phosphatases (Zibaee *et al*., 2011). It may be important to mention that the activities of phosphatases have been disturbed by different plant extracts or secondary metabolites of some botanicals (Diamantino *et al*., 2001; Ottaviani, 2014). However, the detailed mechanism of action was explained (for review, see Senthil-Nathan, 2013).

4.1 Disturbed ACP activity in S. gregaria by Nimbecidine

Diverse, and sometimes contradictory, effects of several botanicals on ACP activity in various insects had been reported since Ghoneim *et al*. (2008) 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*. 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). 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). Coumarin (isolated from of Chicory flower) and Neemix (an azadirachtin formulation) caused significant increase in the activity of ACP in the 4th instar larvae of *S. littoralis* (Gaaboub *et al*., 2012). A significant increase of ACP level was measured in larvae and pupae of the mosquito *Aedes aegypti* by exposure to Neemazal (Koodalingam *et al*., 2014). Inducing effects of both methanol and petroleum ether extracts of *Nigella sativa* seeds on ACP activity had been determined in haemolymph of the nymphs and adults of *S. gregaria* (Ghoneim *et al*., 2016). Results of the present study were in corroboration with some of those reported results, since Nimbecidine significantly promoted the ACP activity in haemolymph of *S. gregaria*, irrespective of the stage, age, or concentration. Also, Nimbecidine exhibited an enhancing effect on the enzyme activity in haemolymph of adult locusts, in a dose-dependent course. In addition, Nimbecidine exhibited a remarkable inducing effect on the enzyme activity in fat bodies of *S. gregaria*, regardless the stage and age, with an exception of the

late-aged nymphs in which the enzyme activity was suppressed. On the other hand, the current results were inconsistent with those reported results of inhibited activity of ACP in larvae or nymphs of some insects after treatment with different botanicals, such as *M. domestica* after treatment with Azadirachtin (Saeed *et al.*, 1987) or Jojoba oil (Ghoneim *et al*., 2008); *S. littoralis* after treatment with Azadirachtin (Ayyangar and Rao, 1990); *Euprepocnemis plorans* after treatment with some neem limonoids (Al-Dali, 2007); *Rhizopertha dominica* after treatment with hexane extract of *Capparis deciduas* (Upadhyay, 2013); *Tribolium castaneum* after treatment with different extracts of *Melia azedarach, Nicotiana tabacum, Azadirachta indica* and *Colosynthus citrullus* (*Ali et al.,* 2015) or with LC_{50} of the garlic oil (Beltagy and Omar, 2016); *Spodoptera litura* after treatment with Andrographolide (a diterpene lactone isolated from *Andrographis paniculata*) (Edwin *et al.,* 2016). Among five tested plants against *Musca domestica*, treatment of 2nd instar larvae with 25% concentration of *Penganum harmala* led to a reduction in the activity of ACP (Zahoor *et al.,* 2020). For interpretation of the induced ACP activity in haemolymph and fat bodies of *S. gregaria* nymphs and adults, after treatment with Nimbecidine in the present investigation, Nimbecidine might exhibit an ecdysone (moulting hormone)-like activity, since this hormone is responsible for increase of lysosome number as a lysosomal ACP enzyme (Bassal and Ismail, 1985). The induced ACP activity could be, also, understood because ACP activity, directly or indirectly, interferes with the digestion, absorption and positive transport of nutrient in the midgut of *S. littoralis* larvae (Senthil Nathan *et al.,* 2004; Smirle *et al.,* 1996).

4.2 Disturbed ALP activity in S. gregaria by Nimbecidine

Few studies have examined the disturbing effects of plant products on ALP activity in insects. For example, feeding of the whitefly (*Bemisia tabaci*) adults on tomato seedlings sprayed with the plant growth regulator 3-indoleacetic acid (IAA) led to increasing activity of ALP (*Di et al.,* 2014). Treatment of the mosquito *A. aegypti* larvae with Neemazal enhanced ALP activity (Koodalingam *et al.,* 2014). Topical application of Biostop Moustiques® (derived from coconut oil) on 4th instar larvae of susceptible and resistant strains of the mosquito *Anopheles gambiae* resulted in a significant increase of ALP activity in both strains (Ahadji-Dabla *et al.,* 2015). In addition, ALP activity was enhanced in different insects by various botanicals, such as *Pieris rapae* larvae by methanolic extract of *Silybium marianum* (Hasheminia *et al.,* 2013); haemolymph of adults of *S. gregaria* by different extracts of *A. visnaga* fruits (*Ghoneim et al.,* 2014); *A. aegypti* larvae by Neemazal (*Koodalingam et al.,* 2014); *Anopheles gambiae* larvae by Biostop Moustiques® (*Ahadji-Dabla et al.,* 2015) and *T.*

castaneum larvae by LC_{50} of the garlic oil (Beltagy and Omar, 2016). Results of the present study were partially in agreement with those reported results, since Nimbecidine exhibited a diverse effect on the ALP activity in *S. gregaria*, depending on the stage and tissue, because it exhibited a predominant reducing effect on the enzyme activity in the haemolymph of nymphs but the enzyme activity was promoted in haemolymph of adults, only at the higher concentration. In respect of the effect of Nimbecidine on ALP activity in fat bodies of *S. gregaria*, the present results indicated that the enzyme activity was significantly enhanced in nymphs but reduced in adults. On the other hand, some of the current results were partially in accordance with the reported results of inhibited enzyme activity after treatment with certain botanicals, such as 4th instar larvae of *S. littoralis* after treatment with Coumarin (isolated from of Chicory flower) and Neemix (Gaaboub *et al.,* 2012); *Rhyzopertha dominica* after treatment with hexane extract of *Capparis deciduas* (Upadhyay, 2013); *T. castaneum* after treatment with different extracts of *Curcuma longa* (Uma devi and Sujatha, 2013) or *Melia azedarach, Nicotiana tabacum, A. indica* and *Citrullus citrullus* on *T. castaneum* adults (Ali *et al.,* 2015); in last instar nymphs of *S. gregaria* after treatment with *A. visnaga* seed extracts (Ghoneim *et al.,* 2014); *Callosobruchus analis* after treatment with LC_{50} of the essential oil from *Acorus calamus* or Biosal (a neem preparation) on (Arif *et al.,* 2015) and *Spodoptera litura* larvae after treatment with Andrographolide (Edwin *et al.,* 2016). Among five tested plants against Musca domestica, treatment of 2nd instar larvae with 25% concentration of Penganum harmala led to a reduction in the activity of ALP (Zahoor *et al.,* 2020). The increasing ALP activity in some tissues of nymphs or adults of *S. gregaria*, in the present study, might indicate the involvement of this enzyme in detoxification process against Nimbecidine (Hasheminia *et al.,* 2013) or denote an increasing capability of *S. gregaria* to detoxify this neem preparation (Sharifi *et al.,* 2013). Also, the increase in ALP activity could be due to a juvenoid effect of Nimbecidine since juvenile hormone leads to increase ALP level in *S. gregaria* (Omar, 2010) or might be due to a disturbance in the physiological balance of midgut (Kamel *et al.,* 2010). In addition, the increased ALP activity could be a protective physiological response against the action of Nimbecidine (Ahadji-Dabla *et al.,* 2015). On the other hand, the reduced ALP activity in some tissues and developmental stages in *S. gregaria* by Nimbecidine, in the present study, might be explicate by some developmental disturbance, as a valuable suggestion of Wu (1990) for the larvae of mosquito *Culex pipiens* after treatment with IGR diflubenzuron. In addition, Nimbecidine might affect the gut physiological events (i.e. transport) causing a prohibition of ALP activity, as well as might affect both juvenile hormone and ecdysone regulation, directly or indirectly, as suggested by

Phillips *et al*. (1988) for *Cnaphalocrocis medinalis*.

5. Conclusion

Because the induction of detoxification metabolic system plays an important role in insect's detoxification mechanism, the enhanced activities of ACP and ALP in the *S. gregaria* nymphs and adults by Nimbecidine, in the present study, denoted an increasing capability of the insect to detoxify it. Depending on our results, Nimbecidine could not be recommended as promising control agent in the integrated pest management of *S. gregaria*.

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