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.

**Keywords**: acid phosphatase, alkaline phosphatase, azadirachtin, fat body, haemolymph.
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 Smaghe, 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, biodegradable, 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 tetrarnortriterpenoid, 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 repellent, 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 (4th) 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 Nimbedicine 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 Nimbedicine, data of the same table revealed a significant promoting effect of Nimbedicine on the enzyme activity, irrespective of the stage, age, or concentration.

| Conc. % | Last instar nymphs | Newly emerged adults |
|---------|-------------------|---------------------|
|         | Early-aged | Mid-aged | Late-aged | Early-aged | Mid-aged | Late-aged |
| LC75 (1.0%) | Mean ± SD | 1137.5 ± 21.7b | 1887.5 ± 57.3d | 1737.5 ± 57.3c | 1850.0 ± 57.3d |
| Change % | +8.3 | +57.3 | +21.9 | +38.3 |
| LC50 (0.3%) | Mean ± SD | 1087.5 ± 57.5a | 1450.0 ± 57.3c | 1675.0 ± 57.3c | 1362.5 ± 21.7a |
| 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, Nimbedicine 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% 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. % | Newly emerged adults | Early-aged | Mid-aged | Late-aged |
|---------|----------------------|------------|----------|-----------|
| LC75 (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 |
| LC50 (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 |

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

| Conc. % | Newly emerged adults | Early-aged | Mid-aged | Late-aged |
|---------|----------------------|------------|----------|-----------|
| LC75 (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 |
| LC50 (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 % | -9.2 | -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 congener). 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 congener). 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 & flip  | Late-aged & flip     |
| LC75 (1.0%) | Mean ± SD         | 23.3 ± 4.0 b         | 8.9 ± 0.5 b          | 3.2 ± 0.6 b         |
|         | Change %           | +79.2                | +30.9                | -33.3               |
| LC50 (0.3%) | Mean ± SD         | 15.3 ± 1.4 a         | 7.4 ± 0.6 a          | 3.1 ± 0.5 b         |
|         | Change %           | +35.4                | +17.7                |
| Controls | Mean ± SD          | 13.0 ± 1.6           | 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 (Zibae 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); *Euprepecnemis 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 LC50 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 ecysone (moultng hormone)-like activity, since this hormone is responsible for increase of lysosome number as a lyosomal 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 (Ahadj-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 *Silybum 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® (Ahadj-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 4$^{th}$ 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 _Citrus 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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