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RESEARCH ARTICLE

Effect of Phytopesticide Nimbecidine on the Biochemical Parameters of the Reproductive Tissues of *Sphaerodema rusticum* (Fabricius)

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ABSTRACT

Present study was aimed to investigate the effect of phytopesticide nimbecidine on oxidative enzymes. The sub-lethal concentration of the phytopesticide nimbecidine (0.00028 ppm 1/10th of LC_{50}) was studied on *Sphaerodema rusticum* for 7, 14 and 21 days of exposure. It reveals significant variation in lactate dehydrogense (LDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH) and glutamate dehydrogenase (GDH) activities. The enzyme activity of SDH and LDH in the fat body, testis and seminal vesicle of nimbecidine treated insects were gradually decreased than the control insects. In contrast, the activity of MDH and GDH in the fat body, testis and seminal vesicle of nimbecidine treated insects were gradually increased than the control insects. These changes were more pronounced in treated tissues than compared to control tissues and it may be due to synergistic effect of nimbecidine.

KEYWORDS

Nimbecidine, *Sphaerodema rusticum*, Oxidative Enzymes, Fat Body, Testis and Seminal Vesicle

INTRODUCTION

Pollution of the environment by pesticide is a dangerous problem around the world. Consequently, so many institutions are engaged in some safe alternative methods of pest control. Botanical insecticides and microbial pesticides are highly effective, safe and ecologically acceptable (Matthews, 1999). Neem components show multiple effects against different insects (Rehimi *et al*., 2011). Azadirachtin is known as phagorepellent natural products from seeds of the neem tree *Azadirachta indica* A. Juss (Meliaceae), which impede the development of insects and sterilize adults (Hummel, *et al.*, 2011). Research on the site and mechanism of action of these components indicates that many terpenoid compounds are involved in insecticidal and insect growth regulation activities. These substances are important enzymatic and metabolic inhibitors (Hammond and Kubo, 1999; Cespedes *et al.*, 2000; Kubo *et al.*, 2003; Panzuto *et al*., 2002). A diverse array of secondary metabolites has different sites of action and different molecular targets when the compounds interact with enzymes and processes of metamorphosis (Calderon *et al*., 2001; Torres *et al*., 2003; Cespedes *et al*., 2004). Increased use of chemical pesticides results in the excess inflow of toxic chemicals, mainly into the aquatic ecosystem (Baskaran *et al.*, 1989; Kalavathy *et al.*, 2001). The aquatic flora and fauna are affected by the toxic substances which eventually enter into their systems or bring about external damages (Pant and Singh, 1983; Hodson, 1988; Johl and Dua, 1995).

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Dehydrogenases are very important tools for the investigation of insect metabolic activities during the course of development. The relative activities of the insect dehydrogenases may be related to the function and energy yielding demands of the tissues (Dickinson and Sullivan, 1975).

Lactate dehydrogenase (LDH) is an important glycolytic enzyme that is present virtually in all tissues (Kaplan and Pesce, 1996). It is involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress (Diamantino *et al.,* 2001).

LDH is, also, a parameter widely used in toxicology and in clinical chemistry to diagnose cell, tissue and organ damage. However, the potential of this enzyme as an indicative criterion in the invertebrate toxicity tests has been scarcely explored (Ribeiro *et al.,* 1999; Senthil Nathan *et al.,* 2006 a & b).

In addition, energy metabolism of reproduction in male insects has not been studied. Investigations were made on the TCA enzymes, SDH, LDH, MDH, and GDH activity in the fat body, testis and seminal vesicle of control and phytopesticide nimbecidine treated adult male insect *Sphaerodema rusticum*.

MATERIALS AND METHOD

The insect, *Sphaerodema rusticum* is aquatic predatory bug. The insects collected from the local ponds and streams were maintained in plastic troughs at the laboratory temperature of 28 ± 3°C with a relative humidity of 80 ± 3ºC percent.

The insects were daily fed with mosquito larvae, pieces of earthworm and aquatic plants and the insects were survived well on these feeds. The troughs were cleaned properly every alternative day and the water was renewed.

Nimbecidine, a neem oil based formulation of Azadirachtin (0.03% EC) of T. Stanes and company Ltd., was purchased from local pesticide agency in Chidambaram.

The enzyme LDH activity was determined according to the method of (Tietz, 1999). The enzyme SDH was assayed by Bernath and Singer (1962) method. The enzyme malate dehydrogenase was assayed by Severo Ochoa (1955) method.

The enzyme GDH activity was assayed by the method of Strecker (1965).

Statistical Analysis

The data were statistically analyzed using one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) and were expressed as mean ± S.D. The values were considered statistically significant if the *p*-value was less than 0.05.

RESULTS

Succinate Dehydrogenase (SDH)

The SDH activity of fat body, testis and seminal vesicle of control insects were 12.8 ± 2.0, 8.0 ± 1.7 and 15.5 ± 2.0 µmoles formazone formed/10mg of protein/hour respectively. Likewise after 7, 14 and 21days of exposure of insect to nimbecidine, the SDH activity of fat body, testis and seminal vesicle were about 11.7 ± 1.4, 6.0 ± 0.5, 13.8 ± 0.6; 9.8 ± 0.94, 4.6 ± 0.5, 11.1 ± 1.3 and 7.5 ± 1.4, 2.2 ± 0.4, 9.7 ± 1.4 µmoles formazone formed/10mg of protein/hour respectively.

The percentage changes were as follows -8.60, -25.0, -10.97%; -23.43, -42.5, -28.38%, and -41.40, -72.5, -37.41% after 7, 14 and 21 days of exposure respectively (Table 1). The SDH activity gradually decreased in the treated insects when compared to control.

Lactate Dehydrogenase (LDH)

The LDH activity of fat body, testis and seminal vesicle of control insects were 1.94 ± 0.2, 4.8 ± 0.8 and 3.7 ± 0.5 µmoles formazone formed/10mg of protein/hour respectively. Likewise after 7, 14 and 21days of exposure of insect to nimbecidine, the LDH activity of fat body, testis and seminal vesicle were about 1.28 ± 0.2, 3.2 ± 0.9, 2.6 ± 0.7; 0.97 ± 0.2, 2.1 ± 0.3, 1.8 ± 0.6 and 0.57 ± 0.1, 1.2 ± 0.2, 1.1 ± 0.2 µmoles formazone formed/10mg of respectively.

The percentage changes were as follows -34.02, -33.33, -29.72; -50.00, -56.25, -51.35 and -70.61, -75.00, -70.27% after the 7, 14 and 21 days of exposure respectively (Table 2).

The LDH activity gradually decreased in the treated insects than compared to control insects.

Malate Dehydrogenase (MDH)

The MDH activity of fat body, testis and seminal vesicle of control insects were 12.5 ± 1.7, 7.1 ± 1.5 and 5.9 ± 1.1 µmoles formazone formed/10mg of protein/hour respectively.

Likewise after 7, 14 and 21days of exposure of insect to nimbecidine, the MDH activity of fat body, testis and seminal vesicle were about 15.0 ± 1.5, 9.6 ± 0.7, 8.3 ± 0.9; 18.1 ± 1.5, 12.1 ± 1.6, 10.9 ± 1.1 and 21.3 ± 1.6, 15.8 ± 1.5, 13.5 ± 1.7 µmoles formazone formed/10mg of protein/hour respectively.

The percentage changes were as follows 20.0, 44.80, 40.68 %; 44.80, 70.42, 84.74 % and 70.40, 122.53, 128.81% after the 7, 14 and 21 days of exposure respectively.

The MDH activity gradually increased in the treated insects than compared to control insects (Table 3).

Glutamate Dehydrogenase (GDH)

The GDH activity of fat body, testis and seminal vesicle of control insects were 9.8 ± 1.4, 13.1 ± 1.5 and 8.6 ± 0.7 µmoles formazone formed/10mg of protein/hour respectively. Likewise after 7, 14 and 21days of exposure of insect to nimbecidine, the GDH activity of fat body, testis and seminal vesicle were about 12.0 ± 1.1, 15.6 ± 1.2, 11.4 ± 1.8; 13.4 ± 1.6, 17.9 ± 0.7, 12.6 ± 2.1and 14.5 ± 1.2, 19.9 ± 1.4, 13.6 ± 1.3 µmoles formazone formed/10mg of protein/hour respectively.

Table 1: Succinate dehydrogenase (SDH) activities in the fat body, testis and seminal vesicle of control and nimbecidine treated adult male insect, *Sphaerodema rusticum*

Tissues	Control µmoles formazone formed/10mg of protein/hour	Sub - lethal exposure duration (Days) µmoles formazone formed/10mg of protein/hour		
		7	14	21
Fat body	12.8 ± 2.0 ^{c}	11.7 ± 1.4 $^{b\,c}$ (-8.60)	9.8 ± 0.94 ^{b} (-23.43)	7.5 ± 1.4 ^{a} (-41.40)
Testis	8.0 ± 1.7 ^{d}	6.0 ± 0.5 ^{c} (-25.00)	4.6 ± 0.5 ^{b} (-42.5)	2.2 ± 0.4 ^{a} (-72.5)
Seminal vesicle	15.5 ± 2.0 ^{b}	13.8 ± 0.6 ^{b} (-10.97)	11.1 ± 1.3 ^{a} (-28.38)	9.7 ± 1.4 ^{a} (-37.41)

Values are mean ± Standard deviation of six individual observations.

Values in paranthesis indicate percentage change over control.

Values that are not sharing a common superscript letter in the same row differ significantly at $p<0.05$

Table 2: Lactate dehydrogenase (LDH) activities in the fat body, testis and seminal vesicle of control and nimbecidine treated adult male insect, *Sphaerodema rusticum*

Tissues	**Control µmoles formazone formed/10mg of protein/hour**	**Sub- lethal exposure duration (Days) µmoles formazone formed/10mg of protein/hour**		
		7	**14**	**21**
Fat body	1.94 ± 0.2 ^{d}	1.28 ± 0.2^{c} (-34.02)	0.97 ± 0.2 ^{b} (-50.00)	0.57 ± 0.1^{a} (-70.61)
Testis	4.8 ± 0.8 ^{d}	3.2 ± 0.9 ^{c} (-33.33)	2.1 ± 0.3 ^{b} (-56.25)	1.2 ± 0.2 ^{a} (-75.00)
Seminal vesicle	3.7 ± 0.5 ^{c}	2.6 ± 0.7 ^{b} (-29.72)	1.8 ± 0.6$^{a\,b}$ (-51.35)	1.1 ± 0.2 ^{a} (-70.27)

Table 3: Malate dehydrogenase (MDH) activities in the fat body, testis and seminal vesicle of control and nimbecidine treated adult male insect, *Sphaerodema rusticum*

Tissues	**Control µmoles formazone formed/10mg of protein/hour**	**Sub- lethal exposure duration (Days) µmoles formazone formed/10mg of protein/hour**		
		7	**14**	**21**
Fat body	12.5 ± 1.7 ^{a}	15.0 ± 1.5^{b} (20.00)	18.1 ± 1.5 ^{c} (44.80)	21.3 ± 1.6 ^{d} (70.40)
Testis	7.1 ± 1.5 ^{d}	9.6 ± 0.7 ^{c} (44.80)	12.1 ± 1.6 ^{b} (70.42)	15.8 ± 1.5 ^{a} (122.53)
Seminal vesicle	5.9 ± 1.1 ^{a}	8.3 ± 0.9 ^{b} (40.68)	10.9 ± 1.1 ^{c} (84.74)	13.5 ± 1.7 ^{d} (128.81)

Table 4: Glutamate dehydrogenase (GDH) activities in the fat body, testis and seminal vesicle of control and nimbecidine treated adult male insect, *Sphaerodema rusticum*

Tissues	**Control µmoles formazone formed/10mg of protein/hour**	**Sub- lethal exposure duration (Days) µmoles formazone formed/10mg of protein/hour**		
		7	**14**	**21**
Fat body	9.8 ± 1.4 ^{a}	12.0 ± 1.1^{b} (22.44)	13.4 ± 1.6 ^{bc} (36.73)	14.5 ± 1.2 ^{c} (47.96)
Testis	13.1 ± 1.5 ^{a}	15.6 ± 1.2 ^{b} (19.08)	17.9 ± 0.7 ^{c} (36.64)	19.9 ± 1.4 ^{d} (51.90)
Seminal vesicle	8.6 ± 0.7 ^{a}	11.4 ± 1.8 ^{b} (32.55)	12.6 ± 2.1 $^{b\,c}$ (46.51)	13.6 ± 1.3 ^{c} (58.13)

Values are mean ± Standard deviation of six individual observations.

Values in paranthesis indicate percentage change over control.

Values that are not sharing a common superscript letter in the same row differ significantly at $p<0.05$

The percentage changes were as follows 22.44, 19.08, 32.55%; 36.73, 36.64, 46.51% and 47.96, 51.90, 58.13% after the 7, 14 and 21 days of exposure respectively. The GDH activity gradually increased in the treated insects than compared to control insects (Table 4).

DISCUSSION

In the present study, the enzyme activity of SDH and LDH in the fat body, testis and seminal vesicle of nimbecidine treated insects were gradually decreased than the control insects. In contrast, the activity of MDH and GDH in the fat body, testis and seminal vesicle of nimbecidine treated insects were gradually increased than the control insects. This observation is in conformity with Sumathi (2002); Rajathi (2004); Ramesh Kumar (2004) Lousia (2010) Riseh *et al*. (2012) and Sameh Mostafa Abd El- Naby and Ehab Wafeek Zidan (2014). In general, any stress inducing substance will affect the respiratory metabolism of insect. Any alteration in the intermediary metabolism due to stress is bound to affect the activity of oxidative enzymes like SDH, LDH etc. Nevertheless, the LDH is an important glycolytic enzyme which is present virtually in all invertebrate tissues (Kaplan and Pesce, 1996).

Dehydrogenases are very important tools for the investigation of insect metabolic activities during the course of development. The relative activities of the insect dehydrogenases may be related to the function and energy yielding demands of the tissues (Dickinson and Sullivan, 1975). Lactate dehydrogenase (LDH) is an important glycolytic enzyme that is present in virtually all tissues (Kaplan and Pesce, 1996; Shekari *et al*., 2008). It is involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress (Diamantino *et al.,* 2001). LDH is, also, a parameter widely used in toxicology and in clinical chemistry to diagnose cell, tissue and organ damage. However, the potential of this enzyme as an indicative criterion in the invertebrate toxicity tests has been scarcely explored (Ribeiro *et al.,* 1999; Senthil Nathan *et al*., 2006b; Riseh *et al*., 2012), in addition to its role as an evidence for an alternative pathway of terminal anaerobic metabolism (Bianconcini *et al.*, 1980).

The present inhibitory effects of the phytopesticide nimbecidine are in agreement with those inhibitory effects of some insecticides and insect growth regulators (IGRs) on the LDH activity in fat bodies of other insects species such as the house fly *Musca domestica* (Hassanein *et al.,* 1996), the silk worm *Bombyx mori* (Nath, 2000), a susceptible strain of the mosquito *C. fatigans* (Azmi *et al.,* 2002), the rice leaf folder *Cnaphalocrocis medinalis* (Senthil Nathan *et al.,* 2006a, 2006b), the cotton leafworm *S. littoralis* (Abdel-Ghaffar and Basiouny, 2007). Investigating the inhibitory effect of certain *A. indica* extracts on LDH in another tissue, mid-gut, of *C. medinalis*, Senthil Nathan *et al.* (2006a) observed a decrease in the enzyme activity denoting a reduced metabolism in the insect and may be due to the toxic effects of neem derivatives on membrane permeability, especially on the gut epithelium (Senthil Nathan *et al.,* 2004, 2005; Smirle *et al.,* 1996).

In insects, two kinds of MDH - cytosolic and mitochondrial are well known (Sacktor, 1975). Cytosolic NADH is oxidized into reduction of oxaloacetate by the action of cytosolic MDH, to yield malate. The malate enters into the mitochondria via, a carrier and is oxidized there into oxaloacetate by the action of mitochondrial MDH. It is evident from the present study that the pattern of activity of the respiratory enzyme MDH, which has been increased in all the reproductive tissues, treated with the sub - lethal concentration of nimbecidine than the control insects. These changes might be due to the supply of energy by the TCA cycle for the treated insects which require energy during nimbecidine intoxication.

Glutamate is the only amino acids for which specific and highly active dehydrogenase exists. This occurs principally through the amino tranferase completed with the action of GDH (Smith *et al.,* 1985). Van Der Berg (1964) has

reported that the formation of α-Keto glutarate from glutamate in the flight muscle of housefly. Deamination of amino acids by GDH is the major route of protein metabolism. The MARGs in insects are known to elaborate proteineous seminal plasm and or spermatophore (Selvisabhanayakam, 1995; Shivaji, 1998; Latha, 1998). Though it was not possible to estimate the TCA cycle metabolites, in view of their highly labile and dynamic nature, an increase of GDH activity during pesticide stress in all the reproductive tissues, suggest that the glutamate may be utilized for the conversion of α-Keto glutarate to augment the energy resource for *S. rusticum* when exposed to sub-lethal concentration of nimbecidine.

The main pathways for the conversion of α-amino acids to the corresponding L - keto acid through the formation of other acid by the enzymes L - amino acids dehydrogenase. Glutamate can be converted into α - Keto glutarate by the transmission with other keto acids. It is also known that deamination of glutarate brought about the action of GDH (NAD^+ dependent) yield ammonia and α- keto glutarate. Osanai *et al.* (1987) have demonstrated the similar metabolic pathway of energy supply to spermatozoa in the spermatophore for *Bombyx mori*. Glutamate dehydrogenase is an enzyme that, in addition to its role in the energy metabolism in mitochondria, is involved in neuromuscular transmission for *Drosophila melanogaster* (Depy Papadopoulou and Christos Louis, 2000). In recent decades many of the investigations for the pest management have been conducted to replace the synthetic chemicals with the natural and economical compounds (Kelm *et al*., 1997; Gahukar, 2010).

In terms of integrative pest control and environmental protection, secondary plant metabolites possessing insecticidal, repellent and/or antifeedant properties are very desirable as a possible means of plant protection (Kosti, *et al*., 2013). On the basis of the observation made in the inhibition of SDH and LDH activities and stimulation of MDH and GDH activities in all the target tissues of the reproductive system of *Sphaerodema rusticum* studied the metabolic pathway has shifted towards anaerobic side rather than aerobic side to meet the increase in energy demand when exposed to sub-lethal concentration of nimbecidine treatment.

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