www.ijrps.pharmascope.org

ISSN: 0975-7538
Research Article

Sub - lethal effect of phytopesticide nimbecidine on biochemical changes in the adult male insect *Sphaerodema rusticum* (Heteroptera: Belostomatidae)

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ABSTRACT

Carbohydrate, protein and lipid are the major components of the body playing important role in the body construction and energy metabolism. Effect of sub -lethal concentration (0.00028 ppm 1/10th of LC_{50}) of the phytopesticide nimbecidine was studied on *Sphaerodema rusticum* for 7, 14 and 21 days of exposure. A significant decline in the contents of glycogen, protein and lipid and an increase of glucose and amino acid contents of fat body, testis and seminal vesicle were observed. It was observed that the biochemical changes in the nimbecidine treated (7, 14 and 21days) fat body, testis and seminal vesicle were significantly differed than control.

Keywords: *Sphaerodema rusticum;* nimbecidine; fat body; testis; seminal vesicle; biochemical changes.

INTRODUCTION

More informations about the environmental pollution on aquatic animals have been obtained from mortality studies. Very little information is available about the damages to different organs or about the disturbed physiological and biochemical processes in an organism. A better understanding of biochemical changes will focus the stress responses in animals exposed to toxicant (Nammalvar, 1984), the understanding of these mechanisms is necessary if we want to predict the potential harmfulness of various chemicals to the environment (Akelarsen *et al*., 1976). Physiological responses like changes in behaviour, biochemical contents, feeding, survival, growth and reproduction to sub-lethal concentration of pesticides, effluents and other chemicals are used as indices of pollution by various workers (Carlson, 1972; Grant and Mehrle 1973 and Jabakumar and Jayaraman, 1988). The indiscriminate use of synthetic pesticides has caused environmental contamination and toxicity to living organisms. This has necessitated the use of ecofriendly products. Ecofriendly biopesticide agents are available abundantly in nature (Swaminathan, 1992). Neem is the most promising potential source of biopesticide of botanical origin (Schmutterer, 1995)

Neem products have also been reported to suppress reproduction (Ludhum and Sieber, 1988). Azadirachtin has antifeedent, anti-ovipositional, growth disrupting and fecundity reducing properties on different insects (Schmutterer, 1990). The environmental hazards posed by synthetic chemical insecticides have necessitated the search for some alternative source of natural origin for applying ecologically viable pest control strategies (Kulkarni and Joshi, 1997). The natural products of phytopesticides can be used for the purpose of controlling insect pests and also have a number of advantages over the conventional chemical insecticides. Carbohydrate, protein and lipid which are the major components of the body play an important role in the body construction and energy metabolism. These constituents are affected by many factors especially by pesticide (Jabakumar and Jayaraman, 1988). Investigations on the effects of pesticides have revealed their interference with carbohydrate metabolism in different species (Ajai Mansingh, 1972 and Babu *et al*., 1988). In most insects carbohydrates reserves are present as glycogen and trehalose which can be readily converted into glucose (Islam and Ray, 1981).

Proteins are the known biological compounds which regulate and integrate several physiological and metabolic processes in the body through hormones, enzymes and nucleoproteins. The protein plays a major role in the synthesis of microsomal detoxifying enzymes and helps to detoxify the toxicants when entering into the animals (Wilkinson, 1976). Wigglesworth (1979) has stated that 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 (Wigglesworth, 1979).

Lipids are the chief form in which energy is stored in insects. The ability to synthesize lipids for storage is widespread, but except for specific item as small amounts, they are not usually essential constituents of

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Received on: 14-09-2010
Revised on: 10-10-2010
Accepted on: 27-11-2010

the diet. Insects utilize lipids and can also synthesize from protein and carbohydrates. Insect growth hormones, pheromones and sex attractants are lipoidal in nature (Gilbert, 1967) and they are also important constituents of cell membrane (Robbins *et al*., 1971). It has been established that lipids provide the energy reserve which can be used during starvation periods or in some insects such as *Schistocerca gregaria* for sustained flight activity (Chapman, 1982). In this context sub-lethal effect of phytopesticide nimbecidine on the biochemical constituents of the fat body, testis and seminal vesicle of aquatic insect *Sphaerodema rusticum* has been studied to assess the pesticide pollution.

MATERIALS AND METHODS

Insects

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. Adult male insects were collected from rearing troughs and vivisected in insect Ringer solution (Ephrussi and Beadle, 1936). 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 following biochemical estimations were made in the tissues of fat body, testis and seminal vesicle. The colorimetric method of Kemp and Kits Van Heijninger (1954) was employed for the quantitative estimation of glucose and glycogen. The colorimetric method of Lowery *et al* (1951) was employed for the quantitative estimation of protein. The colorimetric method of Moore and Stein (1954) was adopted for the quantitative estimation of total free amino acids and lipid content was estimated by the semi micro determination method of Pande *et al*., (1963). Data were analysed using Analysis of Variance (ANOVA), and means separated by Duncan's Multiple Range Tests (DMRT).

RESULT

In the present study, the significant changes in the content of carbohydrate, protein and lipid of fat body, testis and seminal vesicle have been observed in the insects treated with sub-lethal concentration 0.00028 ppm (7, 14 and 21 days) of phytopesticide, nimbecidine. The glycogen content of fat body, testis and seminal vesicle of control insects were 8.3 ± 0.5, 7.0 ± 0.4 and 7.3 ± 0.3 µg/mg respectively. Likewise after 7, 14 and 21 days of exposure of insect to nimbecidine, the amount of glycogen content in the fat body, testis and seminal vesicle were about 6.5 ± 0.5, 5.6 ± 0.6, 5.9 ± 0.5; 4.3 ± 0.4, 3.9 ± 0.7, 3.6 ± 0.5 and 2.3 ± 0.3, 2.0 ± 0.6, 2.2 ± 0.4 µg/mg, respectively. Glycogen content appears to be gradually decreased in the treated insects than compared to control insects (Table 1). The values of mean glycogen content of the fat body, testis and seminal vesicle of treated insects were significantly differed than the control.

The glucose content of fat body, testis and seminal vesicle of control insects were 1.1 ± 0.2, 0.7 ± 0.2 and 0.5 ± 0.2 µg/mg respectively. Likewise after 7, 14 and 21 days of exposure of insect to nimbecidine, the amount of glucose content in the fat body, testis and seminal vesicle were about 1.9 ± 0.4, 0.9 ± 0.2, 0.7 ± 0.1; 2.1 ± 0.3, 1.0 ± 0.3, 1.0 ± 0.2 and 3.0 ± 0.3, 1.7 ± 0.3, 1.7 ± 0.4 µg/mg, respectively. Glucose content appears to be gradually increased in the treated insects than compared to control insects (Table 1). The values of mean glucose content of the fat body, testis and seminal vesicle of treated insects were significantly differed than the control. In the present study, it has been observed that the quantity of glycogen content gradually decreased after 7, 14 and 21 days of nimbecidine exposed fat body, testis and seminal vesicle and the quantity of glucose were increased than the control insects. It indicates possible break down of glycogen into glucose in order to meet the requisite energy demand during the sub-lethal concentration of nimbecidine intoxication.

The protein content of fat body, testis and seminal vesicle of control insects were 42.0 ± 2.3, 26.1 ± 3.2 and 31.7 ± 3.3 µg/mg respectively. Likewise after 7, 14 and 21 days of exposure of insect to nimbecidine, the amount of protein content in the fat body, testis and seminal vesicle were about 35.3 ± 3.1, 21.0 ± 1.3, 29.1 ± 1.8; 25.8 ± 3.6, 19.6 ± 3.5, 22.4 ± 2.7 and 18.7 ± 2.1, 14.3 ± 2.8, 15.6 ± 2.4 µg/mg, respectively. Protein content appears to be gradually decreased in the treated insects when compared to control insects (Table 1). The values of mean protein content of the fat body, testis and seminal vesicle of treated insects were significantly differed than the control. The quantity of protein in all the tissues gradually decreased after 7, 14 and 21 days of the sub-lethal concentration of nimbecidine exposed insects which may be due to intensive proteolysis to meet the extra energy demand when the insects are subjected to nimbecidine stress.

The amino acid content of fat body, testis and seminal vesicle of control insects were 49.4 ± 6.1, 39.8 ± 3.8 and 44.2 ± 4.9 µg/mg respectively. Likewise after 7, 14 and 21 days of exposure of insect to nimbecidine, the amount of amino acid content in the fat body, testis and seminal vesicle were about 61.6 ± 4.9, 41.6 ± 4.8, 50.2 ± 4.9; 64.8 ± 6.5, 49.0 ± 3.8, 57.6 ± 6.8, and 70.6 ± 3.0, 57.8 ± 6.1, 64.8 ± 5.5 µg/mg, respectively. Amino acid content appears to be gradually increased in the treated insects than the control (Table 1). The values of mean amino acid content of the fat body, testis and seminal vesicle of treated insects were significantly differed than the control. It is believed that the high level of amino acids formed by proteolysis in the tissues are transported into the haemolymph and then to

Table 1: Changes in the level of total carbohydrate, protein and lipid content in the tissues of *Sphaerodema rusticum* (µg/mg wet. wt) exposed to sub-lethal concentration (0.00028 ppm) of nimbecidine

Parameters	Tissue µg/mg	Control µg/mg	Sub-lethal exposure duration (Days) µg/mg		
			7	14	21
Glycogen	Fat body	8.3 ± 0.5	6.5 ± 0.5 (-21.69)	4.3 ± 0.4 (-48.19)	2.3 ± 0.3 (-72.29)
	Testis	7.0 ± 0.4	5.6 ± 0.6 (-20.00)	3.9 ± 0.7 (-44.29)	2.0 ± 0.6 (-71.42)
	Seminal vesicle	7.3 ± 0.3	5.9 ± 0.5 (-19.17)	3.6 ± 0.5 (-50.69)	2.2 ± 0.4 (-69.87)
Glucose	Fat body	1.1 ± 0.2	1.9 ± 0.4(72.72)	2.1 ± 0.3 (90.90)	3.0 ± 0.3 (172.72)
	Testis	0.7± 0.2	0.9 ± 0.2 (28.58)	1.0 ± 0.3 (42.86)	1.7 ± 0.3 (142.86)
	Seminal vesicle	0.5 ± 0.2	0.7 ± 0.1 (40.00)	1.0 ± 0.2 (100.00)	1.7 ± 0.4 (240.00)
Protein	Fat body	42.0 ± 2.3	35.3 ± 3.1 (-15.96)	25.8 ± 3.6 (-38.58)	18.7 ± 2.1 (-55.48)
	Testis	26.1 ± 3.2	21.0 ± 1.3 (-19.54)	19.6 ± 3.5 (-24.90)	14.3 ± 2.8 (-45.21)
	Seminal vesicle	31.7 ± 3.3	29.1 ± 1.8 (-8.20)	22.4 ± 2.7 (-29.33)	15.6 ± 2.4 (-50.79)
Amino acid	Fat body	49.4 ± 6.1	61.6 ± 4.9 (24.70)	64.8 ± 6.5 (31.18)	70.6 ± 3.0 (42.91)
	Testis	39.8 ± 3.8	41.6 ± 4.8 (4.52)	49.0 ± 3.8 (23.11)	57.8 ± 6.1 (45.22)
	Seminal vesicle	44.2 ± 4.9	50.2 ± 4.9 (13.58)	57.6 ± 6.8 (30.31)	64.8 ± 5.5 (46.60)
Lipid	Fat body	14.5 ± 2.7	12.3 ± 2.6 (-15.17)	11.0 ± 3.2 (-24.13)	8.7 ± 2.6 (-40.00)
	Testis	12.2 ± 2.3	11.1 ± 1.8 (-9.01)	11.0 ± 3.2 (-22.96)	7.6 ± 1.1 (-37.70)
	Seminal vesicle	8.9 ± 0.7	8.2 ± 0.9 (-7.87)	6.6 ± 0.7 (-25.84)	4.6 ± 0.7 (-48.31)

Values in parenthesis indicate percentage change over control. Values are significantly different at $p < 0.05$

the metabolic pathway by the pyruvate, which directly enter into the TCA cycle in the form of keto-acids to provide extra energy during the stress period.

The lipid content of fat body, testis and seminal vesicle of control insects were 14.5 ± 2.7, 12.2 ± 2.3 and 8.9 ± 0.7 µg/mg respectively. Likewise after 7, 14 and 21 days of exposure of insect to nimbecidine, the amount of lipid content in the fat body, testis and seminal vesicle were about 12.3 ± 2.6, 11.1 ± 1.8, 8.2 ± 0.9; 11.0 ± 3.2, 9.4 ± 1.6, 6.6 ± 0.7, and 8.7 ± 2.6, 7.6 ± 1.1, 4.6 ± 0.7 µg/mg, respectively. Lipid content appears to be gradually decreased in the treated insects when compared to control insects (Table 1). The values of mean lipid content of the fat body, testis and seminal vesicle of treated insects were significantly differed than the control. In the present study, it has been observed that lipid contents in the fat body, testes and seminal vesicle were gradually decreased in the sub-lethal concentration of nimbecidine exposed insects than the control. This finding reveals that lipid content may be utilized for the energy production during nimbecidine stress.

DISCUSSION

Fat body in insects apart from serving as the seat of metabolism also serves in the synthesis of protein, lipid and carbohydrate that serves as precursors for metabolism in other tissues (Keeley, 1985). Kilby, (1963) has reported that carbohydrate, lipids and amino acids are oxidized and utilized for the energy supply. Glycogen is an important nutrient reserve in animal tissue and it is used as an immediate energy source when required by any animals. Therefore, glycogen is an essential component of the normal metabolism (Thunberg and Manchester, 1972). Chattoraj and Sharma (1988) have reported that the pesticide R-20458 when administered to the larvae of *Spodoptera littura*, the carbohydrate was decreased. Dimethoate application also caused decrease in the glycogen content in *Odontopus varicornis* (Jayakumar, 1988). Prakash *et al*. (1990) have reported that the quantity of carbohydrate in the ovary and fat body was decreased in the endosulfan treated *Poecilocerus pictus*. However, the increased glycogen content has been observed in the fat body as well as haemolymph of *Laccotrephes ruber* when exposed to monocrotophos (Ravichandran, 1996). Grant and Schoettger, (1972) have shown that the carbohydrate metabolism is impaired due to various pollutants. The changes appeared may be due to transportation of glucose from the storage organ namely the fat body, which is one of the most fundamental requirements needed for various metabolic activities (Boell, 1965).

Proteins are the most important organic constituents of animal tissues and play an important role in energy production. Normally, tissue proteins in aquatic animals under toxic stress are known to play a role in the activation of compensatory mechanism (Wigglesworth, 1972 and Downer, 1982). The decrease in protein level in liver and muscle might be due to diversion of energy, when animals were under toxic condition (Baskaran, 1980; Manoharan and Subbiah, 1982). Cadmium chloride has been shown to decrease the protein content on the fat body of *Chrysocoris stolli* (Islam and Roy, 1983). Another possible reason is that the protein synthesis is highly inhibited as a result of non-availability of energy for protein synthesis (Saxena *et al*., 1989 and Singh *et al*., 1993). Shanmugavelu (1963) has reported that a decline in protein content in the fat body of *Mylabris pustulata* when treated with heavy metal cadmium. Jayakumar (1988) has reported that the protein content was decreased in the tissues of testes, accessory reproductive glands and fat body of *Odontopus*

varicornis when treated with dimethoate. Pazhanichamy (1997) has reported a sudden depletion of protein content and increased amino acid content in *Laccotrephes ruber* when exposed to heavy metal mercury. Vijayaprabha (1990) has also reported that the protein content was decreased and amino acid content was increased in the brain, fat body and haemolymph of *Catacanthus incarnatus* when exposed to the sublethal concentration of dimethoate.

Lipids play an important role in maintaining integrity of cell structure and functions. Normally all animals depend upon the lipid content to overcome the physiological stress caused by toxicants or environmental contamination (Prakash *et al*., 1990 and Jamil and Hussian 1992). Sastry and Siddiqui (1984) reported some biochemical and histohemical changes in the fresh water teleost *Channa punctatus* exposed to sublethal concentrations of quinolphos. Fluctuation of lipid content in different species of insects treated with different toxicants has been reported by several investigators. Copuzzo and Lancaster (1981) have shown a significant decrease of lipid content in the fat body of *Homarus americanus* when exposed to toxicants. The same trend has been observed in carbohydrate, protein and lipids by *Aspongopus janus* when treated with nimbeciline (Thiruvasagam, 1994), *Periplaneta americana* when treated with *Pongamia glabra* leaf extract (Ramanathan, 1995) and *Laccotrephes ruber* when treated with monocrotophos (Ravichandran, 1996), *Gryllotalpa africana* when treated with endosulfan (Sumathi, 2001), *Laccotrephes ruber* when treated with zinc (Ramesh Kumar, 2004), *Sphaerodema rusticum* when treated with mercury (Rajathi, 2004).

ACKNOWLEDGEMENT

The authors are very grateful to the authorities of Annamalai University and to the Head, Department of Zoology for providing all necessary facilities to complete this work.

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