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Variations in LC_{50} of some pesticides due to physico-chemical parameters and impact of sevin on neurosecretory cells of a fresh water snail, *Thiara lineate*

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ABSTRACT:

Aquatic contamination by pesticides is arising as a result of their extensive use in agriculture and public health programmes. However, indiscriminate use of these pesticides for crop protection causes much damage to the aquatic fauna. The snail an aquatic fauna is economically important which serves as an intermediate host of various trematod parasites, which causes severe diseases to human being as well as domestic animals. The gastropod mollusc, *Thiara lineata* is a filter feeder at the secondary level of fresh water ecosystem. Hence felt necessary to study alteration in the LC_{50} and neurosecretion. The acclimatized, healthy, active and approximately same weight and sized mature adult and immature animals, groups of ten snails each were exposed (in Plastic troughs each containing 1 liter of water) to Organochlorine (Endosulfan), Organophosphate (Nuvacron), synthetic pyrethroids (Cymbush) and Carbamate (Sevin). The LC_{50} values for 24, 48, 72 and 96 hours were calculated. The physico-chemical parameters of water used for holding the animal had temperature between 26-28 ^{0}C , pH 7.1 to 7.3, total hardness 140 to 150 ppm and oxygen content of 5.6 to 7 ml/lit. The physico-chemical factors affecting the LC_{50} like temperature, pH, Salinity and Photoperiodism light and dark hours were studied. During the stress conditions neurosecretions are adversely affected . The snail are exposed to pre determined sub-lethal concentration of sevin(0.913 ppm) for 1,7 and 14 days, simultaneously controls were run. After exposure, It was found that the number of the neurosecretory cells 'A' and 'B' has suddenly discharged neurosecretory material. Neurosecretory material was found to be remaining accumulated in control snails.

Keywords:
Thiara lineata, Sevin, Neurosecretion.

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INTRODUCTION

Many snails serve as an intermediate hosts for a number of parasites causing several diseases to man and live stocks while some snails and slugs act as crop pests affecting agricultural, horticultural, forest and garden plants. The snail, *Thiara lineata* acts as an intermediate host for Schistosome parasites. If the snails and slugs are to be directly controlled, it is essential to understand the ecology and chemical control of slug and snails. Magare and Kulkarni, (1995) studied the Seasonal LC_{50} variations in Paratox exposed freshwater snail, *Indoplanorbis exustus*. Many workers have worked on snails like *L. alte* (Bodhankar, 1984), *Indoplanorbis exustus*(Patil *et.al,*1991; Patil and Mahale, 2010) *Zootecus insularis*(Chaudhari and Kulkarni, 1993), *Thiara lineata* (Patil *et. al.,* 1993; and Chaudhari *et. al.,*1999) studied LC_{50} on the molluscs.

In gastropods, the nervous system is constructed on the same general plan as in the other molluscs undergo. Most of the information is available on the nervous system of gastropods that to mostly of Pulmonates. The nervous system in Prosobaranchs have been studied by a few workers (Baker, 1925, Crofts 1929, Mortan 1956, and Van der Schalie and Dundee, 1956) The most primitive type of nervous system in Prosobranchs is of Haliotis, which has been described by Crofts(1929) Though some extensive studies have been made on the central nervous system of gastropods the endocrinological aspects are rather different in the three subclasses and above all the knowledge we have of them is very scanty .

The essential data concerning the neurosecretion of the Prosobranchs are found in a small number of publications. Many workers have worked on the nervous system of these gastropod viz. *Laevicaulis alte* (Nagabhushanam and Kulkarni, 1971), *Melania scabra* (Muley, 1974; Nagabhushanam and Muley, 1971), *Viviparous bengaiensis* (Utkar, 1982), *I. exustus* (Shinde, 1991) *Cerastus mossonianus* (Magare 1991; Magare and Kulkarni, 1995; Utkar and Kulkarni 2000).

The present study was undertaken with the aim to extend our knowledge of neurosecretion in prosobanchs. The principle objective were to investigate

(1) The presence and distribution of neurosecretory cells in the central nervous system of *Thiara lineata*,

(2) Neuroendocrine regulation of various physiological activities and

(3) Effect of sevin on the neuroendocrine cells of brain

MATERIALS AND METHODS

The fresh water snail, *Thiara lineata* were collected from 'Bori' river near Tamaswadi, Tal-Parola, Dist-Jalgaon, Maharashtra State, India. They were brought to the laboratory, cleaned to remove the fouling algal biomass and mud. and were placed in well aerated fiber troughs containing sufficient water for five days for acclimatization. They were not starved at the time of experimentation. The healthy, active, approximately same sized and mature adult were exposed to pesticide to calculate LC_{50} values for different pesticides viz. Organochlorine (Endosulfan), Organophosphate (Nuvacran), Synthetic Pyrethroid (Cymbush) and Carbamate (Sevin). Values are estimated by the method of Probit Analysis (Finney, 1971). For age related mortality experiments, the snails having length less than 20.00mm were considered to be immature and the snails having length 22.00mm to 25.00mm or more were considered to be mature. Experiments were carried out separately for immature and mature snails.

The adult Snails were exposed to pre-determined sub-lethal concentration of Sevin for 1, 7 and 14 days, simultaneously controls were run, to study the neurosecretion. After exposure period, the central nervous system was quickly and carefully dissected out from the snail and was fixed in aqueous Boins fluid for 24 hours. It was then dehydrated in alcohol by the different grades cleared in xylol and embedded in paraffin wax at 58 -60 ^{0}C (Standard micro-technique) serial sections of 5-6 u thickness were cut and subjected to "Mellory's Triple Staining" (Mallory, 1944) techniques. The physico-chemical parameters of the water were studied by the method of APHA (1981). The observations were made under low and high power microscopic levels.

OBSERVATIONS AND RESULTS

The physico-chemical characters of water used for holding the gastropods had temperature between 26-28^{0}C, pH 7.1 to 7.3, total hardness 140 to 150 ppm and oxygen content of 5.6 to 7 ml/lit.

The LC_{50} values of immature as well as mature snails were calculated at 24, 48, 72 and 96 hours of exposures, to pesticides endosulfan, nuvacran, cymbush and sevin. Summarized in

Table 1. As far as age related mortality is concerned immature snails were more resistant to pesticides as compared to mature snails. While assessing the LC_{50} of pesticides for 24 hours affected by the temperature, pH, salinity and photoperiod on snails, were recorded in the **Table 2.**

Assessing the effect of temperature on the snails exposed to pesticides endosulfan, nuvacran, cymbush and sevin for 24 hours it was seen that an increase in temperature resulted in increasing the mortality rate in all above four pesticides. Similarly increase in pH and salinity, increased mortality of snails.

On evaluating the effects of photoperiod on snails exposed to endosulfan, nuvacran, cymbush and sevin it was revealed that 8L : 16D caused maximum mortality followed by 12L : 12D. Thus increasing dark period caused increased mortality. However, 0L : 24D did not show mortality rate higher than 8L : 16D. This may be because snails remained active for only a limited period according to their circadian rhythm and even when darkness was prevailing for 24 hours, they did not change their rhythm beyond a certain limit.

In order to investigate different type of cells present within the central nervous system of snail, *Thiara lineata* the ganglionic ring was sectioned and stained with Mallory's triple staining technique. The neurosecretory cells are quite distinct from the normal neural cells. There neurosecretory cells bigger with conspicuous nuclei and large amount of cytoplasm. The perikarya and axonic A cells are loaded with fine particles, stained distinctly. These staining peculiarities are characteristic features of neurosecretory cells (Scharrer and Scharrer, 1954) and thus differentiate them from the normal neural cells.

On the basis of their morphological features like shape, size, vacuolization, stainability etc., the neurosecretory cells of *T. lineata* could be classified into two main types (1) "A" cells or Axonic cells and (2) "B" cells or non-axonic cells.

These cells were observed in cerebral, buccal, pleural, parietal, and visceral ganglia and more generally arranged in periphery and at the centre a lumen, neuropile. The extreme periphery and ganglia is supposed to be the neurohaemal region as being observed to be vacuolated. The number, size and shape of these also may vary from ganglion. The characteristics of these cells are given in the **Table 3.**

Cell type "A":

These cells are pyriform in shape and have long axons. The length of the cell body is ranging from 9 to 16 µ in length. The nucleus is oval measuring about 3 to 6 µ in diameter and has one large nucleolus. The nucleus may be either central of eccentric in position. The size and the number are comparatively smaller than B cells. Generally they lie towards the peripheral region of the ganglia. The nucleus generally bears single nucleolus. The cytoplasmic portion of the cell consists of a colloidal neurosecretory material stained deep blue with Mallory triple staining and contains few vacuolar spaces. The neurosecretory materials are elaborated by these cells.

Table: 1 : Age related mortality of *T.* lineata exposed to pesticides:

Name of the Pesticide	Duration of Exposure in hours	LC_{50} of Immature Snails in ppm	LC_{50} ofMature Snails in ppm
Endosulphan (Organochlorine)	24	0.028	0.01182
	48	0.016	0.00360
	72	0.003	0.00134
	96	0.002	0.000142
Nuvacran (Organophosphate)	24	0.038	0.02880
	48	0.027	0.01480
	72	0.016	0.01112
	96	0.013	0.008834
Cymbush (Synthetic Pyrethroid)	24	0.047	0.028113
	48	0.032	0.02244
	72	0.028	0.02078
	96	0.023	0.019615
Sevin (Carbamate)	24	41.37	30.6835
	48	21.47	8.4394
	72	15.47	6.1688
	96	09.02	4.5638

Table : 2 :Effect of Physico-Chemical Parameters on the Percent morality of *T. lineata* exposed to LC_{50} dose of pesticides for 24 hours.

Physico-Chemical Parameters		Pesiticides used			
		Endosulphan	Nuvacron	Sevin	cymbush
Temperature ^0C	25	50	50	50	50
	30	67.50	58	65.00	67.50
	35	87.50	60.50	75.00	77.50
pH	7.5	50.00	50.00	50.00	50.00
	8	57.00	62.00	72.00	72.00
	8.5	74.00	72.00	80.00	81.00
Salinity	.03%	50.00	50.00	50.00	50.00
	.04%	65.50	67.50	71.50	66.00
	.05%	80.50	81.50	78.00	74.00
Photoperiod Light/dark hours	0L-24D	55.00	53.00	55.00	56.00
	8L-16D	65.00	69.00	65.00	71.00
	12L-12D	60.00	68.00	60.00	65.00
	16L-8D	51.50	51.50	52.00	50.00
	24L-0D	37.50	39.00	35.00	38.00

Cell type "B":

These cells are oval or round in shape measuring about 4 to 7.5 µ in diameter. The nuclei are round or oval measuring 1.5 to 3µ in diameter and may be either at the centre or eccentric in position. These cells are characterized by the intensive staining of the cytoplasmic granules. The granules are observed in clumps and stain faint red with Mallory's triple stain. These cells are more common as compared to cell type A and are scattered in all the regions of the ganglia. Table 3 represents some distinct properties of all type A and B cells.

DISCUSSION

The evaluation of age related mortality revealed that the immature snails were more resistant to the action of pesticides used viz. endosulphan, nuvacran, cymbush and sevin as compared to mature snails. This fact is mainly due to the absorptive sole surface (Godan, 1983), Similarly seasonal LC_{50} variations in Paratox exposed freshwater snail, *Indoplanorbis exustus* (Magare, and Kulkarni, 1995). The present investigation reports that during LC_{50} exposure several species of aquatic snails have been used as bioassay for a variety of toxic substances and a broad range of sensitivity has been observed. Allison (1978) expressed in "Current trends in toxicological research" that progress in toxicology has required the combined activity of chemists who have purified and characterized toxic substance and electro physiologists, molecular biologists, and other who have defined their precise mode of action.

The factors affecting the toxicity of a particular pesticide are the animal weight (Pickering *et al*., 1962) its developmental stage

Table : 3: Morphological Characteristic of neurosecretory cell type A and B found in the cerebral ganglion of T. lineata

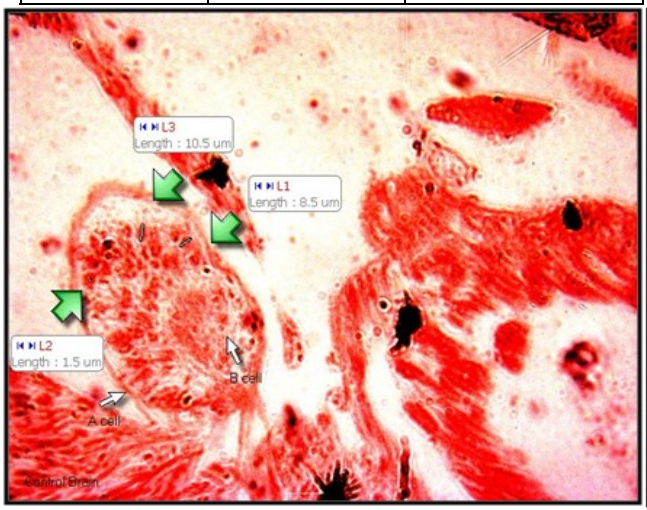
Characteristics/ Descriptions	Cell type "A"	Cell type "B"
Distribution	Mostly along the peripheral region, less in number	Scattered in all the regions of the ganglion abundant in number
Shape of the cell body	Pyriform	Oval
Largest diameter of the cell body	16 u m	7.5 u m
Nucleus	Lobed or rounded	Rounded
Nature of secretary material	Colloidal	Granular in clusters
Vacuoles	Absent	Absent
Staining reaction of cytoplasm	Dark blue and red with Mallory's triple stains.	Blue black and faint red with Mallory's triple stains.

(Kamal deep and Toor, 1977), times of exposure and temperature (Macek *et al*., 1969), pH and hardness of water (Handerson *et al*., 1960) Effect of salinity (Nagabhushanam and Muley, 1976).

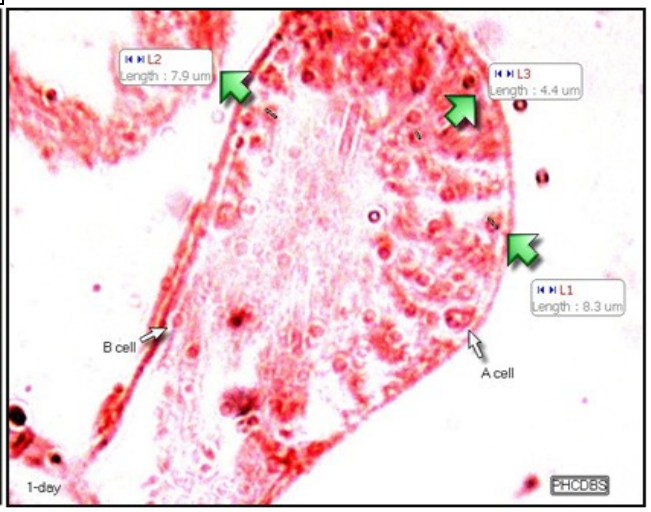
It is a well known fact that the neurosecretory cells control the physiological processes like reproduction and exposure of animals to pesticides interfere with the normal functional process and ultimately create an imbalance in the normal system. Utkar (1982) has found the toxic effect of copper sulphate on the neurosecretion and has reported a decrease in all number cell and all nuclear area, depletion of neurosecretory cells of the freshwater snail, *Viviparous bengalensis*. He also reported that effect of biogenic amines (reserpine and esperpine) on neurosecretory cells and observed that the size of both neurosecretory cells (A and B) was reduced. Bodhankar (1984) observed that there was an decrease in neurosecretory material in both A and B types of cells number of cell types and nuclear areas of these cells of the slug, *Laevicaulis alte* on exposure to five different molluscicides, viz. Malathion, hygro, Tiodon, Sevimol, and Copper

Table : 4 : Variations in the number of stainable neurosecretory cells present in cerebral ganglion of *T. lineata* on exposure to Sevin.

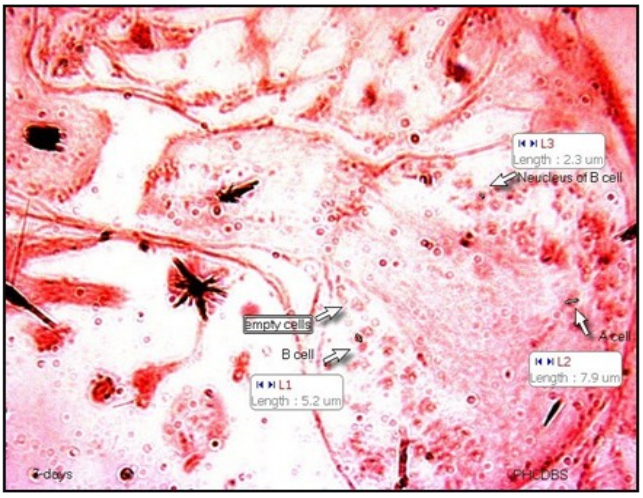
Exposure period	Cells loaded with neurosecretory material (NSM)	
	Cell type "A"	Cell type "B"
Control	22 ±2	56 ±2
1 Day	21 ±1	49 ± 3
7 Days	15 ± 2	38 ± 4
14 Days	12 ± 4	34 ± 8



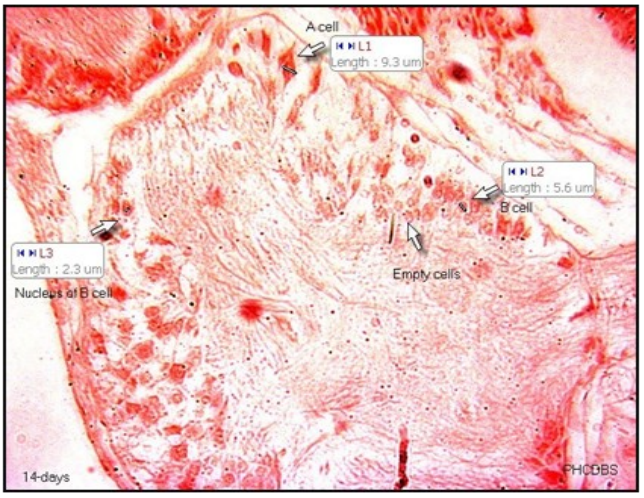
A) Control



B) 1 Day



C) 7 Days



D) 14 Days

Fig: A, B, C, D: T. S. of Brain of *Thiara lineata* after exposure of Sevin on Neurosecretory cells.

sulphate. Bhatlawande (1989) has reported similar phenomenon in the snail, *Cerastus moussonianus*. In the present investigation on exposure of Sevin it was observed that there was an acute cellular degeneration, vacuolization and pronounced decrease in the cell number in cerebral ganglion of *Thiara lineata*. These results are in agreement with the result of Reddy(1982), Utkar(1982), Bodhankar (1984), Bhatlawande(1989).

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