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Impact of dimethoate on brain of the earthworm *Eudichogaster kinneari* (Stephenson): A histological profile

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Abstract

Adult *Eudichogaster kinneari* were exposed to a safe concentration (0.6 ppm) of Dimethoate for twenty days to evaluate the effects on histoarchitecture of cerebral and sub pharyngeal ganglion of brain. Brain was severely affected with exposure of above insecticide, causing degeneration of neurosecretory cells (NSCs). Initially neurosecretory cells exhibited accelerated neurosecretory activity while repeated treatment led exhaustion of neurosecretory material (NSM). The neurosecretory cells lost their normal shape along with many morphological alterations, vacuolization were seen in cytoplasm, nucleoplasm, neurosecretory material and in neuropile, ultimately caused imbalance and lethal effect. Size enhancement in cell area, nuclear diameter, cell length and axon length of neurosecretory cells (p>0.001) were observed significantly.

Key Words*: *Brain, Dimethoate, histomorphology, neurosecretory cells, neurosecretory material, supra pharyngeal ganglion, Sub pharyngeal ganglion

Introduction

Earthworms have been called ecosystem engineers, because they change the structure of their environments. They are important regulators of soil structure and dynamics of soil organic matter. They are a major component of soil fauna communities and comprise a large proportion of macro fauna biomass (Edward and Bohlen, 1996). Earthworms enhance soil nutrient cycling through decomposition of detritus into mineral soil, in their gut, detritus mix with gut mucus and water, which enhances the activity of other beneficial soil micro-organisms (Somniyam and Suwanwaree, 2009). Besides these, earthworms are key components in natural food chains providing food source for many small mammals, birds and fishes and play a key role in the bio magnification processors of several soil pollutants in this way they are important bio indicators of chemical toxicity in the soil ecosystem (Cikutovic *et.al*, 2010; Lionetto *et.al*, 2012; Celine *et.al*, 2104). Fertilizers and insecticides are used for the betterment of agricultural yield. They ultimately persist in soil and decrease soil fertility, causes disturbance in balance between flora and fauna residing in the soil. In this way agro chemicals not only affect the insect but equally damage the soil fauna. The morphology of brain of annelids has been well studied (Herlant-Meewis, 1959; Satija and Garg, 1977; Nagbhushnam and Hanumante, 1977; Kulkarni and Nagbhushanam, 1978; Kodarkar, 1979; Lakhani, 1991). The effect of pesticides and chemicals on the brain of some annelids have been investigated (Nagbhushanam and Hanumante, 1977; Gupta and Verma, 1979; Bhaskar Rao, 1983; Prasad and Pavan Kumar, 1983; Anand, 1984; Kulkarni *et al.*, 1989; Kulkarni, 1989; Sagar, 1989; Lakhani, 1992, 2015) Inspite of this, there is lack of information on the effect of insecticide Dimethoate on histomorphology of neurosecretory cells of brain of earthworm *Eudichogaster kinneari*. Therefore the present work aims to show clearly the changes produced after exposure of safe concentration (0.6 ppm) of Dimethoate for twenty days in brain of *E.kinneari* to evaluate histomorphological abnormalities in their neurosecretory cells of brain.

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Material and Methods

Healthy and sexually matured specimens of *Eudichogaster kinneari* approximately of same

weight [6.5 + 0.001 gm.], length [80-120mm] and diameter [5-7 mm] were collected from the vicinity of Ujjain city, India and acclimated in the laboratory in culture pots with moistened soil, before the commencement of the experiment. 40 earthworms were kept in each pot which was filled with 9000 gm. soil. The earthworms were fed with organic matter, such as decaying leaves, compost manure etc. The market sample of Dimethoate (Rogor 30E Rallis India Ltd) was used for experimental purposes, LC-50 value of these worms, was also determined. The calculated quantity of Dimethoate was taken, and diluted to 500 ml with tap water for preparation of the 0.6 ppm test concentration. The diluted fluid of test solution was sprayed on soil on the first day of experiment and after 10 days. The insecticide was properly mixed with the soil after each spray. The worms were removed before each spray in order to avoid their direct exposure to the spray and afterwards kept in the soil for the next ten days. The control worms were kept in the soil without addition of insecticide. Both control and experimental animals were kept in identical conditions and the experiment was continued for 20 days and the organs were fixed in fixative after 10 and 20 days. Before making the histological preparations, the worms were narcotized and the organs were immersed in saline solution (0.75%) for a few minutes to avoid contractions. The supra and sub pharyngeal ganglion were fixed in aqueous Bouin's fluid.

The fixed ganglions were processed for dehydration and blocks were prepared in paraffin wax, sections were cut at 4-5 µm and stained with Mallory's triple stain ; chrome alum haematoxylin phloxine stain ; Gomori's aldehyde Fuchsine stain for histological details. Statistical analysis of data was carried out by student's 't' test.

Results and Discussion

Control Group:

The brain of *Eudichogaster kinneari* contains one pair of cerebral ganglion, one pair of sub pharyngeal ganglion and circum pharyngeal connectives.

Cerebral Ganglia [Supra Pharyngeal Ganglia]The fused pair of cerebral ganglia lies dorsally between II and III segment. The brain is enclosed in a connective tissue neurolemma and contains a central neuropile surrounded by a mantle of cell. Neurons of various sizes are in association of cells, these are 'A' type, 'B' type and 'C' type of neurosecretory cells.

Sub pharyngeal Ganglia

It is formed by the union of two branches of the circumpharyngeal connectives and swells to form sub pharyngeal ganglion. It lies in the IVth segment at ventral junction of the circumpharyngeal connective. The structure of secretory cells of this ganglion is similar to that of cerebral ganglion.

Depending upon their histological feature (shape, size and stainability etc.), the neurosecretory cells can be classified into three groups, 'A'-cell, 'B'-cell and 'C' cell.

"A" Neurosecretory Cell

These cells are situated beneath the ganglionic capsule. In the Suprapharyngeal ganglion, there appear to be an immense congregation of these cells on the dorsal side along with the anterio-posterior axis and rarely on ventral side. ('Fig'.1).

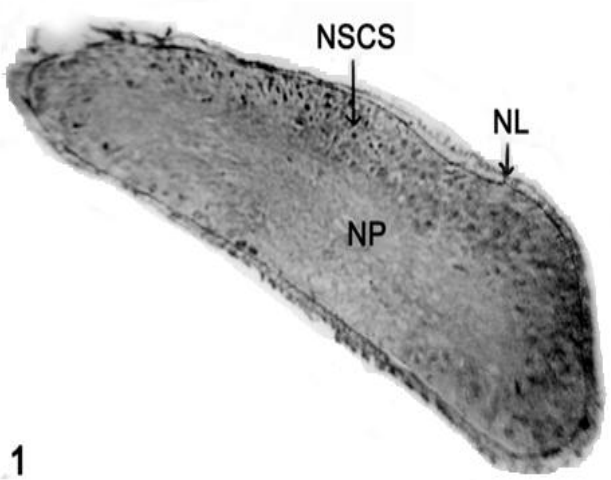


Fig.1. S.S. of Supra Pharyngeal Ganglion showing usual Pattern of NSCs

(Abbreviations: NSCs-Neurosecretory Cells, NP-Neuropile, NL-Neurolemma)

In sub pharyngeal ganglion, these cells occupy chiefly on dorsal and dorsolateral spaces, particularly near the junction of circumpharyngeal connectives. ('Fig'.2).

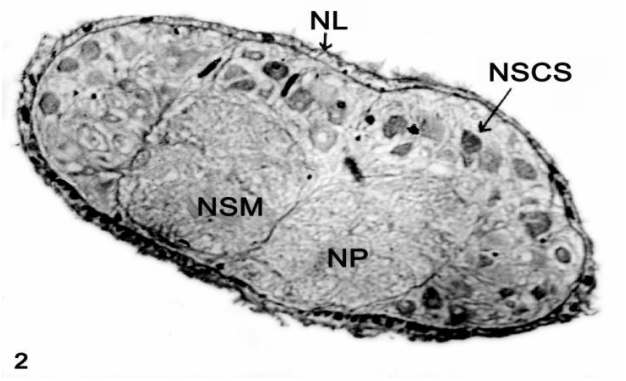


Fig.2. S.S of Sub pharyngeal Ganglion showing usual Pattern of NSCs

These cells are oval or round in shape, having central, sometimes peripheral, oval or rounded nucleus with prominent nucleolus. Size measured 29.12±1.8µm. ('fig'.3, 6).

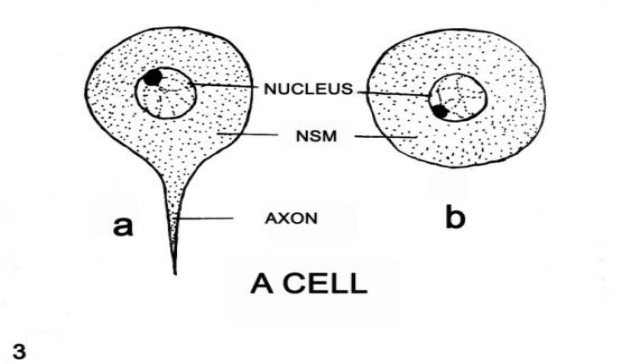


Fig.3. Constructed Diagrarn of 'A' NSC
a)A cell displaying axon
b) A cell in transverse plan wherein axon is not visible

"B" Neurosecretory Cell

These cells are distributed on dorsolateral surface of cerebral ganglion ('Fig'.1) and dorsal and ventrolateral surface of sub pharyngeal ganglion ('Fig'.2). These cells are oblong, elongated or pear shaped, having round nucleus near axonhilock region rarely abaxonal with prominent nucleolus. Size measured 26.6±1.5µm (fig.4, 6).

"C" Neurosecretory Cell

These cells are located in the brain chiefly along the lateral border near circumpharyngeal connectives and rarely on dorsal surface ('Fig'.1, 2).These cells are elongated or elliptical, having spherical ecentric rarely central nucleus with prominent nucleolus, having size16.8±1.2µm. ('fig'.5). These cells do not possesses any cellular processes with warrant their designation as the axons are fewer in number in both the ganglion.

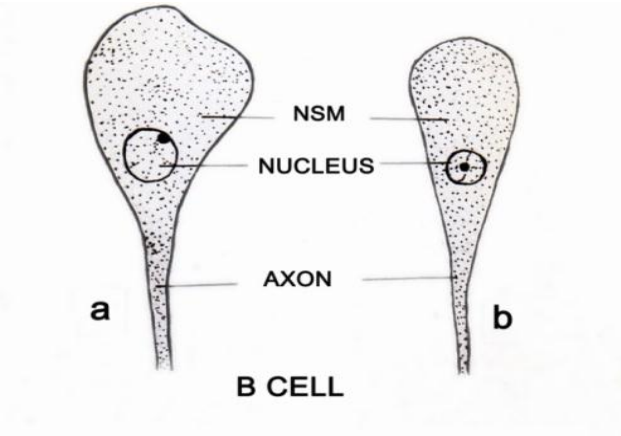


Fig.4. Constructed Diagrarn of 'B' NSC.
a- B cell maximum size
b- B cell normal size

Fig.5. Constructed Diagrarn of 'C' NSC
a- C cell maximum size
b- C cell normal size

Treated Group:

Dimethoate induced profound changes in the histological architecture of the A and B type of NSCs. The "C" cells are fewer in number to beget problem in observations, therefore these cells exempt from any detectable change over the controls. Observations were made by the recording changes in : Histological changes of cytoplasm and nucleus of NSCs, Intensity of NSM, The neurosecretory cell area (µm), The nuclear diameter (µm), The cell length (µm) and, The axon length (µm)

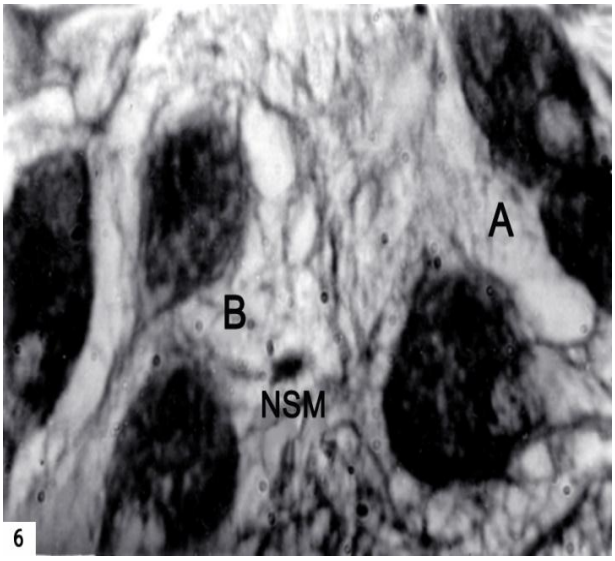
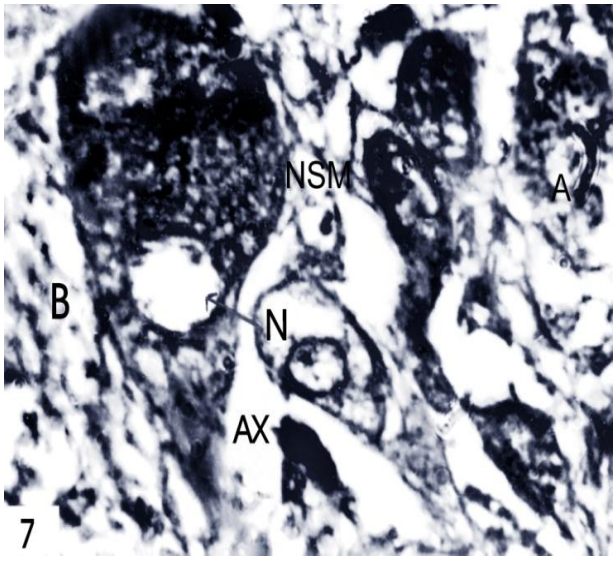


Fig.6. S.S.10 Days control Suprapharyngeal Ganglion Suprapharyngeal or cerebral ganglion



S.S.10 Days Dimethoate treated Suprapharyngeal Ganglion

10 Days Treatment: 10 days exposed ganglion showed enhancement in diameters of NSCs and increased intensity of NSM. Slight vacuolization were seen in cytoplasm and nucleoplasm of NSCs (‘Fig’. 7).

20 Days Treatment : 20 days treated NSCs showed irregular and broken cell membrane, there was an intensive accumulation of NSM near the nuclear region, tremendous vacuolization were seen in neuropile and in nerve fibres, and whole structure exhibited atrophied condition. (Fig. 8). Significantly enhanced diameter ($p>0.001$) of nucleus cell area, cell length and axon length were noticed in both type of NSCs of supra pharyngeal ganglion of brain in *Eudichogaster kinneari* after exposure with Dimethoate in 10 and 20 days duration of experiment (Table -1).

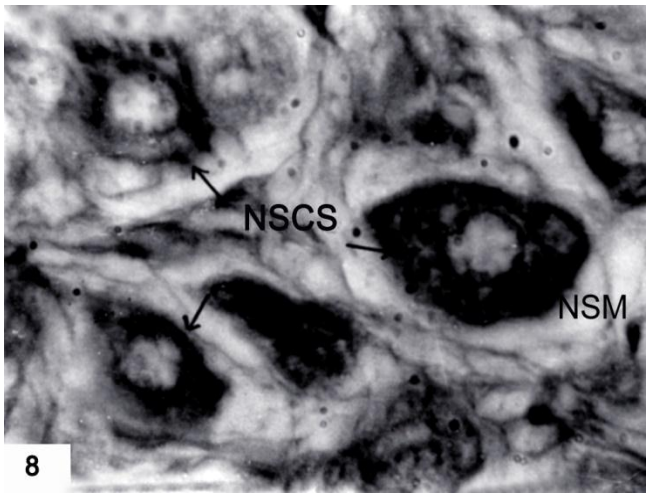
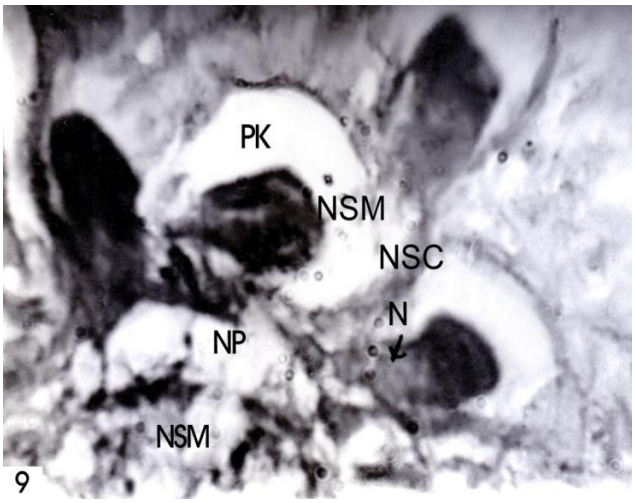


Fig. 7. S.S.20 Days control Suprapharyngeal Ganglion



S.S.20 Days Dimethoate treated Suprapharyngeal Ganglion

Table-1: Diameter of neurosecretory cells of supra pharyngeal ganglion of brain of *Eudichogaster kinneari* when exposed with dimethoate

Days of treatment	Treatment	Sub lethal concentrations used	Supra pharyngeal anglion					
			A Cell			B Cell		
			Cell Area	Nuclear Diameter	Cell Length	Cell Area	Nuclear Diameter	Axon Length
10 Days	Control	-	28.85*** ±2.6	12.5±1.5	37.82±1.7	25.75±1.3	10.20±0.77	27.80±0.55
	Dimethoate	0.6 ppm	34.97±3.3*** +(21.2)	15.0±2.0* +(20.0)	42.62±0.65*** +(12.6)	31.20±2.0*** +(21.1)	15.62±1.35*** +(53.0)	33.07±2.7*** +(18.7)
20 Days	Control	-	29.10±1.8	12.0±1.1	38.10±2.2	26.6±2.5	11.25±1.1	28.0±1.2
	Dimethoate	0.6 ppm	39.62±2.5*** +(36.0)	16.0±1.2*** +(33.3)	44.87±2.3*** +(17.7)	35.25±2.9*** +(32.5)	17.12±1.4*** +(52.17)	35.25±2.9*** +(25.8)

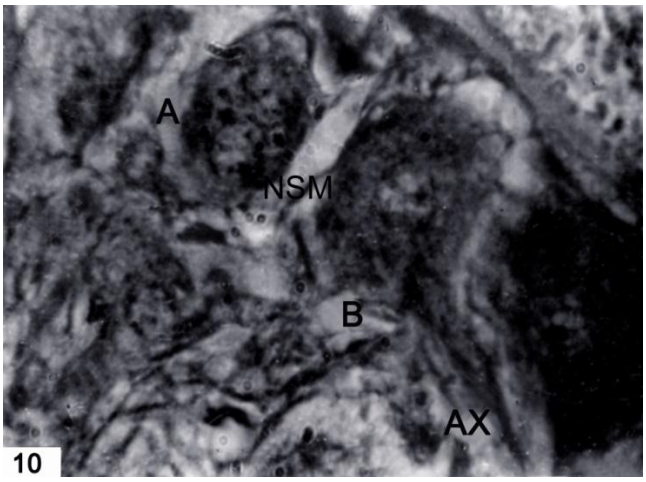
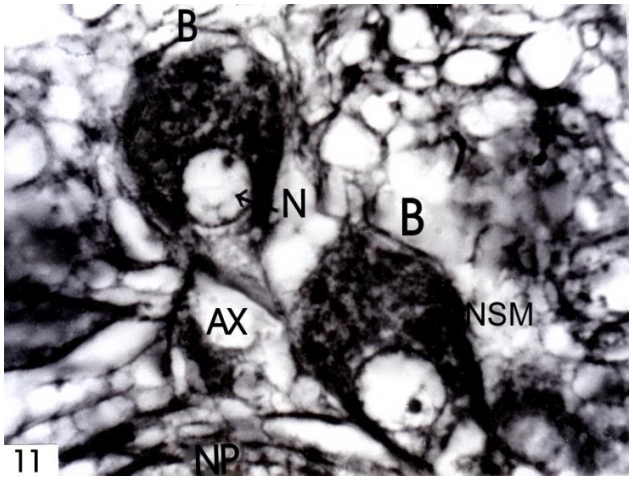


Fig. 8 S.S. 10 Days contro; sub pharyngeal Ganglion



S.S. l0 Da1,s Dimcthoate Trcafcd Sub pharvngral Ganqlion

Sub pharyngeal Ganglion:

10 Days Treatment:

Increased intensity of NSM and size increment of NSCs was observed in Sub pharyngeal Ganglion in 10 days exposure of Dimethoate. ('Fig'. 9).

20 Days Treatment:

20 days Dimethoate treated NSCs showed uneven thickened cell membrane of NSCs, mostly cell perikariya were emptying from NSM and accumulated around the nucleus. NSM became fragmented and vacuolated. Ultimately cellular architecture of NSCS of sub pharyngeal ganglion displayed deterioration ('Fig'. 9). 20 days Dimethoate exposure caused significantly size enhancement (p>0.001) in diameter of cell area, nuclear area, cell length and axon length in both type of NSCs at all intervals of experiment (Table- 2) Numerous endocrine parameters have been studied in earthworm exposed with various insecticides and chemicals, but there is no report on the effect of Dimethoate insecticide on the histomorphology of NSCs of brain of *Eudichogaster kinneari*. The present investigation revealed that Dimethoate at 0.6 ppm concentration arrests neurosecretory activities in the brain of *E.kinneari*. The cellular architecture of NSCs of both the ganglion was severely destructed. Cytoplasm and nuclear abnormalities were observed. The NSCs lost their normal shape.

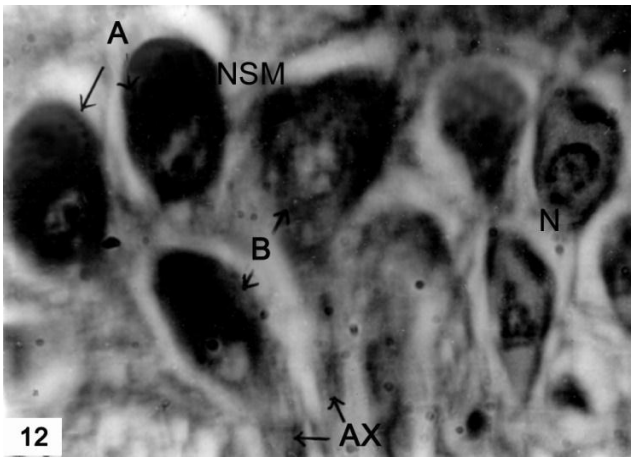
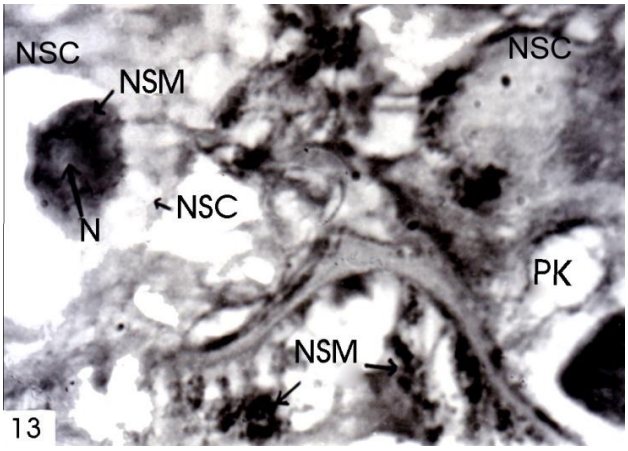


Fig.9. S.S. 20 Days control Sub pharyngeal Ganglion



S.S. 20 Days Dimethoate Treated Sub pharyngeal Ganglion

Abbreviations:
A, B-NSCs, PK- Perikariya, AX- Axon,
NP- Neuropile NSM- Neurosecretory material, N-Nucleus

Table-2: Diameter Of Neurosecretory Cells Of Sub Pharyngeal Ganglion Of Brain Of *Euichogaster kinneari* When Exposed With Dimethoate

Days of treatment	Treatment	Sub lethal concentrations used	Sub pharyngeal ganglion					
			A Cell			B Cell		
			Cell Area	Nuclear Diameter	cell Length	Cell Area	Nuclear Diameter	Axon Length
10 Days	Control	-	20.10±0.87	8.2±1.2	23.80±2.6	20.0±1.5	10.12±1.2	23.21±1.8
	Dimethoate	0.6 ppm	24.5±2.4*** +(21.8)	12.75±1.6*** +(5.4)	27.0±1.2*** +(13.44)	25.5±2.7*** +(27.5)	13.0±1.6*** +(27.4)	28.25±2.5* +(21.7)
20 Days	Control	-	20.60±1.4	8.6±1.0	24.50±1.9	19.75±2.5	10.0±1.2	24.50±1.0
	Dimethoate	0.6 ppm	29.37±2.3*** +(42.5)	15.37±1.6*** +(78.7)	33.12±1.9*** +(35.1)	31.37±0.9*** +(58.8)	15.0±2.0*** +(50.0)	30.62±1.8*** +(24.9)

All Values are expressed as mean± SD: No.=10, Significant levels *, **, *.**

Values in parenthesis are % alterations $ = % increase - = % decrease

Initially accelerated intensity of NSM, was exhausted by repeated treatment and NSM get accumulated around the nucleus, therefore the perikariya and axonal tract of NSCs emptying from NSM. These observations are in good synchronization with the results of Gupta and Verma (1979), which studied on the earthworm *perionyx erassiseptatus* with the effect of chlorpromazine and noticed enhancement in number, diameters of NSCs and increased intensity of NSM in both the ganglion. Om Prasad and Pawan Kumar, (1983) studied in brain NSCs of the earthworm *Eutyphoeus necolsoni* when treated with BHC, Malathion and endrin and reported initially accelerated neurosecretory activity of NSCs while repeated treatment led complete exhaustion of secretory material along with many cytomorphological alterations. Lakhani, (1992;

2015) studied on *E.kinneari* when treated with Endosulfan at 0.003 ppm concentration and Azodrin at 0.5 ppm concentration and noticed decreased diameter of NSCs, decreased intensity of NSM along with many histomorphological alterations, ultimately caused imbalance and lethal effect. Kulkarni, (1989) reported nuclear size increment, decreased cell area, vacuolated neurosecretory cell perikariya in *Lampito marutii* when treated with cyprimenthrin and Fen-Fen insecticides. Anand, (1984) studied on *Hirudo birmanica* and Kulkarni *et.al*., (1989) studied on *Poecilobdella viridis* with the effect of endosulfan, Malathion, Sevin, copper sulphate, mercuric chloride and sodium penta chlorophenate and Sagar, (1989) studied on *P.granulosa* with the effect of endosulfan, Malathion and Sevin. These authors reported cellular degeneration and total emptying of NSM in perikariya of brain NSCs. Nagbhushanam and Hanumante, (1977) studied on *P. excavatus* and observed that insulin induced increase rate of axonal transport while adrenalin caused reduction of NSM.Kulkarni, (1989) reported Ach, AD and 5HF caused 50% increased intensity of NSM in NSCs of supra pharyngeal ganglion while 50% decreased intensity in NSCs of sub pharyngeal ganglion in *L.marutii*. Kodarkar,(1979) reported increased rate of axonal transport of NSM with insulin treatment and pile up of neurosecretory granules in the perikariya and distal tip of the axons of A cells of the lateral group from the brain with amines treatment in *Octochaetoides Sundershensis.* Stenersen, (1979); Kale, (1982); Bharathi and Subba Rao, (1985); Rao, (2004) noticed decreased enzymatic activity of acetyl cholinesterase with Pesticidal treatment in earthworms. Thus this study clearly showed a histological parallelism as other workers reveal that insecticides are toxic to endocrine functions.

Conclusion

The functions of secretions from NSCs of brain of earthworms are responsible for the activities related with physiology , as their secretions play a vital role in activities of egg laying, formation of clitellum, spermatogenesis, oogenesis, cocoon production, effect on estivation and other rhythmic activities (Herlant – Meewis 1959), Regeneration also governed by NSCs secretions (Herlant – Meewis, 1967) ,neurosecretions regulate water and mineral balance in earthworms (Hanumante and Nagbhushnam,1977;Takeuchi, 1980).

Results of above study, concluded that Dimethoate affect histomorphology of NSCs, synthesis and transport of NSM in *E.kinneari*, as impaired neuroendocrine system, affect the above mentioned activities in the *E.kinneari*. Inspite of these results, many other external symptoms such as sluggish movements, body lesions etc. was also caused imbalance, moribund conditions, population depletion and ultimate the death of animals. Direct sprays of insecticides in agricultural fields or accumulated insecticides in the soil, not only affect the earthworm population, but also affect the beneficial biota of soil ecosystem. Therefore it is necessary to minimise the after effects of insecticides in agricultural fields and to save the earthworms.

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