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Thiodan stress on brain neurosecretory cells of the earthworm *Eudichogaster kinneari*: A Histological profile

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Abstract

Adult Eudichogaster kinneari were exposed to a safe concentration (0.003 ppm) of thiodan for twenty days to evaluate the effects on neurosecretory cells of cerebral and sub pharyngeal ganglion of brain. Brain was severely affected by exposure of above insecticide causing denatured neurosecretory cells due to vacuolization and liquification in cytoplasm, nucleoplasm, in neurosecretory material and in neuropile. Irregular shape of neurosecretory cells were seen, viz- uneven stain, disorderly thickened and broken cell membrane was seen at many places. Most of the neurosecretory material became fragmented and accumulated around the nucleus. Ultimate atrophy of whole histological architecture of neurosecretory cells in both the ganglion of brain was seen. Significant reduction was observed in diameter of cell area, nuclear area, cell length and axon length of neurosecretory cells (p< 0.001).

Key Words: Brain, Histomorphology, Neurosecretory cells, Neurosecretory material, Supra Pharyngeal ganglion, Sub pharyngeal ganglion, Thiodan.

Introduction

Agriculture is a backbone of our country. Today agriculture is totally depends on chemical fertilizers and pesticides. Insecticides, molluscides, bactericides, fungicides, nematicides, which are combindly known as pesticides (Ware, 1975).More than 128 pesticides are registered in India (Laxmi, 1992).Tones of synthetic pesticides and fertilizers are applied annually to crops worldwide. It is estimated that less than 0.1% of the pesticides applied to crops reach the target pest (Pimental, 1995). The earthworm is one of the "nature's top soil scientists" which make our soil good enough to grow healthy plants and provide us food. Earthworms decomposes plant residues, they break organic matter and leave behind castings that are a very valuable type of fertilizer, for this they bring down organic matter from the top layer of soil and mixing it with the soil below by involving ingestion, digestion and absorption of castings through the worms metabolic system (Somniyam and Suwanwaree, 2009, Chaudhuri *et al.,* 2009). Earthworms are indicators of soil quality because they respond to and contribute to healthy soil. They benefit soil quality by shredding residues stimulating microbial decomposition improving soil fertility and improving physical properties of soil such as soil aggregation and infiltration. Food availability is the major limiting factor for earthworm numbers. Generally fertilizers increase earthworm numbers by increasing crop residues, especially when pH is neutral. However, some insecticides, nematicides and fungicides are very toxic to earthworms (Edwards and Bohlen 1996). Earthworms are considered as important bio indicators of chemical toxicity in the soil ecosystem and play a key role in the bio magnification processes on several soil pollutants (Cikutovic *et al.,* 1993, 2010; Celine *et al.,* 2014) Soil pollution enormously increased due to intensive use of fertilizers, pesticides and insecticides for 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 agrochemicals not only affect the insects but equally damage the soil fauna. So the pesticides are generally toxic to non–target soil organisms, by causing anatomical and physiological changes in the vital organs and as consequences may hamper proper functioning of

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the soil (Reinecke and Reinecke. 2007) therefore require information on the effect of pesticides on beneficial soil organisms like Earthworms, which play an important role in the soil ecosystem.

The morphology of brain of annelids has been well studied (Herlant-Meewis, 1975; 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 thiodan 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 (0.003 ppm) of thiodan for twenty days in brain of E.kinneari to evaluate histomorphological abnormalities in their neurosecretory cells of brain.

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 Thiodan (Trade name endosulfan, other trademarks are cyclodan, Beosit, malix, Thimul, Thifur, is the adduct of hexachlorocylopentadine and 1, 4 – dihydroxy-2 butane, chlorinated with so_2cl_2 to produce 6,7,8,9,10 Hexachloro -1, 5 , 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodiooxathiepin-3-oxide) was used for experimental purpose, Lc-50 value to these worms , was also determined.. The calculated quantity of thiodan was taken, and diluted to 500 ml with tap water for preparation of the 0.003 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 students 't'test.

Results and Discussion

Control Group:

The brain of *Eudichogaster kinneari* contains one pair of cerebral ganglion,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 IV^{th} 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).

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

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).

"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.

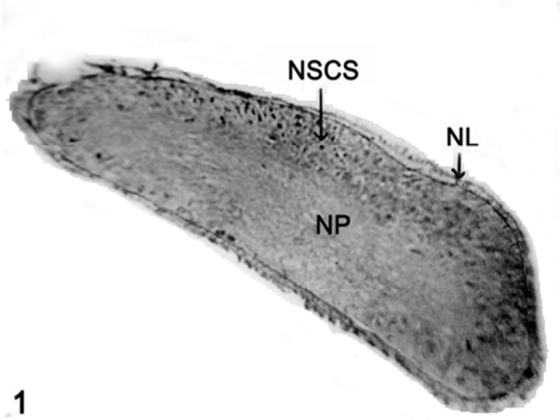

Fig.1. S.S. of Supra Pharyngeal Ganglion showing usual Pattern of NSCs

Abbreviations:

NSCs- Neurosecretory Cells, NP-Neuropile, NL-Neurolemma.

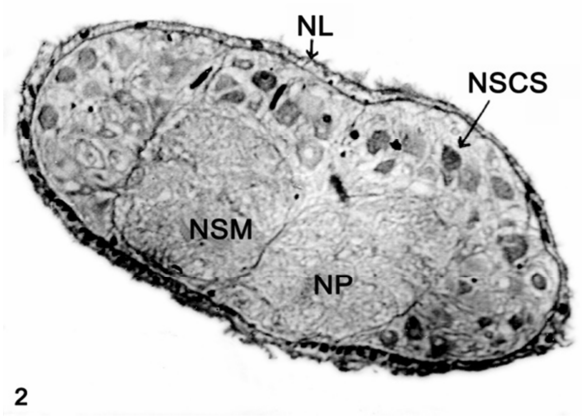

Fig.2. S.S of Sub pharyngeal Ganglion showing usual Pattern of NSCs

Abbreviations:

NSCs-Neurosecretory Cells, NSM-Neurosecretory material, NP-Neuropile, NL-Neurolemma.

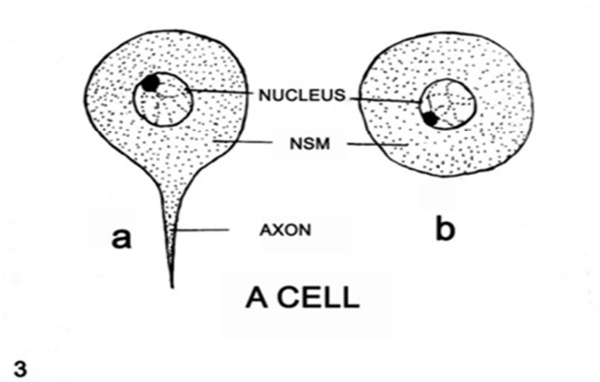

Fig.3. Constructed Diagrarn of 'A' NSC

a- A cell displaying axon

b- A cell in transverse plan wherein axon is not visible

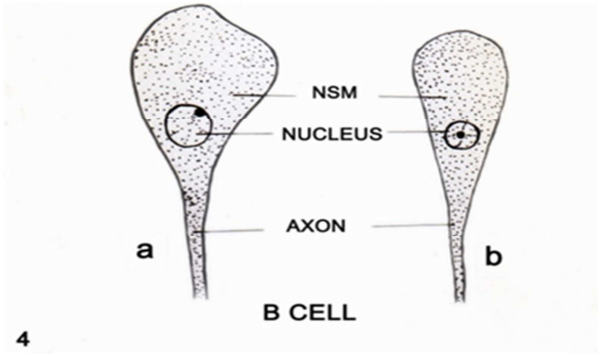

Fig.4. Constructed Diagrarn of 'B' NSC.
a- B cell maximum size
b- B cell normal size

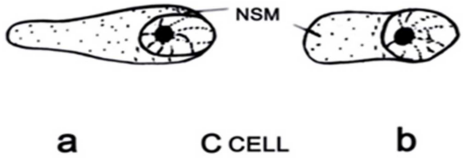

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Fig.5. Constructed Diagrarn of 'C' NSC.
a-C cell maximum size
b- C cell in normal size

Treated Group:
Thiodan 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

i. Histological changes of cytoplasm and nucleus of NSCs.
ii. Intensity of NSM
iii. The neurosecretory cell area (µm)
iv. The nuclear diameter (µm)
v. The cell length (µm) and
vi. The axon length (µm)

Suprapharyngeal or cerebral ganglion
10 Days Treatment:
10 days thiodan exposed ganglion showed decreased diameters of NSCs and decreased intensity of NSM. Vacuolization were seen in cytoplasm and in nucleoplasm of NSCs, at many places NSM were fragmented and accumulated around the nucleus. ('Fig'. 7).

20 Days Treatment
20 days thiodan treated NSCs showed irregular and broken cell membrane, tremendous vacuolization and liquification were seen in cytoplasm, in NSM, in neuropile and in nerve fibres, mostly NSM congregated around the nucleus, finally cells lost their normal shape and became denatured. ('Fig'. 9).

Significantly decreased 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 thiodan in 10 and 20 days duration of experiment (Table -1).

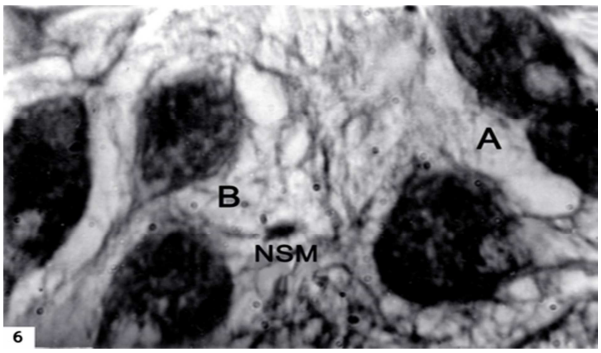

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Fig.6. S.S.10 Days control Suprapharyngeal Ganglion

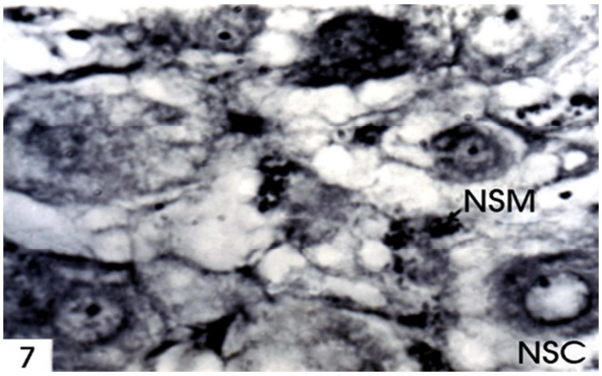

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Fig.7. S.S.10 Days Thiodan treated Supra pharyngeal Ganglion

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

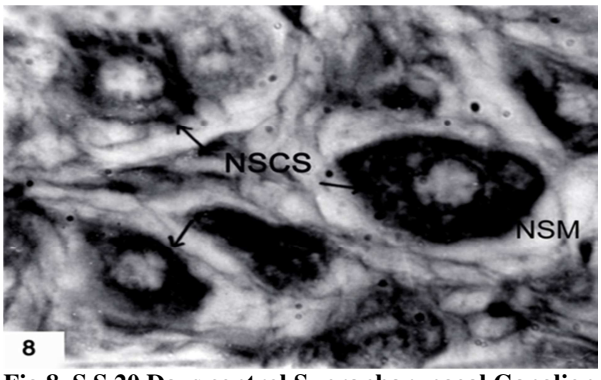

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Fig.8. S.S.20 Days control Suprapharyngeal Ganglion

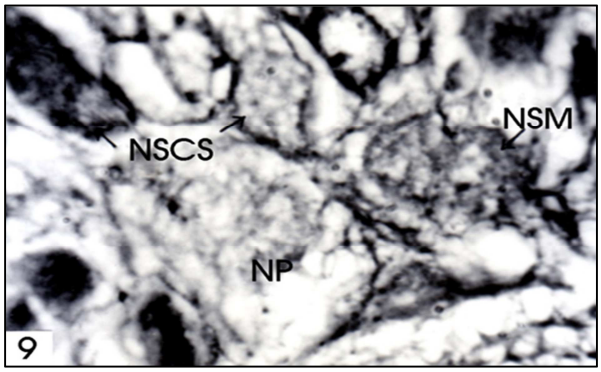

Fig.9. S.S.20 Days Thiodan treated Suprapharyngeal Ganglion

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

Sub pharyngeal Ganglion:

10 Days Treatment:

10 days thiodan treated NSC_S showed tremendous vacuolization in neuropile and in nerve fibres, broken cell boundaries of NSC_S, fragmented NSM was seen('Fig'. 11).

20 Days Treatment:

20 days thiodan treated NSCs showed uneven thickened ,broken and fragile cell boundaries , many empty spaces were seen due to tremendous vacuolization in nucleoplasm, cytoplasm and in neuropile. Less intensity, fragmentation and vacuolization of NSM was observed. Ultimately cellular architecture of NSC_S of sub pharyngeal ganglion displayed deterioration ('Fig'. 13).

Thiodan exposure caused significantly size reduction ($p<0.001$) in diameter of cell area, nuclear area, cell length and axon length in both type of NSCs at 10 and 20 days duration of experiments (Table- 2).

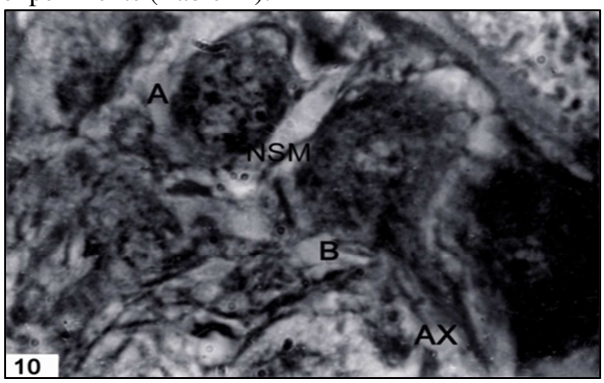

Fig.10. S.S. 10 Days control Sub pharyngeal Ganglion

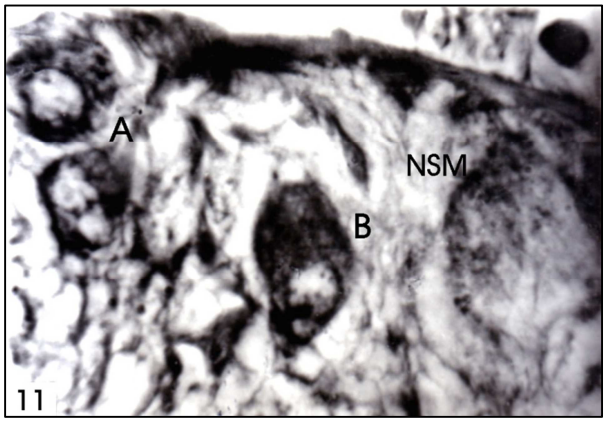

Fig.11. S.S. 10 Days Thiodan Treated Sub pharyngeal Ganglion

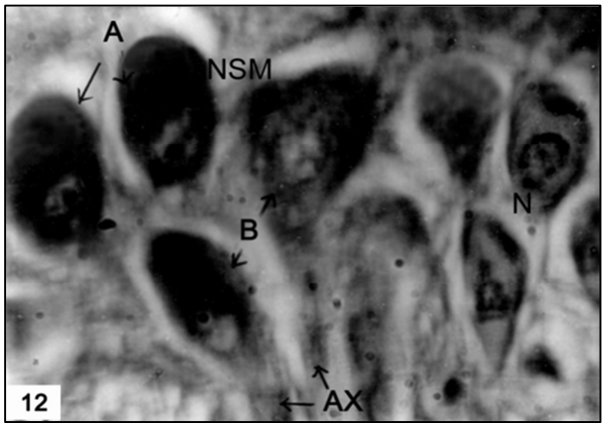

Fig.12. S.S. 20 Days control Sub pharyngeal Ganglion

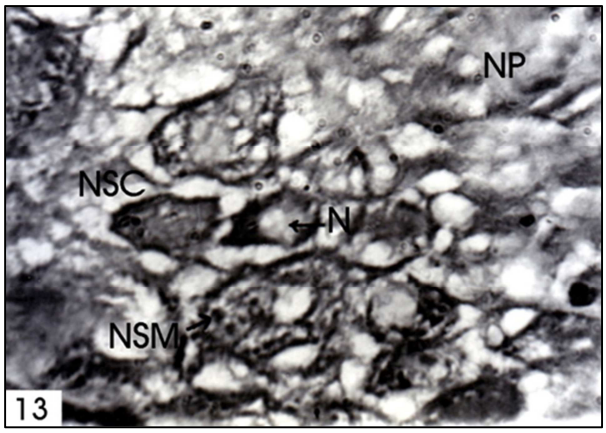

Fig.13. S.S. 20 Days Thiodan Treated Sub pharyngeal Ganglion

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

Table-1: Diameter Of Neurosecretory Cells of Supra Pharyngeal Ganglion of Brain of Eudichogaster kinneari When Exposed with Thiodan

Days of treatment	Treatment	Sub lethal concentrations used	Supra pharyngeal ganglion					
			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
	Thiodan	0.003 ppm	24.62±1.6*** -(14.6)	10.0±0.9*** -(20.0)	33.75±2.68*** -(10.76)	21.75±2.2*** -(15.5)	8.87±0.9*** -(13.0)	20.62±1.82*** -(25.8)
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
	Thiodan	0.003 ppm	19.5±1.7*** -(32.9)	6.12±1.1*** -(49.0)	29.0±2.4*** -(23.8)	15.5±2.8*** -(41.0)	6.12±1.0*** -(45.6)	14.5±2.4*** -(48.2)

Table-2: Diameter Of Neurosecretory Cells Of Sub Pharyngeal Ganglion Of Brain Of Eudichogaster kinneari When Exposed With Thiodan

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
	Thiodan	0.003 ppm	16.25±0.53*** -(19.1)	6.5±0.5** -(20.7)	18.62±2.5*** -(21.7)	17.5±1.3*** -(12.5)	7.62±1.2*** -(24.7)	19.62±1.27*** -(15.4)
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
	Thiodan	0.003 ppm	11.37±1.7*** -(44.8)	5.12±1.0*** -(40.4)	15.37±1.5*** -(37.2)	14.0±1.5*** -(29.1)	4.87±0.9*** -(51.3)	13.25±2.1*** -(45.9)

All Values are expressed as mean± SD; No.=10
Significant levels *, **, *.**
Values in parenthesis are % alterations
+= % increase
-= % decrease

Numerous endocrine parameters have been studied in earthworm exposed with various insecticides and chemicals, but there is no report on the effect of thiodan insecticide on the histomorphology of NSCs of brain of *Eudichogaster kinneari.*

The present investigation revealed that thiodan at 0.003 ppm concentration arrests neurosecretory activities of the brain of *E.kinneari*. The cellular architecture of NSCs of both the ganglion was severely destructed. Cytoplasm and nuclear abnormalities and reduced rate of synthesis of NSM were observed. Similar results were observed by Prasad and Kumar, (1983), who studied on the brain 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; 2015a), studied on *E.kinneari* when treated with Azodrin at 0.5 ppm concentration for 20 days, and noticed decreased diameter of NSCs with azodrin treatment, decreased intensity of NSM along with many histomorphological alterations, ultimately caused imbalance and lethal effect. Gupta and Verma (1979), 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, are in good synchronization with the result of Lakhani, (2015b), studied on *E.kinneari* when treated with dimethoate at 0.6 ppm concentration for 20 days and noticed increased diameter of NSCS, initially acceleration of NSM but repeated treatment caused reduction of secretory material and atrophied NSC_S .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 that 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,(1984) 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 and Krishnamurthy, 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 NSC_S of brain of earthworms are classified with physiological functions; their secretions are responsible for activities of the reproductive organs, egg laying, formation of clitellum, cocoon production, effect on estivation and other rhythmic activities(Herlant,1959).Regeneration also governed by the activities of NSC_S(Herlant, 1967;Morgon,1902;),neurosecretions also help in water and mineral balance(Takeuchi,1980;Hanumante & Nagbhushanam, 1977).Based on these findings it is concluded that thiodan affect histoarchitecture of NSC_S, synthesis and transport of NSM in *E.kinneari* , as impaired neuroendocrine system affect the above mentioned process of earthworms .Besides these effects, predicted signs and symptoms such as sluggish movements, body lesions etc. ultimately caused imbalance ,moribund conditions, reduction of population and death of worms . The accumulated insecticides in the soil or direct exposure of insecticide in the soil, where these animals are live, not only affect the earthworm populations which are old friends of farmers, but also affect the soil ecosystem. Therefore it is necessary to minimize the after effects of insecticides in agricultural fields and to save the earthworms.

References

Anand, C.S.K., 1984. Some aspects of neuroendocrinology of Indian leech*, Hirudo birmanica*, Ph.D. Thesis submitted in Marathawada University, Aurangabad, INDIA.

Bhaskar Rao, A., 1983. Some aspects of Physiology of Indian leech, Ph.D. Thesis submitted in Marathwada University, Aurangabad. INDIA.

Bharathi, C, Subba Rao, B.V.S.S.R., 1985.Inhibition of Ache activity by selected Organophosphorous pesticide in the earthworm *L. mauritii*.***J. Environ. Biol.,*** 6(4): 257-262.

Celine, P., Sebastein, B., Yvan, C., Mickael, H. and Franck, V. 2014. Pesticides and Earthworms. A Review Article. ***Agron. Sustain Dev.,*** 34:199-228. Doi: 10.1007/s 13593-013-0151-Z.

Cikutovic, M.A., Fitzpatrick, L.C., Venables, B.S. and Goven, A.J. 1993. Sperm count in earthworm as a biomarker for environmental toxicology: Effect of cadmium and Chlordane. ***Environ. Pollu.*****8** (12): Pp.123-125.

Cikutovic, M.A., Fitzpatrick, L.C., Goven, A.J., Venables, B, J., Giggleman, M.A. and cooper E.L., 2010. Wound healing in earthworm *Lumbricus terrestris*: A cellular based biomarker for assessing sub lethal chemical toxicity**.** ***Bull. Environ.Contam.Toxicol.*** 62:508-514**.**

Chaudhuri, P.S., Nath, S., Pal, T.K. and Dey, S.K., 2009. Earthworm casting activities under rubber (Herea brasiliensis) plantations in Tripura India, ***World J. Agri. Sci.*****, 5(4)**, 515-521.

Edward, C.A. and Bohlen, P.J. (ed.) 1996. Biology and Ecology of earthworms, 3rd ed. Champman & Hall, London, UK.

Gupta, K.G., Verma, B.R., 1979. Chlorpromazine induced changes in neurosecretory cells of the earthworm *Perionyx erassiseptatus.* ***Indian J. Exp. Biol.,*** 16(10): 1094-1095.

Herlent- Meewis, H., 1959. Phenomenes neurosecretoires et sexualite chez *E.foetida*. ***Comp.Rend. Acad*****, Sci.,** 248:1405-1406.

Herlent – Meewis, H., 1967.Evolution de 1 appareil genital d' *E.foetida* an cours de jeune de la regeneration posterieur et a la suite de 1 ablation de ganglions nerveuse.***Annales de laSociety Royale Zoologique de Belgiqui***, 96: 189-240.

Herlant-Meewis, H., 1975. Neurosecretory phenomena during reproduction in oligochaete: In intersexuality in the animal kingdom. In R. Reinboth (ed.) Springer, Berlin, 57-63.

Hanumante, M.M., and Nagbhushanam, R., 1977. Some aspects of osmotic behaviour of the tropical earthworm. *P. excavatus*. ***J.Animal Morph. Physiol.,*** 24(1): 3-12.

Kale, R.D. and Krishnamurthy, R.V., 1982. Residual effects of seven on the Acetyl cholinesterase activity of the nervous system of earthworm *P. corethurus*, ***curr. Science*****,** 51(8): 885-886.

Kulkarni, G.and Nagbhushanam, R., 1978. Histological and Histochemical features of the brain neurosecretory profile of the Indian leech *Poecilobdella viridis* (Blanchard).***Biology*** III (1):43-45.

Kulkarni, V.D., 1989. Physiological studies of the earthworm *Lampito mauritii* in relation to the impact of some environmental factors, Ph.D. Thesis, submitted to Marathwada University, Aurangabad, India.

Kulkarni, G.K., Bhaskar Rao, A.and Kulkarni, V.D.1989. Effect of some pesticides on the brain neurosecretory profile of a fresh water leech *P. viridis,* ***Environ. Risk Assessment.***:107-121.

Kodarkar, M.S., 1979. Neuroendocrinology of the earthworm *Octochaetoidea sundershensis,* Ph.D. thesis, submitted to Marathwada University, Aurangabad.

Lakhani, L., 1991.Histological and Histochemical studies on the neurosecretory cells of the Oligochaete *E.kinneari*, ***Ind.Inv. Zool. & Aqua. Biol.,*** 3(1):20-27.

Lakhani, L., 1992. Effect of certain insecticides on neuroendocrine system and gonads in an earthworm *Eudichogaster kinneari* (Stephenson), Ph.D. Thesis, submitted to Vikram University, Ujjain, India.

Lakhani, L., 2015. Effect of Azodrin on the brain neurosecretory cells of the earthworm *Eudichogaster kinneari* (Stephenson): A histological profile. ***Life Science Int.Res.J.*** , 2(1):267-275.

Lakhani, L., 2015. Impact of Dimethoate on brain of the earthworm *Eudichogaster kinneari* (Stephenson): A histological profile. ***Environ. Cons. Jour.*** 16(1&2): 1-8.

Laxmi, A., 1992. Pesticides in India, Risk Assessment and Policy. ***Second European Conference on Ecotoxicology,*** Amsterdam, 1-15.

Lionetto, M. G., Calisi, A. and Schettino, T., 2012. Earthworm Biomarkers as tools for soil pollution assessment, Maria C.Hernandez-Soriano (ed.) ISBN 978-953-307-614-0.

Margon, T.H., 1902.experimental studies on the internal factors of regeneration in the earthworm, Arch. Entw-Mech, 14.

Nagbhushanam, R.and Hanumante, M.M., 1977. Histological and Histochemical studies on the neurosecretory cells of the oligochaete *Perionyx excavates*, ***J.Ann. Morph. Physiol.,*** 24(2): 259-268.

Nagbhushnam, R.and Hanumante, M.M.,1977. Effect of different chemicals on the neurosecretory system of the oligochaete worm *P. excavates*, ***Ind. J. Exp. Biol.,***15:70-72

Pimental, D., 1995. Amounts of pesticides reaching target pests, Environmental impacts and ethics. ***J. Agril. Environ Ethics,*** 8:17-29.

Prasad, OM., Kumar, Pavan, 1983. Effect of insecticides on the brain neurosecretory cell of the earthworm *Eutyphoeus nicholsoni*, ***Symp. Nat. Acad. Sci. India,*** 53 Annual Session Goa.

Rao, J.V., 2004. Toxicity of Azodrin on the morphology and acetyl cholinesterase activity of the earthworm *Eisenia foetida*, ***Environ, Res.,*** 96:323-327.

Reinecke, S.A. and Reinecke, A.J., 2007.The impact of organophosphate pesticides in orchards on earthworms in the Western Cape, South Africa, ***Ecotoxicol. Environ. Safety,*** 66**:**244-251.

Sagar, C.R., 1989. Impact of some Pesticidal pollutants on the physiological activities of a
Freshwater leech *Poecilobdella granulosa*, Ph.D. Thesis, submitted to Marathwada University Aurangabad, India.

Satija, R.C., and Garg, D., 1977. Histology of the central nervous system of *P.posthuma*, ***Res.Bull. Punjab Univ. Sci.*** 24(3-4): 137-150.

Somniyam, p. and Suwanwaree, p., 2009. The diversity and distribution of terrestrial earthworms in sakaerat Environmental Research station and adjacent areas, Nakhon Ratchasima, Thailand., ***Word Appl. Sci. J. ,***6(2): 221-226 **.**

Stenersen, J., 1979. Action of pesticides on earthworms, Part I: The Toxicity of Cholinesterase- inhibiting insecticides to earthworms as evaluated by laboratory tests, ***Pestic Science*****,** 10: 66-74.

Takeuchi, N., 1980. Neuroendocrine control of hydration in Megascolecid earthworms, ***Comp. Biochem. Physiol.*****,** 67(3): 341-345.

Ware, G.W., 1975. In Pesticides, Theory and application. Published by ***WH. Freeman and Company San Francisco***,:55-57.