International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **1(4),** 77-80, August **(2012)** *I. Res. J. Biological Sci.*

Short Communication

Effect of Dimethoate on Testicular Histomorphology of the Earthworm Eudichogaster Kinneari (Stephenson)

Lakhani Leena, Khatri Amrita and Choudhary Preeti
Department of Zoology, Govt. Girls PG College, Ujjain, MP, INDIA

Available online at: www.isca.in
Received 11th July 2012, revised 18th July 2012, accepted 30th July 2012

Abstract

Adult Eudichogaster kinneari were exposed to a safe concentration (0.6 ppm) of Dimethoate for twenty days to evaluate the effects on different stages of spermatic follicles. Chronic exposure of above insecticide severely affected the spermatogenesis causing vacuolization and liquefaction of spermatic follicles, uneven arrangement of spermatozoa around the cytophore and ultimate atrophy of spermatic follicles. Size reduction of spermatic follicles ($p<0.001$) as well as changes in histochemical reactions were also observed.

Keywords: Eudichogaster kinneari, histomorphology, dimethoate, testis, insecticide.

Introduction

Earthworms are one of the most important organisms responsible for mechanical mixing of soil and play a major role in maintaining physical soil characteristics and processes such as aeration, water permeability and mineral turnover[1]. Earthworms are key components in natural food chains providing a food source for many small mammals, birds, fishes and prawns[2].

Pesticides are known to produce morphological, anatomical and physiological changes in the vital organs such as reproductive, nervous, respiratory and osmoregulatory of different nontarget animals, such as earthworms and other beneficial organisms[3].

The morphology of gonads of earthworms has been well studied[4]. The effect of pesticides on the reproductive organs of some invertebrates has been investigated[5-10]. However detailed knowledge of the effect of pesticides on the histomorphology of testis is lacking, hence it is intended to study the effect of an Organophosphorus insecticide dimethoate on the histomorphology of testis of an earthworm E.kinneari in an exposure of 20 days.

Material and Methods

Healthy, sexually matured specimens of Eudichogaster kinneari approximately of same weight (6.5 + 0.001 gm), length (80-120 mm) 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 to 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 prepared soil was sprayed with 500 ml of this diluted fluid 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 testes were fixed in aqueous Boin's fluid and 10% formalin. The fixed testes were processed for dehydration and blocks were prepared in paraffin wax, sections were cut at 4-5 µm and stained with Delafield's haematoxylin and eosin and Mallory's triple for histological details and periodic acid Schiff's (PAS) mercuric bromophenol blue (Hg-BPB), luxol fast (LF) best carmine (BC) and Sudan black B (SBB) for histochemical details. Statistical analysis of data was carried out by students' 't' test.

Results and Discussion

Control group: There are two pairs of testes, one on each side of the ventral nerve cord in the 10th and 11th segments. These are creamish or whitish in colour, each testis is attached at its basal end to the septum while the rest part is protected by thread like ligaments, the testes are free and are not enclosed in a testis sac.

The spermatic follicles of testis of E.kinneari were arbitrarily classified into four consecutive developmental stages, depending on the size of spermatic follicles and approximate number of cells per cluster.

Stage-1: Immature- Included small clusters having approximately 1 to 16 cells or fewer cells and measured 29.22+1.2µ. Cells joined together by a small central cytoplasmic bridge, the cytophore. The cells are rounded and contained abundant cytoplasm (figure 1 and 2).

Stage–2: Premature – Included larger clusters with approximately 32-64 cells and measured 39.0±1.7µ. The developing sperm cells are larger and rounded with more prominent cytoplasm and nucleus (figure 1 and 2).

Stage—3: Maturing – Included larger clusters having approximately 64-128 cells and measured 56.75±1.7µ. The developing sperm cells are small, elliptical having a very prominent and much bigger cytophore. The signs of development of sperm tail are evident in some spermatic follicles (figure 1 and 2).

Stage -4 : Fully Mature – Spermatic follicles showed further development compared to those of stage-III, having approximately 128 cells and measured 60.37±1.6µ. The cytophore was larger still having a distinct freely moving sperm tail and the heads attached to a common point (figure 1 and 2).

Treated group: 10 Days Exposure: Exposure of E.kinneari to Dimethoate for 10 days showed vacuolization in immature and premature spermatic cells, uneven arrangement and damaged cells of maturing follicles. Cytophore of mature follicles also showed vacuolization (figure-3). Diameters of spermatic follicles were reduced significantly (P< 0.001) (table 1).

20 Days Exposure: After 20 days exposure, cytoplasm of cells of spermatic follicles showed shrinkage, granulation and vacuolization. Ultimately atrophy of spermatic follicles (figure 5). Decreased intensity with histochemical reactions and significantly reduced size of spermatic follicles (P<0.001) were noticed (table 1).

Table-1
Diameter of Spermatic follicles of Eudichogaster Kinneari exposed to Dimethoate

Days Exposed	Group	Diameter of Spermatic follicles			
		Stage-I	Stage-II	Stage-III	Stage-IV
10 Days	Control	29.05±1.4	38.9±1.1	56.25±1.0	60.5±1.8
	Dimethoate	22.25±1.5***	33.87±1.0***	51.87±1.6***	51.5±1.9***
20 Days	Control	29.12±1.2	40.25±1.7	56.75±1.7	59.5±1.4
	Dimethoate	18.87±1.7***	29.5±1.2***	47.25±1.2***	45.87±1.1***

Each value is mean ± SD , n=10, Significant levels *, **, ***, Values in parenthesis are % alterations., -- =Decrease %

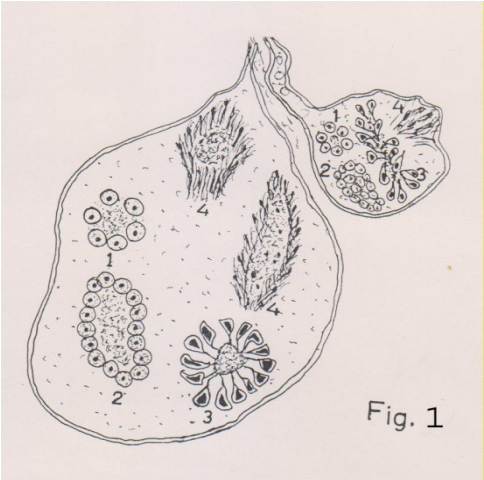

Figure-1
Photograph of Male gonad showing different stages of spermatogenesis of Eudichogaster kinneari.
i. Immature spermatogenic follicles, ii. Premature spermatogenic follicles, iii. Maturing spermatogenic follicles, iv. Fully mature spermatogenic follicles

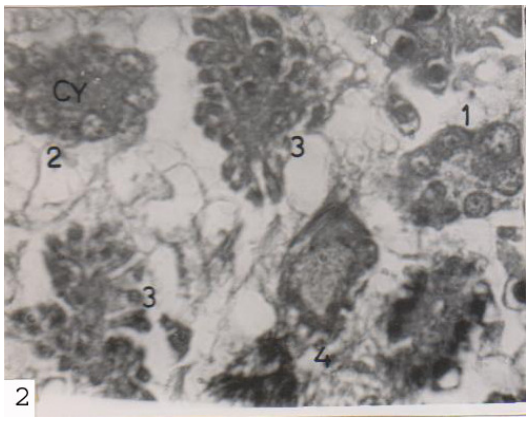

Figure-2
10 days control testis

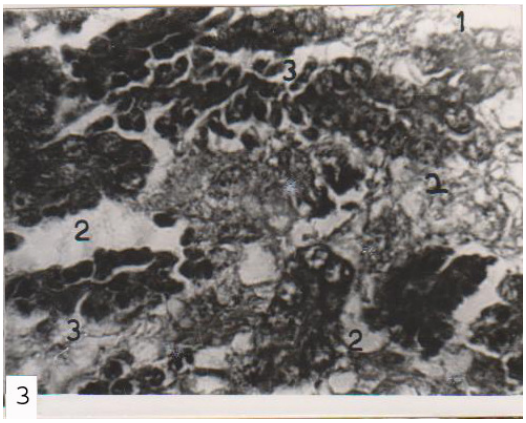

Figure-3
10 days Dimethoate treated testis

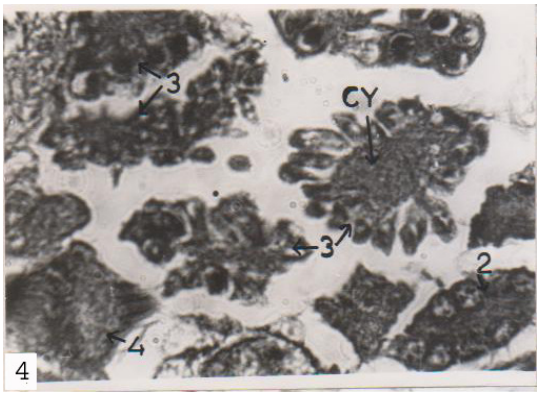

Figure-4
20 days control testis

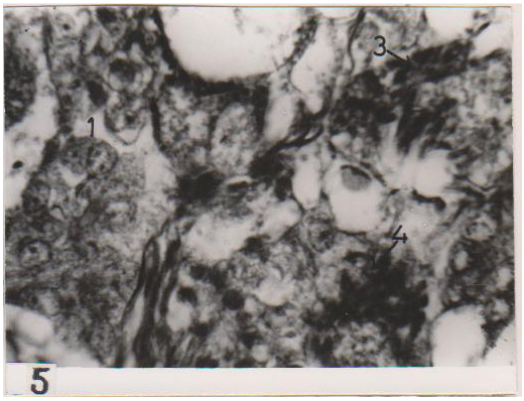

Figure-5
20 days Dimethoate treated testis

Numerous reproductive parameters have been studied in earthworms exposed to various insecticides and chemicals: cocoon, hatching and sperms production, viability of the worms produced, sexual maturation and generotoxicity[11-22].

Several scientist have reported that pesticides influence the reproduction of worms in a dose dependent manner with greater impact of higher concentration of chemicals[12-17].

Available literature indicates that monocrotophos at 0.5 ppm and Endosulfan at 0.003 ppm concentration impaired testicular function in E.kinneari, Cytoplasmic and nuclear abnormalities were also observed in all spermatic follicles. The cellular architecture of all stages of spermatic follicles of testes were severely destructed and showed dissolution and vacuolization, decreased size of spermatic follicles were also noticed[8]. Besides testes, author also studied on ovaries of same species at same concentration and duration by exposure of above insecticides and observed atrophy in cellular architecture and significantly decreased size of oocytes[7-10]. Similar results were observed in gonads of Hirudo birmanica when treated with Endosulfan, malathion and copper sulphate at different concentration for twenty days[5] and in a poecilobdella granulosa with the treatment of Endosulfan, malathion and sevin[6]. When Eisenia foetida treated with malathion, it decreased spermatic viability in spermathica, altering the cell proliferation and modifying the DNA structure of spermatogonia and also has direct cytotoxic effect causing coiling of tail[11].

Pesticides decreased enzymatic activity when studied acetyl cholinesterase activity in Poecilobdella granulosa[6] and Pontoscolex corethurus[20] which in turn affect the process of gametogenesis as regulated by the gonadotropic hormones in the brain of annelids.

Conclusion

The present observations are very important to note that profound changes in the testes after treatment with dimethoate are produced. It is expected that when the earthworms E.kinneari were exposed to dimethoate for 20 days, their cellular enzyme system might have been disturbed, which in turn interfered in the process of normal gametogenesis. The disturbed nervous system might have been affected the release of gonadotropins, which are essential for gametogenesis in E.kinneari.

It can be concluded from the above study that, during the insecticidal use in the agricultural field strict vigil should be

maintained to prevent the accumulation of these hazardous chemicals in the soil and to minimize the after effects on earthworms, the old friends of farmers.

References

1. Barley K.P. and Jennings, A.C., Earthworms and soil fertility, The influence of earthworms on the availability of nitrogen, *Aust. J. Agricultural Research*, **10**, 364-370 (**1959**)

2. Satchell J. E. , Soil biology , Academic press , London and New York , 259-322 (**1967**)

3. Fingerman M., Pollution our enemy-Keynote address, *Proc. Symp. Physiol. Res. Animal pollution*, **1(VI),** (**1984**)

4. Sareen M.L. and Verma V., morphological and cytochemical studies of the vitellogenesis in Eisenia foetida, *Res. Bull. Punjab University*, **27,** 41-49 (**1976**)

5. Kulkarni G.K., Anand C.S.K. and Rao A.B., Effect of some pesticides on the gametogenesis of a fresh water leech Hirudo birmanica, *Proc. Nat. Symp. Ecotoxico.l*, 54-57 (**1987**)

6. Sagar C.R., Impact of some pesticidal pollutants on the physiological activities of a freshwater leech poecilobdella granulosa, Ph.D. Thesis, Marathwada University, Aurangabad, India (**1989**)

7. Lakhani L., Bhatnagar B.S. and Pandey A.K., Effect of monocrotophos on the ovary of the earthworm Eudichogaster kinneari (Stephenson): A histological and histochemical profile, *J. Reprod. Bio. Comp. Endocrinol.,* **3(1),** 39-46 (**1991**)

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

9. Lakhani L., Effect of Endosulfan on ovary of the Earthworm Eudichogaster kinneari (Stephenson): A histological and histochemical profile, *Res. Hunt*, **2(II),** 24-31 **(2007)**

10. Lakhani L. and Khatri A., Effect of Rogor on the ovary of the Earthworm Eudichogaster kinneari, A histological and histochemical profile, *J.E.R.D.*, **2(3),** 378-385 (**2008**)

11. Cikutovic M.A., Fitzpatrick L.C., Venables B.S. and Goven A.J., sperm count in earthworms as a biomarker for environmental toxicology: Effect of cadmium and chlordane, *Environmental pollution,* **81(2),** 123-125 (**1993**)

12. Espinoza-Navaroo O. and Bustos E., Sublethal doses of malathion after male reproductive parameters of Eisenia foetida, *Int.J.Morphology*, **22**, 297-302 (**2004**)

13. Sorvour J. and Larink O., Toxic effects of benomyl on the ultrastructure during spermatogenesis of the earthworm Eisenia foetida, *Ecotoxicol. and Environ. safety*, **50(3)**, 180-188 (**2001**)

14. Bustos-Obregon E. and Goicochea R.I., Pesticide soil contamination mainly affects earthworm male reproductive parameters, *Asian J. Andrology* **4(3)**, 195-199 (**2002**)

15. Navaroo O. and bustos-obregon E., Effect of malathion on the male reproductive organs of earthworm Eisenia foetida, *Asian J. Andrology*, **7(1)**, 97-101 **(2005)**

16. Zohu Q.X., Zhang Q.R. and Laing J.D., Toxic effects of acetochlor and metha midophos on earthworm Eisenia foetida, *J.Enviorn. sciences*, **18(4)**, 741-745 **(2006)**

17. Yasmin S. and D'souza D., Effect of perticides on the reproductive output of Eisenia foetida, *Bull. Environ. Contam. Toxicol*., **79(5)**, 529-532 **(2007)**

18. Yasmin S. and D'souza D., Effect of pesticides on growth and reproduction of earthworm: A Review, Applied and Environmental soil science **(2010)**

19. Gupta S.K. and saxena P.N., carbaryl induced behavioural and reproductive abnormalities in the earthworm Metaphire posthuma: a sensitive model, Alternatives to laboratory animals**, 31(6),** 587-5 **(2010)**

20. Correia F.V. and Moreira J.C., Effect of glyphosate and 2,4-D on earthworms Eisenia foetida, *Bull. Environ. Contam. Toxicol*., **85(3)**, 264-8 **(2010)**

21. Santos M.J, Ferreira M.F, Cachada A., Duarte A.C., and Sousa J.P., Pesticide application to agricultured fields: Effect on the reproduction and avoidance behaviour of folsomia candida and Eisenia andrej**,** *Ecotoxicol*., **(2012)**

22. Wang J.H., Zhu L.S., Liu W., Wang J. and Xie H., Biochemical responces of earthworm E. foetida to pesticide chlorpyrifos and fenvalerate**,** *J.Toxocol. Mech. Methods*, **22(3),** 236-41 **(2012)**

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