EFFECT OF THREE COMMONLY USED INSECTICIDES ON HISTOMORPHOLOGY AND HISTOCHEMISTRY OF TESTIS OF EARTHWORM EUDICHOGASTER KINNEARI (ANNELIDA: OLIGOCHEATA)

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Abstract:

Adult Eudichogaster kinneari were exposed to 0.6 ppm concentration of Dimethoate, 0.5 ppm concentration of Azodrin and 0.003 ppm concentration of Thiodan for twenty days to evaluate profound changes in the histomorphology and histochemistry of testis. Spermatogenesis was severely affected with exposure of above insecticides causing reduced testicular activity by destruction of cellular architecture observed by vacuolization of cells in different stages of spermatic follicles and in cytophore, necrosis, lesions, congregation of spermatogenic material, and uneven arrangement of spermatozoa around the cytophore and ultimate atrophy of spermatic follicles. Decreased intensity with histochemical reactions and reduced size of spermatic follicles ($p<0.001$) were observed. The present study indicates that among the three insecticides tested, thiodon is most toxic to earthworm E.kinneari, than azodrin and dimethoate respectively. The intensity of deterioration were noticed more toxic in thiodan > Azodrin > Dimethoate respectively.

Keywords:

KEY WORDS: Eudichogaster kinneari, Insecticide, testis, histomorphology, histochemistry.

Introduction:

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 bioindicators of chemical toxicity in the soil ecosystem

and play a key role in the biomagnifications 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. Inspite of this, there is lack of information on the effect of three commonly used insecticides dimethoate, azodrin and thiodan on the testicular histomorphology and histochemistry of earthworm Eudichogaster kinneari. Therefore the present work aims to show clearly the changes produced after exposure of safe concentration of dimethoate (0.6 ppm), azodrin

(0.5 ppm) and thiodan (0.003 ppm) for twenty days in the testis of an earthworm Eudichogaster kinneari to evaluate histomorphological and histochemical abnormalities in their testis.

Material and Method:

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), Azodrin (monochrotophos,

"Nuvacron" shell development co.) and Thiodan (Endosulfan, Southern minerals limited Haryana) were used for experimental purposes, Dimethoate and Azodrin are organophosphorous and Thiodan is organochlorine insecticide. Lc-50 value of these insecticides for Eudichogaster kinneari was determined. The calculated quantity of dimethoate, azodrin and thiodan was taken and diluted to 500 ml with tap water for preparation of the 0.6 ppm test

concentration for dimethoate, 0.5 ppm concentration for azodrin and 0.003 ppm concentration for thiodan. The prepared solution was sprayed on soil and mix with soil properly on the first day and on the 10th day of experiment. 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 fixatives after 10 and 20 days. Before making the histological and histochemical 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 Bouin‟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 student„s„t‟ test.

Result 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 color, 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 spermatic follicles: 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 (fig. 1 and 2). Stage 2: Premature spermatic follicles: 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 (fig. 1 and 2). Stage 3: Maturing spermatic follicles: 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 (fig. 1 and 2). Stage 4: Fully mature spermatic follicles: 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 with cytophore (fig.1 and 2). Histochemically all spermatic follicles showed mild reactions with periodic acid Schiff"s (PAS) technique, all spermatic follicles showed mild reactions which suggest the presence of least quantity of carbohydrates. Mercuric Bromophenol blue (Hg-BPB) test revealed moderately positive results, indicating the presence of proteins. Lipids have been traced in minute quantities and phospholipids in sufficient quantities evidenced by Sudan black B (SBB) and Luxol fast (LF) techniques. Presence of glycogen was also observed in less quantity with Best Carmine (BC).Table 1. TREATED GROUP: 10 DAYS EXPOSURE: Exposure of E.kinneari to dimethoate for 10 days showed vacuolization in spermatic follicles (Fig.3). Azodrin treated spermatic follicles showed dissolution at many places, cells and cytophore of spermatic follicles exhibited vacuolization (fig.4). Thiodan treatment depicted vacuolization due to shrinkage of spermatogenic components, thickened lining of spermatic follicles were also noticed (fig.5). Decreased intensity with histochemical reactions (Table 1) and significantly reduced diameter of spermatic follicles (Table 2) and (fig.10) were seen. 20 DAYS EXPOSURE: 20 days treatment of dimethoate treated spermatic follicles showed shrinkage, granulation and vacuolization in cytoplasm of their cells (Fig.7). Azodrin treatment showed broken spermatic follicles, due to which cells scattered everywhere within the sac. Almost all follicles showed clumping, vacuolization in their cells and in their cytophore, ultimately cellular

architecture showed atrophied condition (Fig.8). 20 days thiodan treatment showed drastic effects in all spermatic follicles caused complete degeneration of tissues with broken follicles, destruction of cellular architecture caused by necrosis, lesions and congregation of spermatogenic material (Fig.9). Histochemically all stages of spermatic follicles exhibited less intensity (Table-1) and significantly reduced size of ($p<0.001$) spermatic follicles were seen (Table-2) and (fig.10) when exposed with above insecticides.

Numerous reproductive parameters have been studied in earthworms, exposed to various insecticides and chemicals viz: cocoon production, a reduced mean and maximum number of hatchlings per cocoon, sperm production, cytotoxicity, generotoxicity. Scient

Conclusion:

Based on the observations of the present study and previous studies, it can be concluded that the reproductive parameters of earthworms affected by above insecticides seems to be useful bioindicators of soil pollution and indicate negative impact of pesticides on earthworm‟s reproduction. It is expected that when the earthworms E.kinneari were exposed to above three insecticides for 20 days at safe concentrations, their cellular enzyme system might have been disturbed, as the disturbed nervous system might have been affected the release of gonadotropins, which are essential for gametogenesis in E.kinneari. As we know that earthworms are old friends of farmers, it is necessary to minimize the after effects of insecticides in agricultural fields as to save the earthworms. Application of insecticides should be restricted to needed places only, especially during breeding time when the earthworms are near to soil surface. The products which are used in agriculture fields should be least injurious to earthworms.

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TABLE1: HISTOCHEMISTRY OF TESTIS OF E. KINNEARI EXPOSED WITH INSECTICIDES

Days of treatment	Treatment	Sublethal concentrations used	Histochemical Test				
			PAS	Hg-BPB	LF	SBB	BC
10 Days	Control		$$	$$$	$$$	$$	$$$
	Dimethoate	0.6 ppm	$$	$$$	$$$	$$	$$$
	Azodrin	0.5 ppm	$$	$$$	$$$	$$	$$$
	Thiodan	0.003ppm	$$	$$$	$$$	$$	$$$
20 Days	Control		$$	$$$	$$$	$$	$$$
	Dimethoate	0.6 ppm	$	$	$	$	$
	Azodrin	0.5 PPM	±	$	$	±	$
	Thiodan	0.003 ppm	±	$	$	±	$

PAS-Periodic Acid Schiff"s, Hg-BPB- Mercuric Bromophenol blue, LF- Luxol Fast, SBB- Sudan black B, BC-Best Carmine +++,++ Positive reactions, + Mild Positive reactions, ± Not clear

TABLE2: DIAMETER OF SPERMATIC FOLLICLES OF E. KINNEARI EXPOSED WITH INSECTICIDES

Days of treatment	Treatment	Sublethal concentrations used	Diameter of Spermatic Follicles			
			Stage 1	Stage 2	Stage 3	Stage 4
10 Days	Control	-	29.05±1.4	38.9±1.1	56.25±1.0	60.5±1.8
	Dimethoate	0.6 ppm	22.25±1.5*** -(24.5)	33.87±1.0*** -(29.9)	51.87±1.6*** -(7.7)	51.5±1.9*** -(14.8)
	Azodrin	0.5 ppm	21.5±1.6*** -(27.1)	32.75±1.8*** -(15.8)	50.87±1.2*** -(9.5)	51.5±1.9*** -(14.8)
	Thiodan	0.003ppm	17.24±1.6*** -(41.5)	29.5±1.2*** -(24.1)	47.25±1.2*** -(19.0)	44.75±1.6*** -(26)
20 Days	Control	-	29.12±1.2	40.25±1.7	56.75±1.7	59.5±1.4
	Dimethoate	0.6 ppm	18.87±1.7*** -(35)	29.5±1.2*** -(26)	47.25±1.2*** -(16.7)	45.87±1.1*** -(22.9)
	Azodrin	0.5 ppm	17.24±1.6*** -(40.7)	28.22±1.2*** -(29.8)	45.12±1.8*** -(20.0)	45.37±2.6*** -(23.74)
	Thiodan	0.003ppm	12.75±1.3*** -(56.2)	21.47±1.8*** -(46.6)	40.62±1.5*** -(28.42)	37.0±1.8*** -(37.8)

All Values are expressed as mean+ SD: No.=10 Significant levels *,**,***.
Values in parenthesis are % alterations

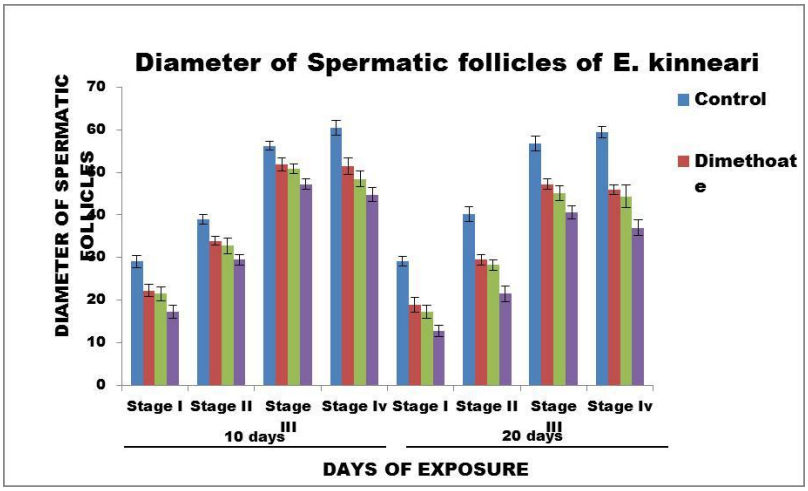

FIGURE 10

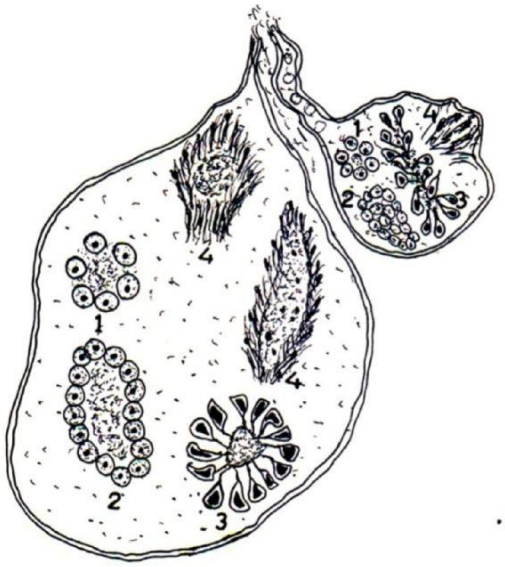

FIGURE 1

PHOTOGRAPH OF T.S. MALE GONAD OF E. KINNEARI SHOWING DIFFERENT STAGES OF SPERMATOGENESIS

1. Immature spermatic follicles 2. Premature spermatic follicles
3. Maturing spermatic follicles 4. Fully mature spermatic follicles

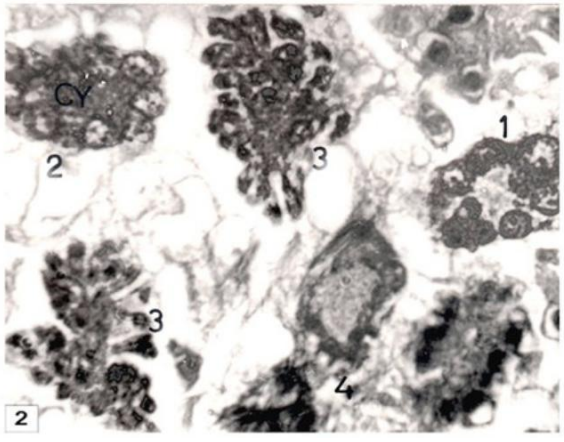

FIGURE 2

10 DAYS T.S. CONTROL TESTIS

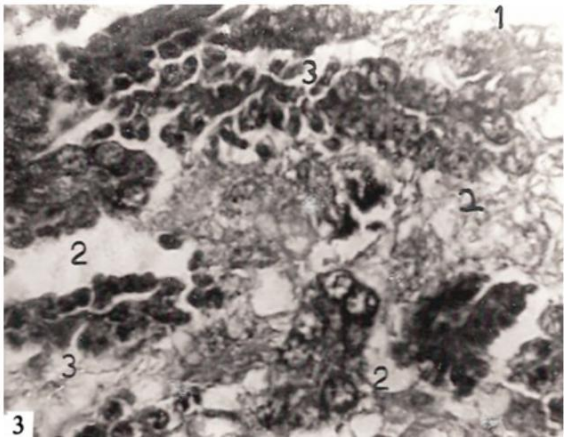

FIGURE 3

10 DAYS T.S DIMETHOATE TREATED TESTIS

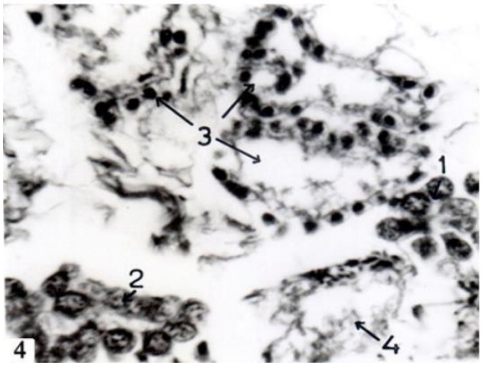

FIGURE 4
10 DAYS T.S AZODRIN TREATED TESTIS
1. Immature spermatic follicles
3. Maturing spermatic follicles

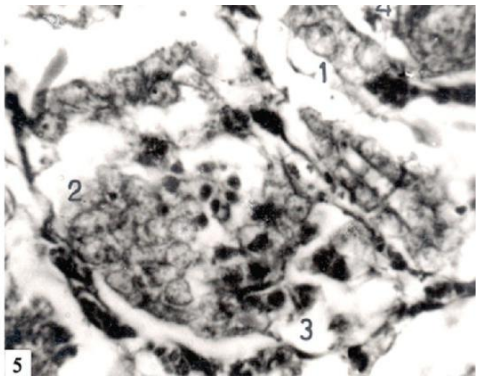

FIGURE 5
10 DAYS T.S. THIODAN TREATED TESTIS
2. Premature spermatic follicles
4. Fully mature spermatic follicles

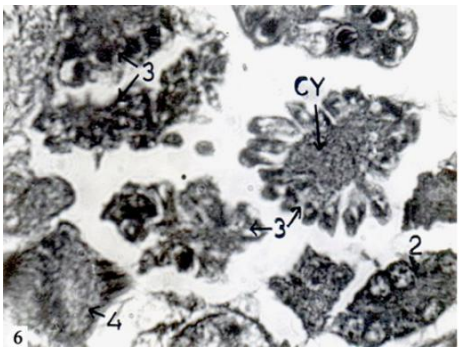

FIGURE 6
20 DAYD CONTROL TESTIS

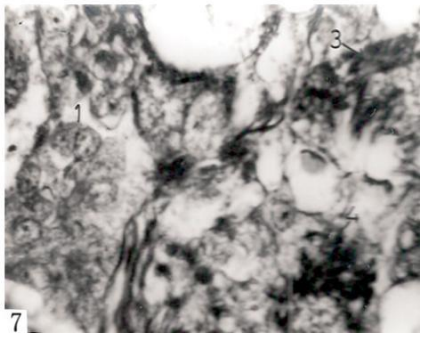

FIGURE 7
20 DAYS T.S. DIMETHOATE TREATED TESTIS

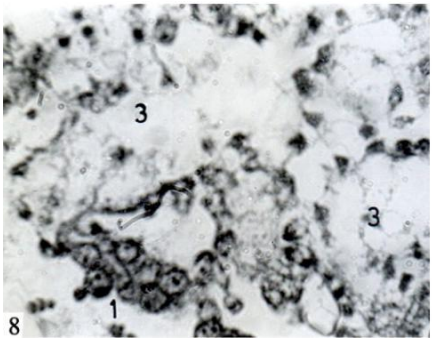

FIGURE 8
20 DAYS T.S. AZODRIN TREATED TESTIS

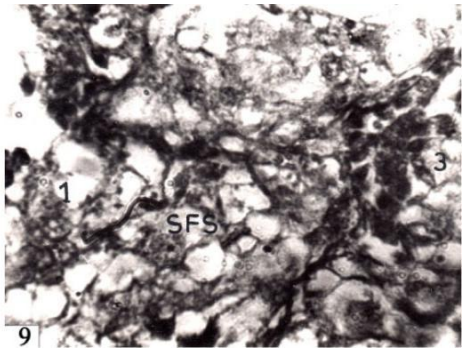

FIGURE 9
20 DAYS T.S.THIODAN TREATED TESTIS

1. Immature spermatic follicles 2. Premature spermatic follicles
3. Maturing spermatic follicles 4. Fully mature spermatic follicles