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Effect of Azodrin on the Testes of the Earthworm Eudichogaster kinneari (Stephenson): A Histological and Histochemical Profile

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Abstract

Adult earthworms Eudichogaster kinneari were exposed to a safe concentration (0.5 ppm) of Azodrin for twenty days to evaluate the effect on different stages of spermatic follicles. Spermatogenesis was severely affected by exposure of above insecticide causing degeneration in tissues of spermatic follicles due to clumping, vacuolization and necrosis. Changes in histochemical reactions and significant reduction in size of spermatic follicles ($p<0.001$) were also observed.

Keywords: Eudichogaster kinneari, testes, pesticides, fertilizers, histomorphology, histochemistry, azodrin.

Introduction

Earthworms, the "Golden bough of our agricultural history" are the terrestrial oligocheates. They are of immense ecological importance to mankind, particularly in his agricultural endeavors. Aristotle draws attention to their role in turning over the soil. He called them as "intestines of the earth". They are very important soil organisms which help in breaking down of dead plants and animal material in soil and plant litter, i.e. Thatch layer, and in recycling of nutrients. Their burrowing habit makes tunnels, which helps to enter oxygen and water in soil more easily. Earthworms make significant contribution to improve soil fertility and air porosity. Their castings are rich in N, P, K and other nutrients; this helps soil to make it more fertile and healthy. These are known as beneficial gardeners and industrial ploughmen; they have played an important role in agricultural aspect of the world[1-3].

Thatch is a layer of living and dead roots, stems and organic matter, accumulate at the soil surface, its accumulation becomes higher, when the rate of decomposition is much lower than the rate of grass growth. Thatch layer becomes thick by the use of pesticides because beneficial organisms, such as earthworms are killed by these pesticides. Excessive thatch reduces penetration of water and other materials, such as fertilizers. Earthworms help to break the thatch layer and pull organic matter into the soil.

Earthworms being terrestrial invertebrate animals generally live in upper layer of the soil, because this is the place where food and moisture is most abundant. The worms ingest soil and organic matter which is swallowed and grind in the gizzard. The ejected materials, castings are used to line the burrow. Activities of the Earthworms depend directly on soil moisture, PH and temperature. These way earthworms are of enormous ecological importance. They contribute in the recycling of organic wastes and production of organic fertilizers, hence are helpful in maintenance of soil structure, aeration and fertility[4].

Expansion of agriculture is heavily relying on the use of pesticides. 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 the soil[5-7], 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 gonads of earthworms has been well studied[8-9]. The effect of pesticides on the reproductive organs of some invertebrates has been investigated[10-12]. However detailed knowledge of the effect of insecticides on the histology and histochemistry of testes is lacking, hence present study is aimed to study the effect of an organophosphorus insecticide Azodrin on the histomorphology and histochemistry of testes of an earthworm Eudichogaster kinneari in an exposure of twenty days.

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 Azodrin (monocrotophos, "Nuvacron" shell development co., formerly 3- (dimethoxyphosphinyloxy) N-methylisocrotonamide) was used for experimental purposes, Lc-50 value to these worms, was also determined. The

calculated quantity of Azodrin was taken and diluted to 500 ml with tap water for preparation of the 0.5 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 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.

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 Azodrin for ten days, showed vacuolization in all stages of spermatic follicles, follicles became dissolved at many places, therefore showed uneven thickening, cytophore also exhibit vacuolization (figure 3). Histochemical reactions showed mildly positive intensity and significantly reduced size of spermatic follicles ($p<0.001$), (table 1 and 2).

20 Days exposure : 20 Days Exposure of E.kinneari to Azodrin profoundly affect all stages of spermatic follicles by causing appearance of less number of follicles, follicles broken at many places, due to which arrangement of cells around cytophore became uneven and cells scattered every where within the sac. Almost all follicles showed clumping, vacuolization in their cells and in cytophore (figure5). Histochemical reactions showed less intensity when compared with control (table 1). Diameters of all stages of spermatic follicles showed significantly reduction. (table 2)

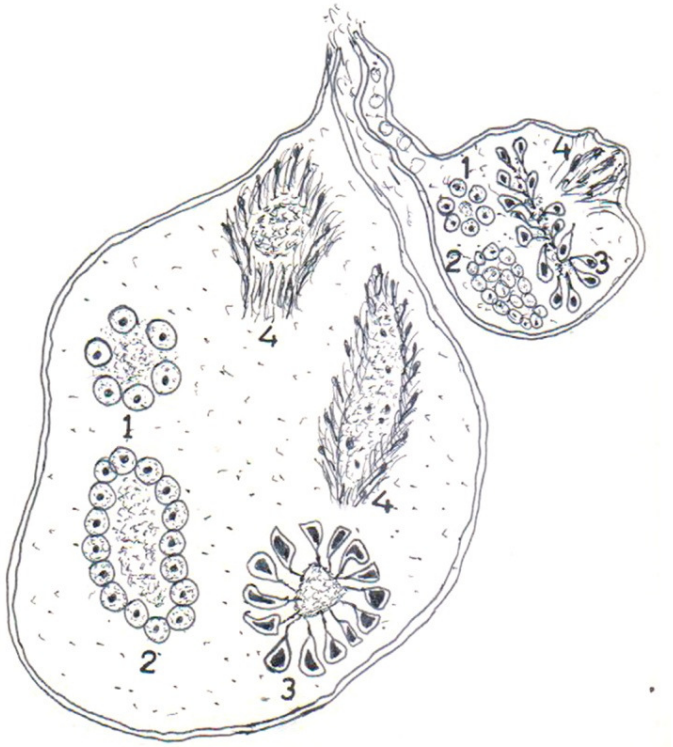


Figure -1
Photograph T.S. of Male gonad showing different stages of spermatogenesis of Eudichogaster kinneari. 1. Immature spermatogenic follicles, 2. Premature spermatogenic follicles, 3. Maturing spermatogenic follicles, 4. Fully mature spermatogenic follicles

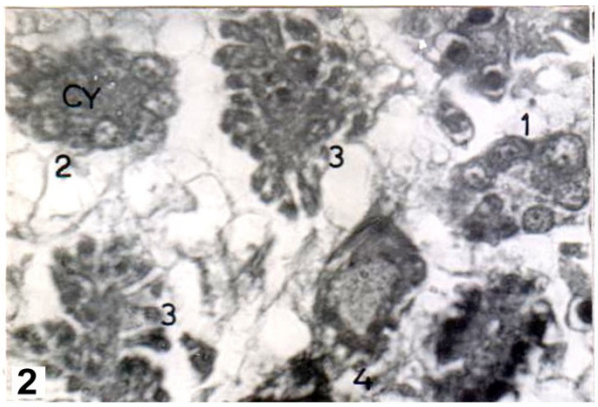


Figure – 2
10 Days control testis (T.S.)

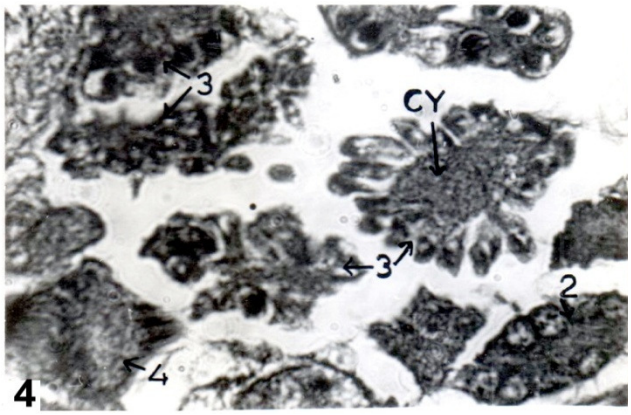
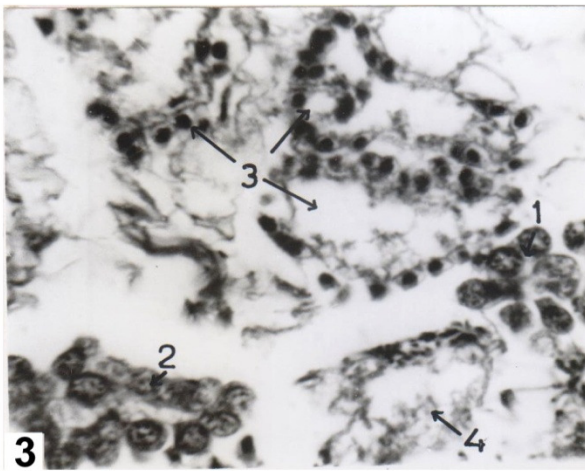


Figure – 4
20 Days control testis (T.S.)



Figure–3
10 Days Azodrin treated testis (T.S.)

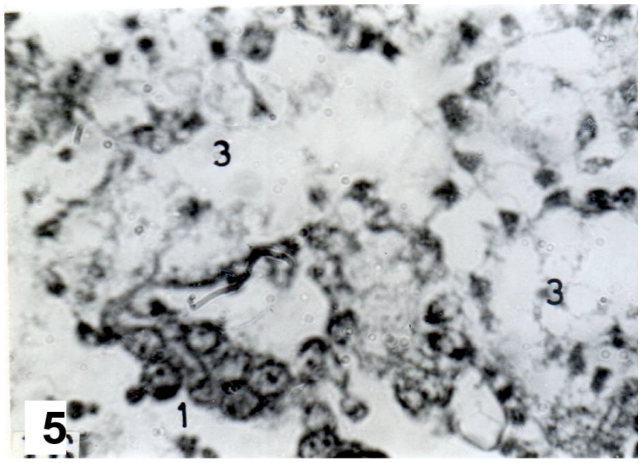


Figure – 5
20 Days Azodrin treated testis (T.S.)

Table- 1
Histochemistry of spermatic follicles of testis of E. kinneari, exposed to Azodrin

Days Exposed	Group	TEST				
		PAS	Hg-BPB	BC	LF	SBB
10 Days	Control	++	+++	++	+++	+++
	Azodrin	+	++	+	++	++
20 Days	Control	++	+++	++	+++	+++
	Azodrin	±	+	±	+	+

PAS = Periodic Acid Schiffs: Hg-BPB = Mercuric Bromophenol Blue; BC = Best Carmine ; LF = Luxol Fast; SBB = Sudan Black B in ethanol; ++, +++ = Positive reactions, + = mildly positive, ± = Not clear.

Table-2
Diameter of spermatic follicles of testis of Eudichogaster kinneari exposed to Azodrin

Days Exposed	Group	Diameter of spermatic follicles			
		Stage-I	Stage-II	Stage-III	Stage-IV
	Initial control	28.22±1.2	39.0±1.8	56.75±1.7	60.37±1.6
10 Days	Control	29.05 ±1.4	38.9 ±1.1	56.25 ±1.0	60.5 ±1.8
	Azodrin	21.5 ± 1.6*** – (27.1)	32.75 ±1.8*** – (15.8)	50.87 ±1.2*** – (9.5)	51.5 ± 1.9*** – (14.8)
20 Days	Control	29.12 ±1.2	40.25 ±1.7	56.75 ±1.7	59.5 ±1.4
	Azodrin	17.24 ± 1.6*** – (40.7)	28.22 ± 1.2*** – (29.8)	45.12 ±1.8*** – (20.0)	45.37 ± 2.6*** – (23.74)

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

Numerous reproductive parameters have been studied in earthworms exposed to various insecticides and chemicals: cocoon, hatching, sperms production, viability of the sperms produced, sexual maturation and generotoxicity and cytotoxicity. Several scientists have reported that pesticides influence the reproduction of worms in a dose dependent manner with greater impact of higher concentration of chemicals[13-30].

Available literature indicates that Dimethoate at 0.6 ppm and Endosulfan at 0.003 ppm concentration impaired testicular function in E.kinneari, Cytoplasm 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, vacuolization, decreased size of spermatic follicles were also noticed[14,17]. 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[13-16]. Similar results were observed in gonads of Hirudo birmanica when treated with endosulfan, malathion and copper sulphate at different concentration for twenty days[10], and in a poecilobdella granulosa with the treatment of endosulfan, malathion and sevin[11]. Pesticides carbendazim, dimethoate and glyophosate affect growth and reproduction in Eisenia fetida by negative impact and carbendazim and dimethoate affect severely than glyophosate[19]. Depressed reproduction was noticed in Perionyx excavatus when treated with formulated carbafuran than its pure compound and decreased toxicity of three chemicals in the order carbafuran > chlorpyriphos>mancozeb were observed[24].

Pesticides decreased enzymatic activity when studied acetyl cholinesterase activity in Poecilobdella granulosa[11] and Pontoscolex corethurus[20,] which in turn affect the process of gametogenesis is regulated by the gonadotropic hormones in the brain of annelids.

Conclusion

The present observations are very important to note that profound changes in histology and histochemistry of the testes after treatment with Azodrin are produced. It is expected that when the earthworms E.kinneari were exposed to Azodrin 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 insecticide uses in the agricultural fields, to reduce detrimental effects of insecticides on earthworms: i. Application of Insecticides should be restricted only in needed areas, especially in the rainy season when earthworms are near the soil surface. ii. Those selected products should be used which are least injurious to earthworms. iii. If possible, treat only infested areas.

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