UTTAR PRADESH JOURNAL OF ZOOLOGY

43(17): 73-82, 2022
ISSN: 0256-971X (P)

TOXIC EFFECT OF LAMBDA CYHALOTHRIN AND PRETILACHLOR ON BIOMASS AND HISTOLOGY OF TESTIS OF *Lampito mauritii* (KINBERG)

G. INDHUMATHI [aφ], V. KAVITHA [a*#] AND R. ANANDHAN [a#]

[a] Department of Zoology, Annamalai University, Annamalai Nagar - 608 002, Tamil Nadu, India.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2022/v43i173157

Editor(s):
(1) Dr. Juan Carlos Troiano, University of Buenos Aires, Argentina.
Reviewers:
(1) Sangeetha Naveen, University of Malaya, Malaysia.
(2) Cenk Kılıç, Turkiye.

Received: 05 July 2022
Accepted: 09 September 2022
Published: 17 September 2022

Original Research Article

ABSTRACT

Lambda cyhalothrin and pretilachlor is widely used to control agricultural pest but also affect non target organisms like earthworms. The present laboratory study was conducted to determine the effect of Lambda cyhalothrin and Pretilachlor on biomass and histology of testis of *Lampito mauritii*. Lower and higher sub-lethal concentrations of Lambda cyhalothrin (T1 and T2) and Pretilachlor (T3 and T4) was mixed with soil substrate. Biomass was observed once in 10 days up to 90 days. 10 non clitellate *L.mauritii* was introduced into each treatment. Soil substrate without lambda cyhalothrin and pretilachlor served as control. The present study was revealed that lambda cyhalothrin and pretilachlor was highly affected the growth of *L. mauritii.* For histopathology study, four adult *L. mauritii* were introduced in to C, T1, T2, T3 and T4. After 5^{th}, 15^{th} and 30^{th} day, testis was dissected out. The results reported that histology of testis was highly damaged on 5^{th} and 15^{th} day of experiment than 30^{th} day.

Keywords: Earthworm; *Lampito mauritii*; lambda cyhalothrin; pretilachlor; biomass; testis.

1. INTRODUCTION

Our nation father GandhiG told that agriculture is a backbone of India. The growing human population is expected to be 9.1 billion by 2050 (UN [1]). It needs more food production. So, chemical fertilizers and pesticides are used in the agricultural field to avoid increasing demand for food which leads to soil contamination. India is the largest manufacturer of pesticides. More than 128 pesticides are registered in India Laxmi [2]. They are not only destroy pest and boostup plant growth also affect farmer's friend as

[φ]Research Scholar;
[#]Assistant Professor;
**Corresponding author: Email: kavisamikshaa@gmail.com;*

earthworm. Earthworms constitute about 60-80% of total soil biomass. They play an important role in improving structure and fertility of soil ecosystems Bartllet et al. [3] by modifying soil organic matter both chemically and physically, facilitating the formation and stabilization of soil aggregates and improving soil porosity. Therefore they act as good bioindicators of soil providing safety thresholds for insecticide applications Lourenco et al. [4]. Fertilizers and insecticides are used for the betterment of agricultural yield. They ultimately persist in soil and decrease soil fertile nature, causes flora and fauna residing in the soil. Lambda-cyhalothrin is a pyrethroid insecticide that kills insects and mites by disrupting cellular functions in peripheral and central nervous systems. Pretilachlor is a pre-emergence herbicide that belongs to choloroacetamide group herbicide, which affectively controls most of the weeds in rice crops. It inhibits protein biosynthesis, resulting in growth inhibition, cell disruption, and death of target weed. Hence the present study was aimed to study the effect of lambda cyhalothrin and pretilachlor on biomass and histology of testis of *L.mauritii*.

The review of literature reported that many researchers found that toxic effect of various pesticides on earthworms. They were: Hundal et al. [5] observed that "effect of chlorofyrifos on survival, growth and reproductive performance of *Eudrillus eugeniae"*. Yasmin and Dsouza [6] found that "effect of pesticide on the growth and reproduction of earthworm". Anshu et al. [7] observed that "individual and combined toxic effect of herbicides on growth parameters and fecundity of *Eisenia fetida*. But, no one studied the effect of lambda cyhalothrin and pretilachlor on biomass and histology of testis of *L.mauritii*. A very few studies have been reported on the histopathological effects of pesticides on earthworms. Histology is the most useful tool for determining the influence of agricultural pesticide, industrial pollutants, organic wastes etc., at tissue level of an organism as it provides useful informationon on soil contamination". Suneel Kumar and Satyendra Singh [8] studied the toxic effects of phorate on morphological and structural integrity of the tissues. The morphology of gonads of earthworms has been well studied Joshi and Dabral [9]. Lakhani Leena [10] observed "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". The present work aims to show clearly the changes produced after exposure of lambda cyhalothrin and pretilachlor on biomass and histology of testis of *L.mauritii.*

2. MATERIALS AND METHODS

Materials:

Soil and cowdung: Clay loam soil and fresh cowdung were obtained from Annamalai University, Agricultural Farm and Dairy-yard.

Pesticides: Lambda cyhalothrin 5% EC (Insecticide) and Pretilachlor 50% EC (herbicide) (made in India) was purchased from an agro-center in Thanjavur, Tami Nadu, India.

Experimental organism: *L. mauritii* was widely distributed in South India. Hence it was selected as test organisms in this study. The body of *L.mauritii* is long, cylindrical and tubular. The ventral surface is pale in colour and the dorsal surface is light brown to yellow in colour with purplish blackish band like tinge. Adult worms are 8-21 cm long, 3-5 mm in diameter, and weight 0.8-1.5 g with 165-190 segments. *L.mauritii* was obtained from Annamalai University dairy yard in Annamalai Nagar, Tamil Nadu and India. They were acclimatized to the laboratory conditions by growing in cow dung.

Experimental Setup:

Biomass study: Sun-dried and powdered cow dung (a nitrogen- rich natural food for earthworms) was mixed with soil (low nitrogen) in a ratio of 1:3 (vol/vol). It was termed the "soil substrate" and used throughout this study. For chronic toxicity study, sublethal concentrations were selected from 96 h LC50 values of both the pesticides. Experimental setup was C, T1, T2, T3 and T4. In control, soil substrate was mixed only with water. Lower and higher sublethal concentrations (1.78 ppm/kg^{-1} and 4.45ppm/kg^{-1}) of lambda cyhalothrin mixed with 1Kg of soil substrate using 300 ml of water. It was considered as T1 and T2. Similarly lower and higher sublethal concentrations of pretilachlor (2.68 ppm/kg^{-1} and 6.7ppm kg^{-1}) was mixed with 1Kg of soil substrate. It called as T3 and T4. After taking initial weight 10 non-clitalate *L.mauritii* was introduced into each experiments conducted in troughs. The troughs were covered by nylon nets and maintained at room temperature (28±2ºC) with 60-70% moisture content. Three replicates were maintained for every experiment. The wet weight of earthworms, were observed once in 10 days up to 90 days.

Histopathology study: Experimental media preparation for the histopathological study was similar biomass study. But well clitellated *L. mauritii* were introduced in to C, T1, T2, T3, and T4. *L. mauritii*

were collected from control and treatments (C, T1, T2, T3 and T4). Three *L. mauritii* were removed from each experimental period (5th, 15th and 30th day) then to minimize their movement while easily dissecting them, they were kept in a freezer for about 1 h before dissection. Later the animals were dissected and two pairs of testis were removed and fixed for 24 h in Bouin's fixative. After tissue processing, paraffin sections of testis were cut at 5µm thickness and stained with hematoxylin-eosin, and it was analysed for light and electron microscopy.

3. RESULTS AND DISCUSSION

Chronic Toxic Effect of Lambda cyhalothrin and pretilachlor on Biomass of *L. mauritii*:

The effect of lower and higher sublethal concentrations of lambda cyhalothrin and pretilachlor exposure on the biomass of *L. mauritii* are presented in Tables 1 and 2. In control the initial weight (56.5 ±0.40 mg) of *L. mauritii* increased continuously up to 90th day. Instead of the results in T_1, growth was decreased up to 40th day (26.4 ± 0.55 mg) thereafter slowly started to gain weight at the end of the experiment (41.4±0.58 mg). Similarly in T2, biomass was drastically inhibited up to 40th day (15.4±0.52 mg) after that gradually increased (26.6±0.062 mg). In the experiment the biomass was significantly decreased ($P<0.01$) over control in all the periods of study. The growth of *L.mauritii* was exceedingly obstructed by higher concentration of lambda cyhalothrin (T2) than T1. Likewise in T3, biomass was reduced up to 40th day (34.36 ± 0.55 mg) thereafter slowly increased up to 90th day (56.3±0.53 mg). Identically in T4, biomass was highly inhibited up to 40th day (25.9±0.26 mg^{-10}) thereafter slowly increased (48.46±0.52 mg). Compared with control, growth of *L.mauritii* was severely affected by T4 than T3, T2 and T1.

The present results were supported by various pesticides on other species of earthworms. Maboeta et al. [11] and Inouye et al. [12] reported that long term exposure to metals and organochlorine pesticides affected the cocoon and hatchling production. Espinoza-Navarro and Bustos-Obregon [13] has been reported that high concentration of malathion reduced body weight of *E. fetida*. Zhou et al. [14] reported that "chlorpyrifos affected growth and reproduction of earthworms, but this is dependent on pesticide concentration and exposure period". Jordaan et al. [15] reported "the effect of pesticide azinphos-methyl on maturation, growth, reproduction, burrowing activity, and ChE inhibition in *E. Andrei"*. Zhou et al. [16] reported "a decrease growth and reproduction in earthworm *E. fetida andrei* when exposed to concentration of 5 mg/kg of mixture of cypermethrin and chlorpyrifos".

"After exposed to endosulfan, an isopod had decreased growth rate. It was explained by a reduced feeding that may have been a strategy to avoid the pesticide" Ribera et al. [17]. Kavitha et al. [18] observed that "organophosphorus insecticide monocrotophos highly inhibited growth and reproduction of *L. mauritii"*. Sherwan Taeeb Ahmed [19] was studied that "the impact of four pesticides on the earthworm *Lumbricu sterrestris*. The results indicated that the pesticides reduced weight, signs and symptoms of toxicity such as coiling, swollen body, sluggish movements and discharge of coelomic fluid. Many investigators have reported that high toxicity of

Table 1. Weight changes in *Lampito mauritii* exposed to sublethal lower (LC) and higher (HC) concentrations of Lambda cyhalothrin for 90 days

Lambda cyhalothrin	Mean weight per earthworm (mg)		
Exposure (days)	C	T1	T2
1 (Initial)	56.5±0.40	56.53±0.65	54.3±0.53
10	60.23±0.55	44.5±0.46	38.9±0.21
20	67.9±0.26	36.43±0.70	28.4±0.58
30	71.4±0.66	32.46±0.77	22.3±0.46
40	76.4±0.58	26.4±0.55	15.4±0.52
50	82.0±0.15	29.0±0.37	18.36±0.61
60	86.2±0.56	31.36±0.58	20.2±0.55
70	90.5±0.46	36.16±0.28	23.23±0.42
80	95.2±0.37	37.46±0.37	25.5±0.52
90	97.5±0.69	41.4±0.58	26.6±0.62

Values are mean of 10 observations ± S.E; C = Control (soil substrate alone); T1– 1.78 ppm (LC); T2– 4.45ppm (HC) The values are represented as mean ± SE of three replicates and were found to be statically significant at P < 0.05 and P < 0.01 (Based on one way ANOVA)

Table 2. Weight changes in *Lampito mauritii* exposed to sublethal lower (LC) and higher (HC) concentrations of pretilachlor for 90 days

Exposure (days)	Mean weight per earthworm (mg)		
Pretilachlor	C	T1	T2
1 (Initial)	56.5±0.40	54.23±0.49	56.63±0.76
10	60.23±0.55	45.43±0.52	43.3±0.53
20	67.9±0.26	41.2±0.63	36.4±0.52
30	71.4±0.66	38.1±0.72	32.1±0.80
40	76.4±0.58	34.36±0.55	25.9±0.26
50	82.0±0.15	37.4±0.62	29.0±0.26
60	86.2±0.56	43.8±1.93	30.96±0.24
70	90.5±0.46	46.0±0.29	37.63±0.46
80	95.2±0.37	52.63±0.52	45.1±0.51
90	97.5±0.69	56.3±0.53	48.46±0.52

Values are mean of 10 observations ± S.E; C = Control (soil substrate alone); T1– 2.68 ppm (LC); T2– 6.7 ppm (HC)
The values are represented as mean ± SE of three replicates and were found to be statically significant at P < 0.05 and P < 0.01 (Based on one way ANOVA)

Chlorpyrifos and Cypermethrin insecticides on many species of earthworms: moderate mortality to *Perionyx exacavtus"* Chakravorty and Kaviraj [20] and decreased body weight of *Eisenia fetida* Yasamin and Dsouza [6]. Booth et al. [21] observed "loss of weight of *Aporrectodea caliginosa* after exposure to organophosphate pesticides in field and laboratory also". Farrukh and Ali [22] clearly showed that "dichlorovos insecticide caused decrease in body weight of all groups of earthworms". Vinothini et al. [23] observed that "sublethal concentrations of thiacloprid insecticide decreased *L.mauritii* biomass, gut bacterial and fungal population, digestive enzymes such as cellulase, α amylase, β amylase, protease and lipase level at 5th, 15th, and 30th day of exposure than control". Capowiez and Berard [24] and Gomez-Eyles et al. [25] stated that the reason for reduced body weight is avoidance of feeding behaviour due to presence of unwanted substances in soil. Those authors noted that worms excavate less when exposed to imidacloprid, which means that they feed less. Alves et al. [26] reported that "high concentrations of insecticides such as imidacloprid, fipronil, and thiametoxam, and fungicides captan and carboxin+ thiram affected survival, reproduction, and behavior of *Eisenia Andrei".* The above author's findings evidenced to our results ie., reduced body weight of L.mauritii by the application of lambda cyhalothrin and pretilachlor.

Histopathology:

Histology of testis

Testes: 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. According to Hanumante [27] "the testes are ventral from the alimentary canal, on both sides of the nerve chord". Hanumante [27] described "the testes as white, lobed organs with a compact base carrying four to eight arms of spermatogonial cells". Lakhani Leena [10] reported that "spermatic follicles of testis of *L. mauiritii* were arbitrarily classified into four consecutive developmental stages depending on the size of spermatic follicles and approximate number of cells per cluster". Immature spermatic follicles: 1 to 16 cells or fewer cells are found in small clusters. the size of the cells, 29.22+1.2µ. Cells joined together by a small central cytoplasmic bridge, the cytophore. The cells are rounded and contained abundant cytoplasm. 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. 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. 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.

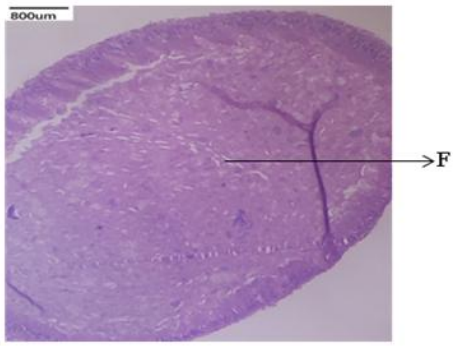

Fig. 1.

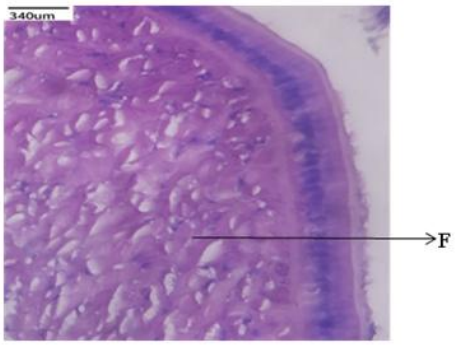

Fig. 2.

Figs. 1 and 2. Control testis showing normal tissue organization (x100), F=Follicle

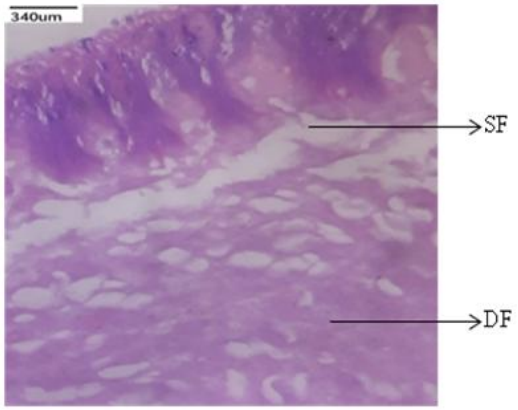

Fig. 3. Section of testis exposed to lambda cyhalothrin for 5th day (x100), SP= Space formation, DF= Disintegrated follicles

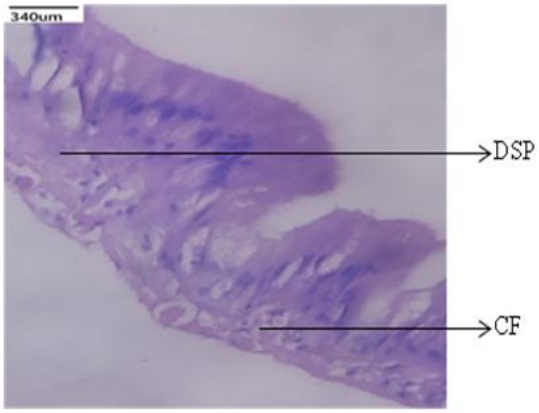

Fig. 4. Section of testis exposed to lambda cyhalothrin for 15th day (x100), DSF= Disintegrated spermatic follicles, CF=Clumped follicles

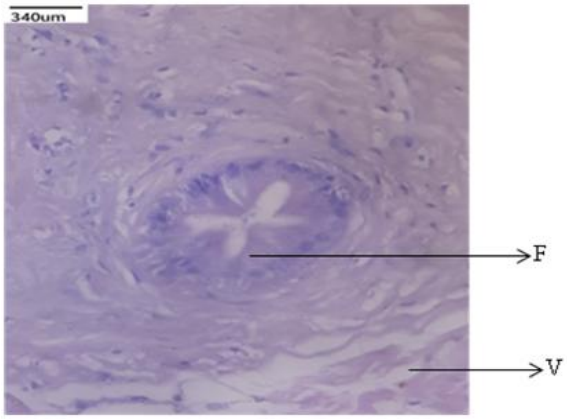

Fig. 5. Section of testis exposed to Lambda cyhalothrin for 30th day (x100), F= Follicles, V= Vacuoles

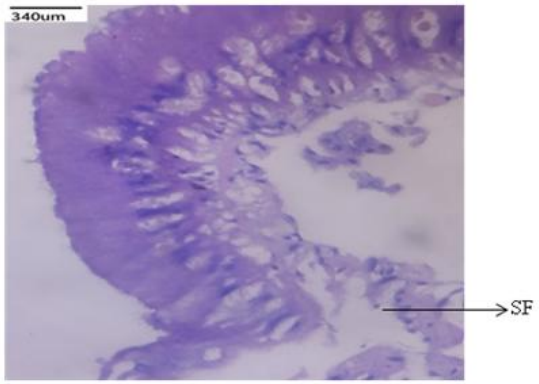

Fig. 6. Section of testis exposed to pretilachlor for 5th day (x100) , VF=Space formation

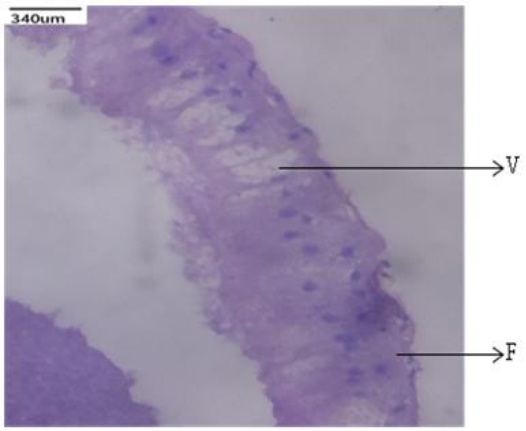

Fig. 7. Section of testis exposed to pretilachlor for 15th day (x100), V= Vacuoles, F=Follicles

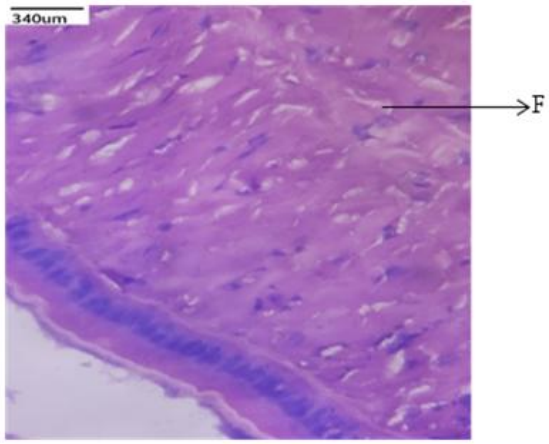

Fig. 8. Section of testis exposed to pretilachlor for 30th day (x100), F=follicles

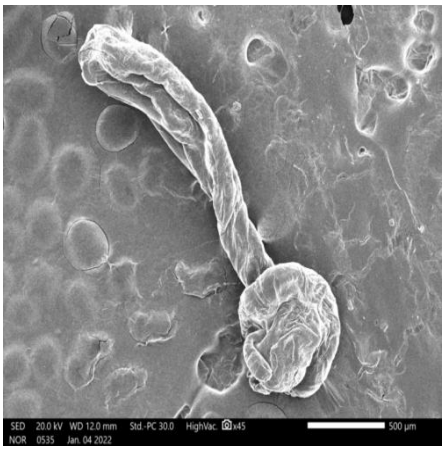

Fig. 9.

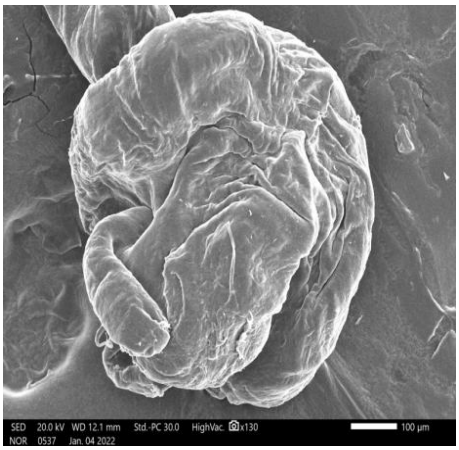

Fig. 10.

Figs. 9 and 10. SEM study of *Lampito mauritii* testis (Control). (500 µm and 100 µm)

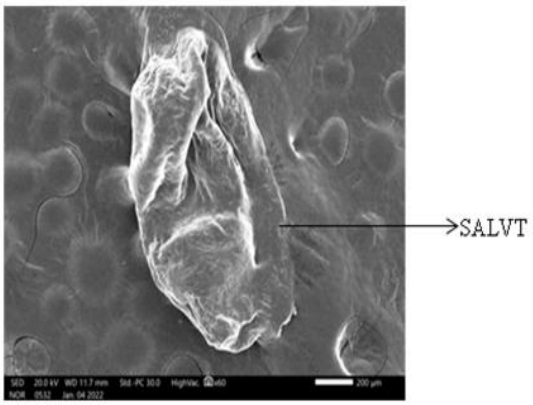

Fig. 11. SEM study of *Lampito mauritii* testis exposed to lambda cyhalothrin 5th day (200 µm) SALVT= Shrinked and large vacuolated testis

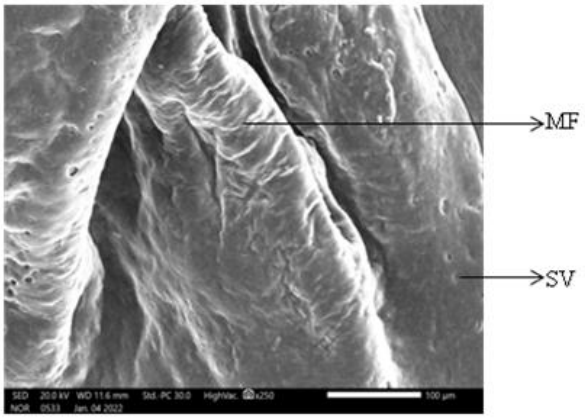

Fig. 12. SEM study of *Lampito mauritii* testis exposed to lambda cyhalothrin 15th day (100 µm) MF=Many folding, SV= Small Vacuoles

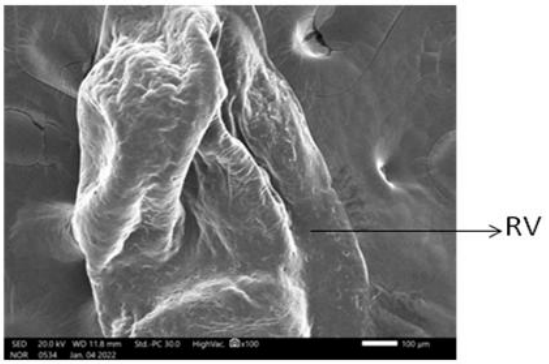

Fig. 13. SEM study of *Lampito mauritii* testis exposed to lambda cyhalothrin 30th day (100 µm), RV=Recovered Vacuoles

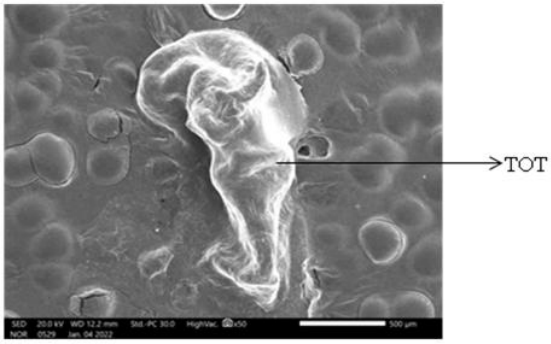

Fig. 14. SEM study of *Lampito mauritii* testis exposed to pretilachlor 5th (200 µm and 100 µm), TOT= Thickness of tissues

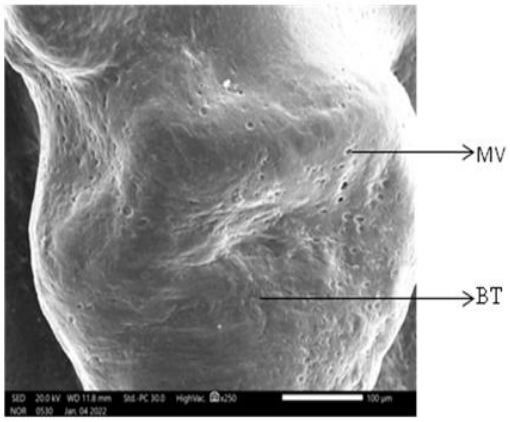

Fig. 15. SEM study of *Lampito mauritii* testis exposed to pretilachlor 15th day (100 µm) MV=More Vacuoles and BT= Bulged tissues

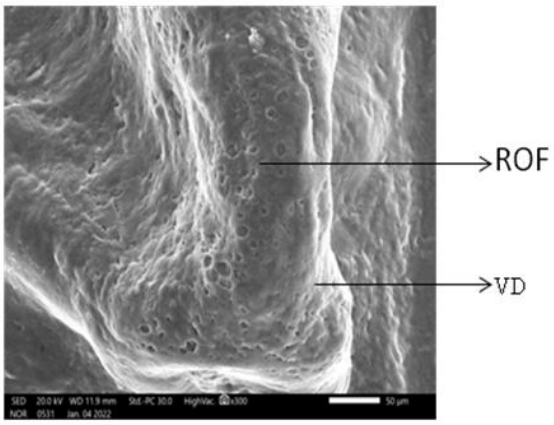

Fig. 16. SEM study of *Lampito mauritii* testis exposed to pretilachlor 30th day (50 µm) ROF=Regeneration of follicles, VD=Vacuoles decreased

The Figs. 1 and 2 showed histology of testis. 5 days lambda cyhalothrin exposed testis showed vacuoles formation and disintegrated follicles (Fig. 3). On 15th day, disintegrated spermatic follicles and vacuolization, follicles broken at many places clumped follicles were observed (Fig. 4). 30 days of exposure showed follicles development were slowly started, vacuolization also decreased (Fig. 5). Recovered testis were observed. Vacuoles formation and broken follicle cells were reduced.

After exposed to pretilachlor, on 5th day testis showed tissue damages, vacuoles formation and damaged spermatic follicles (Fig. 6). On 15th day, displacement and damaged cells were observed (Fig.7). On 30th day, recovery was observed ie., regeneration of follicles, less vacuoles (Fig. 8).

Electron microscopic images (Figs. 9 and 10) indicated that the untreated (control) earthworm species have well definite structure of testis. On 5th day exposure, due to toxic effect shrinked and a large vacuolated testis was found (Fig. 11). On 15th day, many foldings and small vacuoles were observed (Fig. 12). Instead of this, due to degradation of Lambda cyhalothrin, recovered above observed damages (Fig.13) ie, decreased foldings and vacuoles. Pretilachlor exposed testis showed thickened tissues on 5th day, more vacuoles and bulged tissues on 15th day and the undulations were decreased on 30th day exposure (Figs. 14, 15 and 16).

Many researchers studied that effect of pesticides on various tissues of earthworms. Their results were supported to our results. Any authors not had done the effect of lambda cyhalothrin and pretilachlor on testis of *L. mauritii*. Dimethoate at 0.6 ppm and endosulfan at 0.003 ppm concentrations impaired testicular function of *E. kinneari* Leena Lakhani et al. [28]. Reddy and Rao [29] reported that organophosphate

profenofos altered histology of *Eiesenia fetida*. Mazdak Razi1 [30] reported that "Glyphosate (GP) caused adverse effects on testicular tissue, spermatogenesis, sperm viability and abnormality which potentially can cause infertility on rat". Leena Lakhani [31] observed that Dimethoate insecticide severely affected *Eudichogaster kinneari* histomorphology of NSCs of brain. Suneel Kumar and Satyendra M. Singh [8] suggested that phorate exert its toxic effects on morphological and structural integrity of body wall of both earthworms *Metaphire posthuma* and *Lampito mauritti*. Gobi Muthukaruppan et al. [32] observed "glandular cell enlargement in the intestinal region of *perionyx ansibaricus* after exposure to butachlor and suggested that which may massively affect food intake and which in turn may indirectly inhibit the earthworms' growth and reproductive capacity". Sherwan Taeeb Ahmed [19] showed that four pesticides Cyren, Ridomil, Triplen and Mamba significantly decreased sperm numbers of Lumbricus terristris. Sophie et al. [33] showed that "low concentration dieldrin caused structural damage, especially to the nucleus of the sperm which may cause several changes in morphology, motility, and sperm density". Kavitha et al. [34] reported that "organophosphate insecticide, monocrotophos was affected microbial population and histology of intestine upto 15 days due to toxicity of monocrotophos. Thereafter, they were recovered by presence of some of the pesticide degrading bacteria and fungi in the gut".The results of the study showed that insecticide lambda cyhalothrin highly inhibited the biomass and histology of testis than herbicide pretilachlor.

4. CONCLUSION

The present observations are very important to note that profound changes were observed in biomass and histology of testes of *L. mauritii* after exposure of lambda cyhalothrin and pretilachlor. Biomass and histology is best indicator tools to find out the of pesticides on earthworm population in soil. Many researchers reported that pesticides highly affected growth, reproductive ability, digestive and antioxidants enzymes level, microbial population in gut and soil and also histology of various organs of *L.mauritii*. So we should be avoid these harmful pesticides application in agricultural land thereby protect our soil fertility.

ACKNOWLEDGEMENT

The authors are thankful to the Head of the Department of Zoology, Annamalai University, Annamalai Nagar, 608 002, Tamil Nadu, India for providing the necessary facilities to carry out this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. United N. World Population Prospects The 2004 Revision Highlights. World Population Prospects. The 2004 Revision. Highlights. 2005.
2. Laxmi A. Pesticides in India, Risk Assessment and Policy. In Second European Conference on Ecotoxicology. 1992;1-15.
3. Bartlett MD, Briones MJ, Neilson R, Schmidt O, Spurgeon D, Creamer RE. A critical review of current methods in earthworm ecology: from individuals to populations. European Journal of Soil Biology. 2010;1;46(2):67-73.
4. Lourenço JI, Pereira RO, Silva AC, Morgado JM, Carvalho FP, Oliveira JM, Malta MP, Paiva AA, Mendo SA, Gonçalves FJ. Genotoxic endpoints in the earthworms sub-lethal assay to evaluate natural soils contaminated by metals and radionuclides. Journal of Hazardous Materials. 2011;15; 186(1):788-95.
5. Hundal SS, Kaur R, Kaur A. Effect of chlorpyrifos on survival, growth and reproductive performance of Eudrilus eugeniae (Kinberg). Journal of Applied and Natural Science. 2016 ;1;8(3):1618-22.
6. Yasmin S, D'Souza D. Effects of pesticides on the growth and reproduction of earthworm: a review. Applied and Environmental Soil Science. 2010;1;9.
7. Anshu , Singh D, Mahima and shewatha .Individual and Combined Toxic Effects of Herbicides on Growth Parameters and Fecundity of Eisenia fetida. International Journal of Current Microbiology and Applied Sciences. 2020; 9 :12
8. Kumar S, Singh SM. Morpho-histopathological response of phorate-an organo-phosphorous pesticide on the integumentary musculature of an epigeic earthworm, Eisenia fetida. International Journal of Current Microbiology and Applied Sciences. 2017;6(4):2048-53.
9. Joshi N, Dabral M. Life cycle of earthworms Drawida nepalensis, Metaphire houlleti and Perionyx excavatus under laboratory controlled conditions. Life Science Journal. 2008;5(4): 83-6.

10. Leena L. Effect of Azodrin on the testes of the earthworm Eudichogaster kinneri (Stephenson): a histological and Histochemical profile. International Research Journal of Biological Sciences.2013;2(9):54-8
11. Maboeta MS, Reinecke AJ, Reinecke SA. Effects of low levels of lead on growth and reproduction of the Asian earthworm *Perionyx excavatus* (Oligochaeta). Ecotoxicology and Environmental Safety. 1999;1;44(3):236-40.
12. Inouye LS, Jones RP, Bednar AJ. Tungsten effects on survival, growth, and reproduction in the earthworm, Eisenia fetida. Environmental Toxicology and Chemistry: An International Journal. 2006;25(3):763-8.
13. Espinoza-Navarro O, Bustos-Obregón E. Effect of malathion on the male reproductive organs of earthworms, Eisenia foetida. Asian Journal of Andrology. 2005;7(1):97-101.
14. Zhou SP, Duan CQ, Hui FU, Chen YH, Wang XH, Yu ZF. Toxicity assessment for chlorpyrifos-contaminated soil with three different earthworm test methods. Journal of Environmental Sciences. 2007;1;19(7):854-8.
15. Jordaan MS, Reinecke SA, Reinecke AJ. Acute and sublethal effects of sequential exposure to the pesticide azinphos-methyl on juvenile earthworms (*Eisenia andrei*). Ecotoxicology. 2012;21(3):649-61.
16. Zhou S, Duan C, Michelle WH, Yang F, Wang X. Individual and combined toxic effects of cypermethrin and chlorpyrifos on earthworm. Journal of Environmental Sciences. 2011; 23(4):676-80.
17. Ribera D, Narbonne JF, Arnaud C, Saint-Denis M. Biochemical responses of the earthworm Eisenia fetida andrei exposed to contaminated artificial soil, effects of carbaryl. Soil Biology and Biochemistry. 2001;1;33 (7-8):1123-30.
18. Kavitha V, Ramalingam R, Anandi V. Effect of endosulfan on the bacterial and fungal populations in the gut of the Indian earthworm Lampito mauritii (Kinberg). J Sci Trans Environ Technov. 2008;2(2):78-81.
19. Ahmed ST. The impact of four pesticides on the earthworm Lumbricus terrestris (Annelida; Oligochaeta). International Journal of Current Research and Review. 2013;1;5(21):1.
20. Das Gupta R, Chakravarthy pp, Kaviraj A. Studies on relative toxicities six insecticides on epigeic earthworm, Perionyx excavates. Bulletin of Environmental Contamination and Toxicology. 2010;85(1):83-6.
21. Booth LH, O'Halloran K. A comparison of biomarker responses in the earthworm Aporrectodea caliginosa to the organophosphorus insecticides diazinon and chlorpyrifos. Environmental Toxicology and Chemistry: An International Journal. 2001; 20(11):2494-502.
22. Farrukh S, and Ali. Effects of dichlorovos organophosphate on growth, reproduction, and avoidance behavior of earthworm Eisenia foetida. Iranian Jouranal of Toxicology 2011; 5(14):495- 501.
23. Vinothini M, Kavitha V, Monisha J, Ramya RR, Anandhan R. Toxicity of thiacloprid (Insecticide) on biomass, gut microbial population and digestiveenzymes activity of an Indian Earthworm, *Lampito mauritii* (Kinberg) (Annelida: Oligocheta) International Journal of Entomology.2022; 2455-4758.
24. Capowiez Y, Bérard A. Assessment of the effects of imidacloprid on the behavior of two earthworm species (*Aporrectodea nocturna* and *Allolobophora icterica*) using 2D terraria. Ecotoxicology and Environmental Safety. 2006;1;64(2):198-206.
25. Gomez-Eyles JL, Svendsen C, Lister L, Martin H, Hodson ME, Spurgeon DJ. Measuring and modelling mixture toxicity of imidacloprid and thiacloprid on Caenorhabditis elegans and Eisenia fetida. Ecotoxicology and Environmental Safety. 2009;72(1):71-9.
26. Alves PR, Cardoso EJ, Martines AM, Sousa JP, Pasini A. Earthworm ecotoxicological assessments of pesticides used to treat seeds under tropical conditions. Chemosphere. 2013; 90(11):2674-82.
27. Hanumante M.M. On the anatomy of the reproductive system of the earthworm: Perionyx excavatus. A1arathwada University.!. Sci. (lJio! SCi.) 1975;15(8): 193-197.
28. Lakhani L. Effect of certain insecticides on neuroendocrine system and gonads in an earthworm Eudichogaster kinneari (Stephenson) (Doctoral dissertation, Ph. D. Thesis, submitted to Vikram University, Ujjain, India).
29. Reddy NC, Rao JV. Biological response of earthworm, Eisenia foetida (Savigny) to an organophosphorous pesticide, profenofos. Ecotoxicology and Environmental Safety. 2008;1;71(2):574-82.
30. Razi M, Najafi G, Feyzi S, Karimi A, Shahmohamadloo S, Nejati V. Histological and histochemical effects of Gly-phosate on testicular tissue and function. Iranian Journal of Reproductive Medicine. 2012;10 (3):181.
31. Lakhani L. Impact of dimethoate on brain of the earthworm Eudichogaster kinneari

(Stephenson): A histological profile. Environment Conservation Journal. 2015;18; 16(1-2):1-8.

32. Muthukaruppan G, Janardhanan S, Vijayalakshmi G. Sublethal Toxicity of the Herbicide Butachlor on the Earthworm Perionyx sansibaricus and its Histological Change. Journal of Soils and Sediments. 2005;5(2):82-6.
33. Sophie A, Reinecke AJ, Froneman ML. The effects of dieldrin on the sperm ultrastructure of the earthworm Eudrilus eugeniae (Oligochaeta). Environmental Toxicology and Chemistry: An International Journal. 1995;14(6):961-5.
34. Kavitha V, Anandhan R, Alharbi N S, Kadaikunnan S, Khaled J M, Almanaa T N, Govindarajan M.. Impact of pesticide monocrotophos on microbial populations and histology of intestine in the Indian earthworm *Lampito mauritii* (Kinberg). Microbial Pathogenesis. 2020;139. Available:https://doi.org/10.1016/j.micpath.2019.103893.