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Effects of Pesticide Lambda-Cyhalothrin on the Histo-Architecture of Earthworm- *Eudrilus eugeniae*

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Abstract: Agricultural expansion and increased use of pesticides often lead to affect the soil ecosystem. Most of the pesticides are non-specific and affects the non-target organisms. Such pesticides cause morphological, behavioural, and physiological changes in many soil dwelling organisms like earthworms. The pesticide like Lambda-Cyhalothrin was used to study its effect on the histo-architectural changes in the earthworm *E. eugeniae.* An investigational study was conducted for a period of 3 months from June-2022 to August-2022 in the laboratory conditions. The histological study was performed by exposing the adult worms to the soil spiked with different concentrations of pesticide in separate containers for a period of 120 h and 15 days observation. It was observed that all the worms exposed to the treated soil were alive, but their histo-architecture of mid gut and clitellum regions were damaged, and their average body weight reduced by 0.02 g. It is suggested to use this insecticide in an eco-friendly safe limit.

Keywords: Lambda-cyhalothrin, *Eudrilus eugeniae*, Chlorogogenous tissue, Histo-architectural, Pesticide

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Introduction

The extensive use of pesticides to protect the agricultural crops from insects, fungi, and other pests often leads to a loss of biodiversity (Hole *et al*., 2005). The application of pesticides causes morphological, behavioural, and physiological changes in reproductive, nervous, respiratory and osmoregulatory organs of many soil organisms and contaminate the soil which exerts a harmful impact on various invertebrates (Fingerman, 1984; Mangala *et al*., 2009; De Silva, 2009). Insecticides are found to be the most lethal toxic class of pesticides to non-target organisms (Aktar *et al*., 2009; Mahmood, 2016). As the earthworms are incredible source for the fertility of soil, they

also play a significant role as indicator in determining the health of the soil. Such earthworms are being exposed to various pesticides applications and have immensely been affected- histologically, behaviourally, reproductively and developmentally (Booth and O'Halloran, 2001; Muthukaruppan *et al*., 2005; Oluah *et al*., 2010; Rani *et al*., 2017; Latha and Basha, 2019). Different types of synthetic pesticides have extensively been used to protect ragi, paddy, jowar, chilli, ground nut etc. from pests/insects infestation in the agricultural field around Tumakuru district. To find the impact of one such pesticide -- Lambda-Cyhalothrin on earthworm, the present study was conducted in Tumakuru district.

Materials and Methods

Test organism: The earthworm *Eudrilus eugeniae*, was selected for the experiment purpose based on its availability, size, prolific breeding and surface-dwelling nature. The vermiculture of the selected species was maintained in the earthen pot measuring 77x36.5x29 cm^3 (L x W x H) containing the mixture of soil and farmyard manure in 3:1 with moisture content of 35 to 40%. The worms were exposed to regular 12 h day and night cycle every day. The experiment was conducted in the laboratory at Department of Studies and Research in Zoology (DOSR), Tumkur University between June-2022 to September 2022 at constant room temperature and moisture.

The matured worms from the culture were utilized for finding LC_{50} and histo-architectural studies upon exposure to Lambda-Cyhalothrin 5% EC.

Pesticides: For experimentation, Lambda-Cyhalothrin 5% EC, a synthetic pyrethroid insecticide was selected. It is in EPA toxicity class II, it starts working immediately upon contact or ingestion, resulting in fast insect knock-down and kill.

Toxicity Test (*LC_{50}*): Acute toxicity for earthworm was determined for the selected pesticide by following OECD- Earthworm, Acute toxicity Tests-207 Guideline by adapting simple paper contact toxicity test (OECD-1987) on *Eudrilus eugeniae*.

The Lambda-Cyhalothrin was diluted with distilled water at different concentrations and sprayed to the blotting paper taken in watch glass and the degutted worms were left and kept for 24 h observations. The behaviour and morphological changes were observed regularly. The experiments were repeated by exponential increase in their concentration till 50% of the worms dead. Each concentration of three replicates with 6 animals in each replica was maintained. Finney's (1971) probit method using graphical analysis was followed to calculate the LC-50 value. In this study, the LC_{50} value was 0.25 ml/l.

Soil preparation and histo-pathological studies: The mixture of 1 kg soil and dry cow dung powder (3:1) spiked with different concentrations of 0.04 ml (0.2 ml/l), 0.05 ml (0.25 ml/l), and 0.06 ml (0.3 ml/l) Lambda-Cyhalothrin diluted with 200 ml of water was taken in separate plastic boxes measuring 21x10x8 cm^3 (L x W x H) of 2 replicates each. The degutted 10 earthworms were left in each of these replicas. The control soil mixture was also maintained with 10 earthworms under laboratory conditions.

Tissue preparation and processing: Earthworms from each of the spiked soil and control soil were removed randomly from the culture boxes after 120 h and 15 days of pesticide exposure. Later the worms were degutted and dissected out the midgut and clitellum for tissue preparation. The sections were cut and stained with the haematoxylin-eosin for histological studies.

The prepared histological slides were scanned for intact and even staining sections of midgut and clitellum region by using Lawerence-Mayo digital microscope and photomicrography was done and analyzed for changes in histo-architecture.

Results

Toxicity test- LC_{50}: The effect of toxic chemical

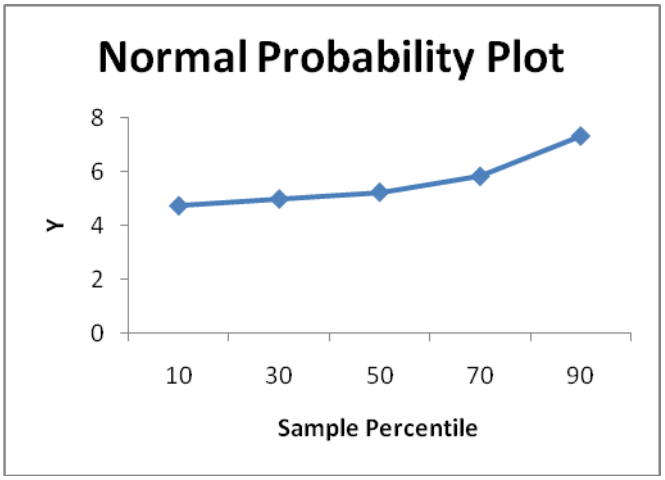

Fig. 1: Probit regression R^2 value for LC_{50} of Lambda-Cyhalothrin.

on the biological system is close-related. In these experiments, the concentration of the insecticide that kills 50% of the animals is called as LC_{50} (Lethal concentration 50). The lower the LC_{50} value the more lethal is the pesticide. LC_{50} is calculated under controlled laboratory condition by administration of the specific dose within a particular time.

To estimate the toxicity of the pesticides on an organism toxicity test was conducted. In Lambda-Cyhalothrin its lethality is shown at the concentration of 0.25 ml/l for 24 h. The LC_{50} concentration for the pesticide was further calculated by using probit regression R^2 analyses – IBM SPSS statistics version 26 software (IBM Corp, 2019) (Fig. 1).

LC_{50} studies revealed that the earthworms are highly susceptible to the selected pesticide causing immobility, rigidity and a significant effect on body weight reduction, growth and reproduction by disrupting various physiological activities.

Effects of Lambda-Cyhalothrin on body weight of E. eugeniae: On exposing the earthworm *E. eugeniae* to the pesticide Lambda-Cyhalothrin, no mortality was observed at any of the selected concentrations -- 0.2 ml/l, 0.25 ml/l, and 0.3 ml/l.

Worm growth at various exposures over the 15 days of observation is presented in Table 1. The average weight of 10 worms before the experiment in control group was 0.355 g and the average weight of 10 worms that were considered for further treatment was 0.3 g, 0.29 g, and 0.37 g under different concentrations of 0.2 ml/l, 0.25 ml/l and 0.3 ml/l Lambda-Cyhalothrin, respectively.

At the end of the experiment, the average weight of the control group worms was shown to be increased by 0.02 g. After 15 days of exposure to Lambda-Cyhalothrin, the average weight of each set of 10 worms at different concentrations was reduced. The decreased body weight difference was found to be 0.01 g, 0.02 g and 0.04 g for 0.2 ml/l, 0.25 ml/l and 0.3 ml/l concentrations, respectively. The more body weight reduction was noticed at the Lambda-cyhalothrin concentration of 0.3 ml/l. As the concentration of Lambda-cyhalothrin increases the more reduction in the average body weight of the *E. eugeniae* was recorded in the order of 0.3>0.25>0.2 ml/l of concentration (Table 1, Fig. 2). The experimental result has shown that the Lambda-Cyhalothrin negatively influenced on the growth of *E. eugeniae* by reducing its average body weight.

Study of histo-architectural changes: Changes in the histo-architecture of mid gut and clitellum of *E. eugeniae* on exposure to different concentrations

Table 1: Average body weight of earthworm before and after treatment with Lambda-Cyhalothrin

Concentration of pesticides	Average weight of worms before treatment (10 worms)	Average weight of worms after treatment (10 worms)
Blank(control)	0.35	0.37
0.2 ml/l	0.3	0.29
0.25 ml/l	0.29	0.27
0.3 ml/l	0.37	0.33

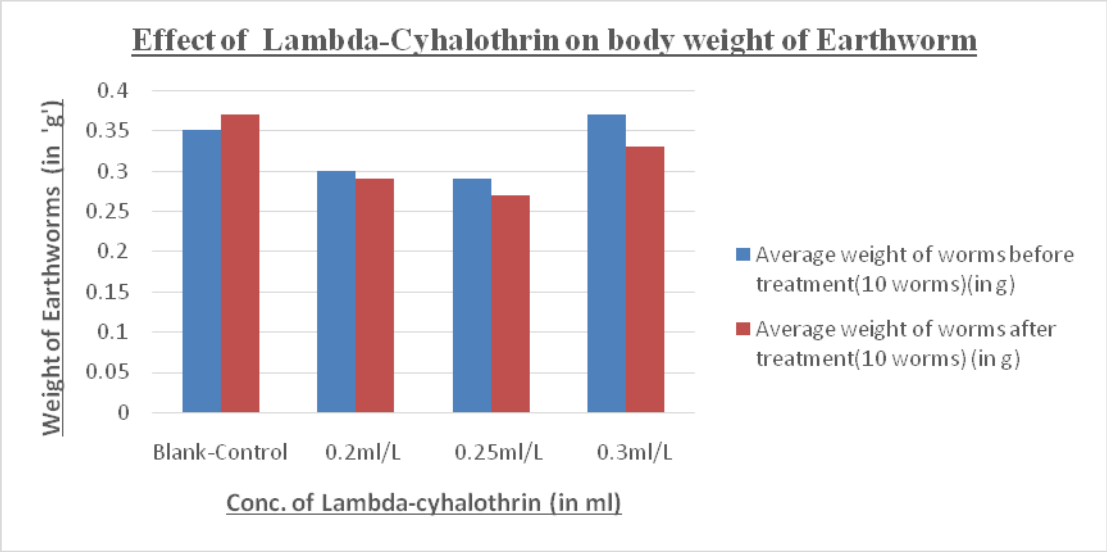

Fig. 2: Body weight of *E. eugeniae* before and after treatment with Lambda-Cyhalothrin.

of Lambda- Cyhalothrin were recorded. Epidermal tissues of midgut are slightly damaged at lower concentration and seen to be greatly damaged as the concentration of pesticide increases. Intestine has shown to be less damaged to greatly reduced in size with distorted shape of lumen with blood sinuses on increased concentration of the selected pesticide and prolonged exposure (0.3 ml/l for 15 days) (Figs. 4, 5, 6). Chlorogogenous tissue was also noticed to be highly distorted at higher concentration and prolonged exposure to the treated soil. Epidermal mucus glands of clitellum were enlarged, longitudinal muscles were splitted and intestinal epithelium was highly constricted at higher concentration (0.25 ml/l and 0.3 ml/l) of treated soil (Figs. 4, 5, 6) than the control soil (Fig. 3).

In the present study, extensive alterations in tissues of the body wall, intestinal tract and clitellum structures of the earthworm were noticed on exposure to different concentrations of Lambda-Cyhalothrin. The severity of alterations in the tissues of organisms increased as the concentration and exposure period to insecticide increases. The extensive histo-architectural alterations of mid gut and clitellum regions were noticed at LC_{50} (0.25 ml/l) and its above concentrations (0.3 ml/l). The earthworms exposed to sub-lethal dose were least damaged.

Discussion

The applied pesticides/insecticides in the agriculture field enter the non-target organisms such as earthworm body via direct feeding of

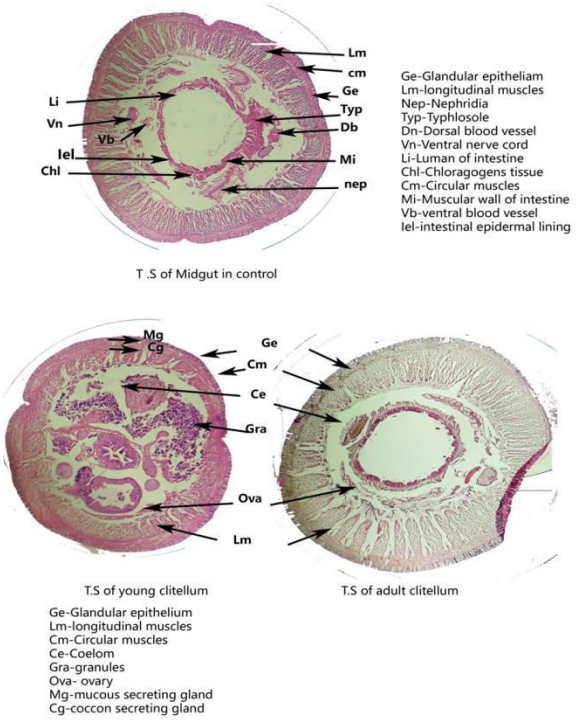

Fig. 3: Showing histo-architecture of *E. eugeniae* exposed to control soil.

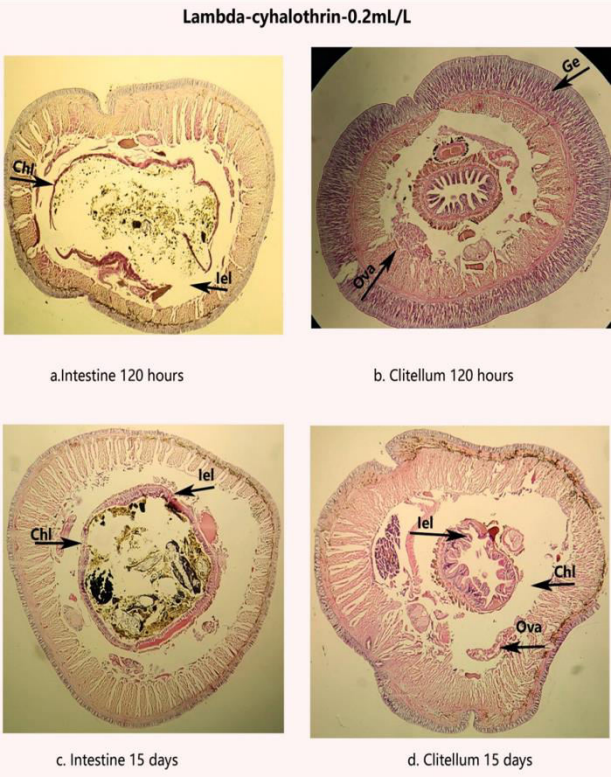

Fig. 4: Histo-architectural changes when exposed to 0.2 ml/l of Lambda-Cyhalothrin.

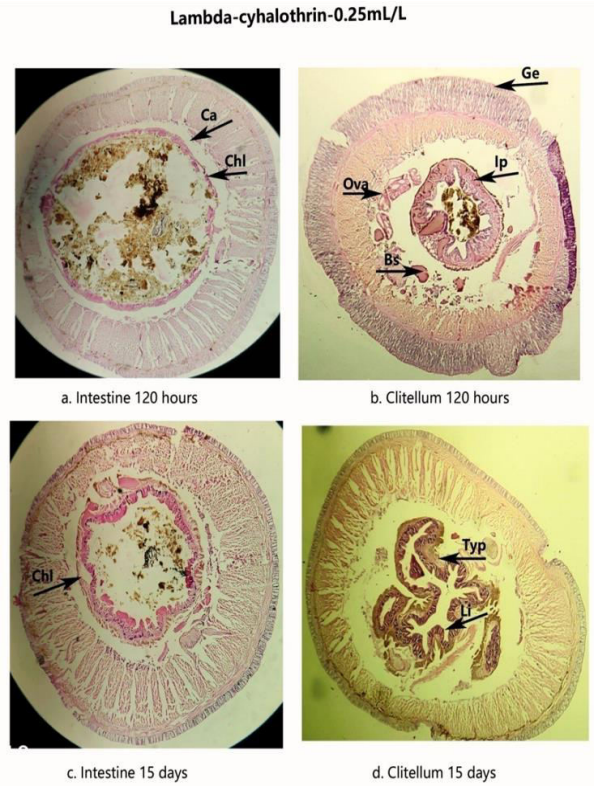

Fig. 5: Histo-architectural changes when exposed to 0.25 ml/l of Lambda-Cyhalothrin. Arrow indicates histological changes.

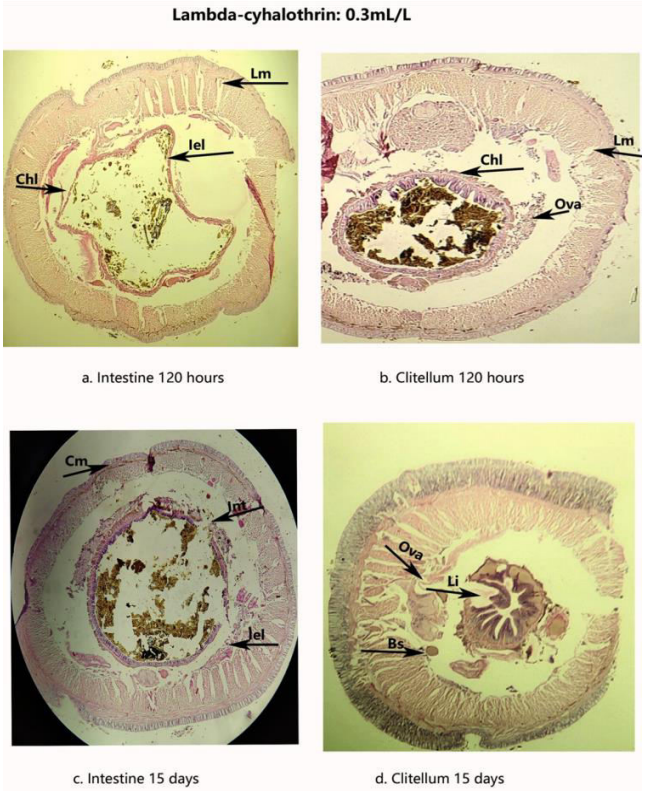

Fig. 6: Histo-architectural changes when exposed to 0.3 ml/l of Lambda-Cyhalothrin. Arrow indicates histological changes.

treated dead plant parts as well as through pesticide amended soil. Earthworms are impacted by pesticides at all organization levels i.e., pesticides disrupt enzymatic activities, increase individual mortality, decrease fecundity and growth, change individual behavior such as feeding rate and decrease the overall community biomass and density (Celine *et al*., 2013).

The body weight loss may indicate a reduced food intake, by which earthworms regulate intake of pesticides and lead to growth inhibition (Mosleh *et al*., 2003). Earthworms are affected by pesticides either through skin contact or by feeding on contaminated litter in soil. Primarily, these toxicants passing through the skin have direct contact to the contaminated soils and skin is considered as a significant route to uptake of toxicants (Saxe *et al*., 2001; Jager *et al*., 2003; Vijver *et al*., 2003). Epidermis and cuticle represent a primary barrier that protects earthworm's body from environment and responsible for transport of ions. In this study, the histo-pathological evaluation revealed that the cuticular membrane and ectodermal layers were disintegrated and profusion of glandular epithelium. It is evident from earlier reports that the morphological and histological changes were prominent when earthworms were exposed to different toxic pesticides. Earthworms ingest large amounts of soil and therefore elective non-target organisms exposed to pesticides through their external and internal surfaces. Thus, morphological or anatomical changes in these organisms are one of the suitable indicators for monitoring the effectiveness of soil pollutants (Amaral *et al*., 2006).

Gupta and Sundaram (1988) reported carbaryl induced changes in the chloragocytes of *Pheretima posthuma*. Chloragogen tissues are food reserves and may accumulate Ca, P, S, Cl, Zn, K and Fe (Prento, 1979; Fisher and Trombitas, 1980; Morgan, 1981; Morgan and Winters, 1982), but the amounts and ratios of the elements are variable. In the current study, the necrosis of chloragogenous tissue of *E. eugeniae* on exposure to different concentrations of Lambda-Cyhalothrine was observed. The necrosis is characterized by cytoplasmic swelling, mitochondrial damage and an excessive loss of osmotic regulation caused by the loss of cellular energy reserves (Bowen and Lochschin, 1981). Our results are in congruent with the findings of Gobi Muthukarappan *et al*. (2005) on *Perionyx sansibaricus* exposed to the herbicide- Butachlor for over 60 days.

Intestinal villi increase the absorption area of food. The anomalies of intestinal villi tend to reduce the absorptive area of food. In the present study, the intestinal villi were found to be shrinked and damaged on exposure to the selected pesticide. Similar observations were noticed by Anu *et al.* (2018) on exposure of earthworms to Fipronil. Oluach *et al.* (2010) also reported the destruction of chloragogenous layer and inner epithelium of intestine of *Nsukkadrilus mbae* exposed to soil treated with Atrazine. The presence of Lambda-Cyhalothrine in *E. eugeniae* may decrease the area of food digestion, which tend to reduce the average body weight and cocoon production of the treated worms.

Conclusion

In this study the worms were significantly affected by pesticide on their average body weight and histo-architecture of midgut and clitellum. The average body weight of the treated worms was decreased as compared to control group. The Lambda-Cyhalothrin treated soil affected the internal tissues of midgut and clitellum significantly by necrosis. Our study concludes that the Lambda-Cyhalothrin has proved to be more toxic on *E. eugeniae* with least LC_{50} (0.25 ml/l) and cause not much mortality in the treated worms but on the other hand it inhibited the growth and reproductive capacity of the worms under laboratory conditions. However, the morpho-logical and histological changes observed in *E. eugeniae* cannot be assessed lonely by the influence of the selected insecticide- Lambda-Cyhalothrin in the natural field conditions. But these changes may also be influenced by the

edaphic factors, temperature and species of the earthworm. Temperature and soil types also play a significant role in degree of toxicity of pesticides (Bindesbol *et al*., 2009; De Silva *et al*., 2009). It needs further investigation under natural field conditions to assess the toxicity of insecticides on earthworms.

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