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**TOXICOLOGICAL STUDY OF DELTAMETHRIN AND ITS METABOLITES
ON EARTHWORM (*EISENIA FETIDA*)**

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ABSTRACT

Deltamethrin, a synthetic pyrethroid is used in agricultural to control pests on variety of crops. It is a broad spectrum insecticide that acts mainly on the nervous system of vertebrates and invertebrates. Earthworms are common soil organisms that play an important role in improving texture, structure and soil aggregation, physical and chemical properties of the soil with improved fertility. However, they are sensitive and susceptible to agrochemicals because they lack hard cuticle around their body. The present paper deals with the toxicological studies of deltamethrin and its metabolites benzene dicarboxylic acid, benzene and propane that were produced by a newly isolated strain of *Paracoccus siganidrum* APM1. The toxicity was assessed by 48 hrs filter paper contact test, 14 days soil test and histopathological methods. The results of filter paper contact test revealed that the deltamethrin and its degraded metabolites vary in their contact toxicities. Earthworms were more susceptible to deltamethrin than their metabolites. The LC₅₀ value of deltamethrin was 7 ppm while LC₅₀ value of metabolites was 30 ppm. Fourteen days soil test are showed that at 7 ppm concentration of deltamethrin, half numbers of earthworms were died after 14 days and with the increase in the concentration, mortality was increasing. At 10 ppm concentration, all earthworms were died. However, half numbers of earthworms were died after 14 days at the metabolites concentration of 30 ppm. The histopathological results also indicated the adverse effects of deltamethrin and very little effect of metabolites on the morphological properties and structural integrity of the tissues. Thus, it was concluded that the metabolites of deltamethrin are less toxic to earthworm than the deltamethrin.

KEYWORDS: deltamethrin, metabolites, histopathology, filter paper contact test, 14 days soil test.

1.INTRODUCTION

Synthetic pyrethroids occur constantly in some environments due to their wide use and repeated application. Due to their extremely hydrophobic properties, pyrethroids toughly bind to soil constituents and organic material. This permits pesticides to leach into the ground water and to form residues. This in turn is harmful to the ecosystem. Deltamethrin has been included in Type II pyrethroid. It induces long lasting inhibition of the sodium channel activation gate by its α -cyano group which causes extended penetrability of the nerve to sodium (Joy R M., 1994).

Earthworms are common soil organisms that play a major role in increasing texture, structure and soil aggregation, physical and chemical properties of the soil (Wang *et al.*, 2012).

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They represent up to 60 to 80% of the total animal biomass in soil (Olette *et al.*, 2008; Jouquet *et al.*, 2010). Earthworms are sensitive and susceptible to soil chemicals especially agrochemicals because they lack hard cuticle around their body (Lanno *et al.*, 2004; Nahmani *et al.*, 2007). Pyrethroids cause significant reduction in earthworm populations (Lukowicz - Ratajczak J. and Krechniak J., 1992). Earthworms are also highly susceptible to changes of ecological influences, mostly those intrinsic to the soil and earthworm behavior can therefore imitate soil contamination. It is known that earthworms reflect changes taking place in the soil, particularly changes of soil physical, chemical and biological properties, as well as changes in the water, air and thermal systems (Suthar *et al.*, 2008). Globally earthworms are used as bio-indicators for investigating chemical environmental pollution.

There is a vital advantage of using microorganisms for degradation of pesticides. This is due to their diversity, wide dispersal and adaptation of variable metabolic pathways. The gene clusters are involved in microbial degradation. Many bacteria have potential to degrade the pyrethroid pesticides including deltamethrin. In the present study, deltamethrin and its metabolites such as benzene dicarboxylic acid, benzene and propane produced by a newly isolated strain APGM1 of bacteria *Paracoccus signidrum* was used to determine the toxicity of deltamethrin and its metabolites to earthworm.

2.MATERIALS AND METHODS

Earthworms:

Earthworms (*Eisenia fetida*) used in the experimental work were obtained from the earthworm culture farm in Kolhapur, India. Healthy adult worms with a well developed clitellum (average weight, 200-250 mg) were used for study.

48 hours Contact Filter Paper Test:

Different concentrations of deltamethrin pesticide (1 to 10 ppm) were prepared in distilled water. Similarly, different concentrations of metabolites (1 to 10 ppm and above) from ethyl acetate extract of degradation medium were prepared. Filter paper pieces were treated with the different concentrations of the pesticides as well as metabolites and placed in a petridish. Earthworms were placed on the top of filter papers. The set up was replicated three times for each of the concentration and a similar design was set up using distilled water as a control. The dishes were incubated in the dark at $20 \pm 1^{\circ}\text{C}$ for 48 hours and mortality was recorded at 12 hrs of time intervals.

14 Days Soil Test:

The natural soil was obtained from the local field, it was homogenized, air dried and sieved through 2 mm mesh. It was mixed with different concentration of pesticide (deltamethrin) (1 to 10 ppm) and its metabolites. 600 g of pesticide mixed soil was taken in the plastic container of 3.5 liter capacity and 10 earthworms were placed on each container and allowed to borrow. The earthworms

National Conference On Sustainable Agriculture Proceeding, 2019 www.rjlbpcs.com RJLBPCS JOURNAL were fed with urine free dried and grinded cattle manure (cow dung's) throughout the period of the experiment. This set up was repeated in three replicate for all the concentrations and control were also prepared using distilled water. Mortality of earthworms was evaluated on daily basis to determine the LC₅₀ of the pesticide. To check the mortality, the test containers were emptied on a clean tray and earthworms were separated from the soil. Earthworms were judged to be dead when they fail to respond to gentle mechanical stimulations with a blunt probe.

Statistical Analysis:

Probit analysis had been used to determine the LC₅₀ value at 95 % Confidence level using SPSS.

Histopathological study of earthworm:

Live earthworms from each treatment including the control, metabolites of pesticide and pesticide were taken and washed with distilled water. They were transferred into jars containing agar gel and left for another 96 hours to facilitate the removal of the sand content of the intestine, as agar is easily eaten by earthworm (Pokarzheyskii *et al.*, 2000; Gobi *et al.*, 2004).

By using routine paraffin method the histology of intestine of earthworm was performed (Humason, 1979). Intestine of earthworm, dissected out from the control and experimental animals, were blotted free of mucus, washed thoroughly in physiological saline, cut into pieces of desired size and fixed in Bovines fluid fixative immediately after autopsy. Fixation was carried out at room temperature for 24h, after which the tissues were transferred to 70% alcohol. Several changes of 70% alcohol were given until the yellow colour disappeared from the tissues. The tissues were then dehydrated by passing through ascending grades of alcohol, cleared in xylene, infiltrated with molten paraffin, and finally embedded in paraffin wax (58⁰C MP).

Tissue sections of 5- μ m thick transverse sections were obtained using a rotary microtome (Leica, Germany). The sections obtained were stained in Harris hematoxyline and eosin, dehydrated using alcohol, cleared in xylene and mounted using dihydroxy phthalate xylol (DPX). The stained slides were observed in a CarlZeiss (Germany) Axio-2 Plus research microscope.

3.RESULTS AND DISCUSSION

48 hours Filter Paper Contact Test (LC₅₀ determination):

The results of filter paper contact test are presented in Tables 1. It shows that the deltamethrin and its degraded metabolites varied in their contact toxicities. Earthworms were more susceptible to deltamethrin than their metabolites. The LC₅₀ value of deltamethrin was 7 ppm while LC₅₀ value of metabolites was 30 ppm. Hence metabolites of deltamethrin degradation are less toxic to earthworm than deltamethrin.

Table 1: LC₅₀ Determination

Concentration of deltamethrin (ppm)	No. of exposed Earthworm	Mortality	Concentration of metabolite (ppm)	No. of exposed Earthworm	Mortality
1	10	1	1	10	0
2	10	2	2	10	0
3	10	2	3	10	0
4	10	3	4	10	0
5	10	3	5	10	0
6	10	4	6	10	0
7	10	5	7	10	0
8	10	8	8	10	1
9	10	9	9	10	2
10	10	10	10	10	3
-	-	-	20	10	4
-	-	-	30	10	5
-	-	-	40	10	7
			50	10	10
Control Distilled Water	10	0	Control D/W	10	0

For deltamethrin

Spearman Karber Trim : 10.00%

LC50 : 7 ppm

95% Lower confidence : 0.02

95 % Upper confidence : 0.03

For Metabolites

Spearman Karber Trim : 10.00%

LC50 : 30 ppm

95% Lower confidence : 0.03

95 % Upper confidence : 0.04

**Fig. 1 Filter paper contact test**

Fourteen days soil test (LC₅₀ determination):

The results of soil test are as presented in Table 2. It shows that at 7 ppm concentration of deltamethrin, half numbers of earthworms were died after 14 days and with the increase in the concentration, mortality is increasing. At 10 ppm concentration, all earthworms were died.

The metabolites of degraded deltamethrin were also used to check its toxicity on earthworm where it is found that at concentration of 30 ppm of metabolites (LC₅₀ value of 30) half numbers of earthworms were died after 14 days.

Table 2: LC₅₀ Determination

Conc. of Deltamethrin (mg kg ⁻¹)	No. of exposed Earthworm	Mortality After 14 days	Conc. of metabolite (mg kg ⁻¹)	No. of exposed Earthworm	Mortality After 14 days
1	10	1	1	10	0
2	10	2	2	10	0
3	10	3	3	10	0
4	10	3	4	10	0
5	10	4	5	10	0
6	10	4	6	10	0
7	10	5	7	10	0
8	10	8	8	10	1
9	10	9	9	10	2
10	10	10	10	10	3
			20	10	4
			30	10	5
			40	10	7
			50	10	10
Control D/W	10	0	Control D/W	10	0

For deltamethrin

Spearman Karber Trim : 10.00%

LC50 : 7 mgkg⁻¹

95% Lower confidence : 0.02

95 % Upper confidence: 0.03 95 %

For Metabolites

Spearman Karber Trim: 10.00%

LC50 : 30 mgkg⁻¹

95% Lower confidence : 0.03

Upper confidence : 0.04

Histopathological study of earthworm:



Fig. 2 C, P and M: Histology of earthworm intestine (C-control, P- Effect of deltamethrin pesticide, M-Effect of deltamethrin metabolites)

Histopathological examination of intestinal sections of control earthworm showed normal structure of wall and intact nature of circular and longitudinal muscles (Fig. 2C). In the earthworms exposed to deltamethrin pesticide at LC_{50} (7 ppm), a circular and longitudinal muscle damage and tissue erosion was observed at 48h of exposure. It may lead to the fragmentation of the body with swelling of longitudinal muscles. The structural integrity in longitudinal muscles was lost. There was a total damage body wall of the *Eisenia fetida* due to the internal and ectodermal tissue erosion (Fig. 2P). However, earthworms exposed to deltamethrin metabolite at LC_{50} (30ppm) for 48h, revealed normal structure of body wall and expansion of spaces between the longitudinal muscles with proliferation of glandular cells (Fig. 2M). These results indicate the adverse toxic effects of deltamethrin and very little effect of metabolites of deltamethrin on the morphological properties and structural integrity of the tissues. Contact filter paper test is reported to be an excellent screening technique to assess the relative toxicity pesticides to the earthworms. It is one of the simpler, cheaper and quick method. It is designed in such a way that the earthworms are exposed to the toxicant both by contact and in the aquatic phase (Edwards and Bohlen, 1996). According to the regulation of environmental risk assessment for agricultural pesticides, the suggested standard of toxicity are $LC_{50} < 1 \text{ mg kg}^{-1}$ for highly-toxic pesticides, $1-10 \text{ mg kg}^{-1}$ for medium-toxic pesticides, and $> 10 \text{ mg kg}^{-1}$ for low-toxic pesticides (MEPPRC, 1990). With this standard, the acute toxicity of deltamethrin on earthworm was found to be medium, while acute toxicity of degraded metabolites was found to be low. Yuguda *et al.* (2015) also assessed the toxicity of some pesticides on earthworms (*lumbricus terrestris*) using 48 hours contact filter paper test and 14 days soil test. They found that all the pesticides were toxic to earthworms based on LC_{50} values of 48 hours contact filter paper test while difference in toxicity profile in fourteen days soil test that was based on the pesticides classes. Soil test is a more representation of natural environment of earthworms and the pesticides are mainly absorbed by gut in this method (De Silva and Van Gestel, 2009, Udovic and

National Conference On Sustainable Agriculture Proceeding, 2019 www.rjlbpcs.com RJLBPCS JOURNAL Lestan, 2010). Therefore the soil test is more adequate when toxicity of pesticides to earthworms is to be evaluated (Wang *et al.*, 2011).

Many previous reports on earthworms also suggest the morphological and histopathological changes on exposure of different toxic metals and organophosphate pesticides. Disintegrations of cuticular and ectodermal membranes were recorded by Amaral *et al.*, 2006. Landrum *et al.*, (2006) discussed the survival rate of *Eisenia fetida* when exposed to Perchlorate in the form of disintegration of the peritoneum (chloragogenous layer) and the epithelium.

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