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TOXICICOLOGICAL STUDY OF CYPERMETHRIN AND ITS METABOLITES ON EARTHWORM (*EISENIA FETIDA*)

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ABSTRACT: *Cypermethrin, 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 cypermethrin and its metabolites 2-propionic acid, benzoic acid and chlorine that were produced by a newly isolated strain of Paracoccus siganidrum APGM1. 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 cypermethrin and its degraded metabolites vary in their contact toxicities. Earthworms were more susceptible to cypermethrin than their metabolites. The LC_{50} value of cypermethrin was 6 ppm while LC_{50} value of metabolites was 20 ppm. Fourteen days soil test are showed that at 6 ppm concentration of cypermethrin, 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 20 ppm. The histopathological results also indicated the adverse effects of cypermethrin and very little effect of metabolites on the morphological properties and structural integrity of the tissues. Thus, it was concluded that the metabolites of cypermethrin are less toxic to earthworm than the cypermethrin.*

Keywords: *Cypermethrin, metabolites, histopathology, filter paper contact test, 14 days soil test.*

I. 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. Usually cypermethrin exists as a combination of *cis* and *trans* isomers (Kidd and James, 1991). The *cis* isomers are more active than *trans* isomers. There is no much more difference between the photo degradation rates of these two isomers in soil (Takahashi *et al*., 1985).

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). They represent up to 60 to 80% of the total animal biomass in soil (Olette *etal.,* 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 bioindicators 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 cypermethrin. In the present study, cypermethrin and its metabolites such as 2-propionic acid, benzoic acid and chlorine produced by a newly isolated strain APGM1 of bacteria *Paracoccus siganidrum* was used to determine the toxicity of cypermethrin and its metabolites to earthworm.

II. MATERIAL 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 cypermethrin 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 ± 1^0C 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 (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 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 LC_{50} 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 LC_{50} value at 95 % Confidence level using SPSS.

Histopathalogical 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 (58^0C MP).

Tissue sections of 5-µm thick transverse sections were obtained using a rotary microtome (Leica, Germany). The sections obtained were stained in Harris hematoxylene 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.

III. RESULTS AND DISCUSSION

48 hours Filter Paper Contact Test (LC_{50} determination):

The results of filter paper contact test are presented in Tables 1. It shows that the cypermethrin and its degraded metabolites varied in their contact toxicities. Earthworms were more susceptible to cypermethrin than their metabolites. The LC_{50} value of cypermethrin was 6 ppm while LC_{50} value of metabolites was 20 ppm. Hence metabolites of cypermethrin degradation are less toxic to earthworm than cypermethrin.

Table 1: LC_{50} Determination

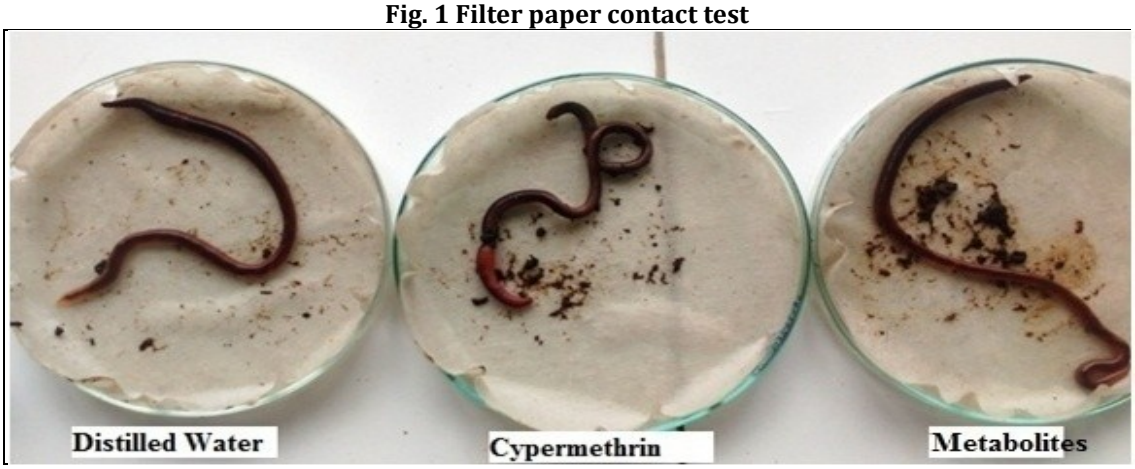
Concentration of Cypermethrin (ppm)	No. of exposed Earthworm	Mortality	Concentration of metabolite (ppm)	No. of exposed Earthworm	Mortality
1	10	1	1	10	0
2	10	2	2	10	0
3	10	2	3	10	0
4	10	3	4	10	0
5	10	4	5	10	0

6	10	5	6	10	0
7	10	6	7	10	0
8	10	8	8	10	1
9	10	9	9	10	2
10	10	10	10	10	3
-	-	-	20	10	5
-	-	-	30	10	8
-	-	-	40	10	10
Control Distilled Water	10	0	**Control D/W**	10	0

For cypermethrin
Spearman Karber Trim : 10.00%
LC50 : 6 pmm
95% Lower confidence : 0.02
95 % Upper confidence : 0.03

For Metabolites
Spearman Karber Trim : 10.00%
LC50 : 20 ppm
95% Lower confidence : 0.03
95 % Upper confidence : 0.04

Fig. 1 Filter paper contact test



Fourteen days soil test (LC_{50} determination):

The results of soil test are as presented in Table 2. It shows that at 6 ppm concentration of cypermethrin, 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 cypermethrin were also used to check its toxicity on earthworm where it is found that at concentration of 20 ppm of metabolites (LC_{50} value of 20) half numbers of earthworms were died after 14 days.

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 cypermethrin, disintegration of ectodermal layer as well as circular and longitudinal muscles was found. It may be due to the necrotic effect of cypermethrin pesticide at LC50 (6ppm) for 48 h. There was a total damage of body wall of the *Eisenia fetida* due to the internal and ectodermal tissue erosion (Fig. 2P). However, earthworms exposed to cypermethrin metabolite at LC50 (20ppm) for 48 h, 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 cypermethrin and very little effect of metabolites of cypermethrin 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 mg kg^{-1} for highly-toxic pesticides, 1–10 mg kg^{-1} for medium-toxic pesticides, and > 10 mg $kg^{-1}$for low-toxic pesticides (MEPPRC, 1990). With this standard, the acute toxicity of cypermethrin 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 Lestan, 2010). Therefore the soil test is more adequate when toxicity of pesticides to earthworms is to be evaluated (Wang *et al.,* 2011).

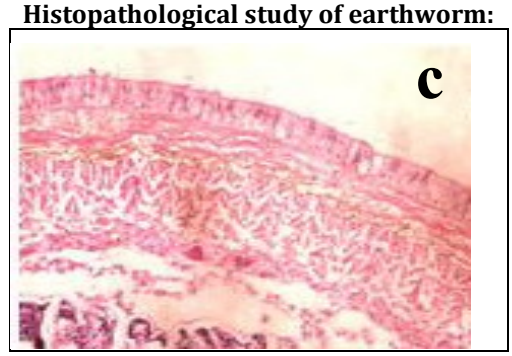
Table 2: LC_{50} Determination

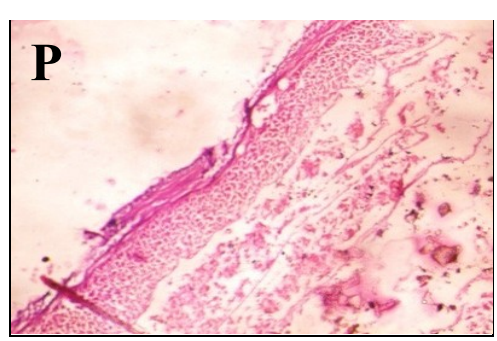
Concentration of Cypermethrin in soil ($mgkg^{-1}$)	No. of exposed earthworms	Mortality after 14th days	Concentration of metabolites ($mgkg^{-1}$)	No. of exposed earthworm	Mortality after 14 th days
1	10	1	1	10	0
2	10	2	2	10	0
3	10	2	3	10	0
4	10	3	4	10	0
5	10	4	5	10	0
6	10	5	6	10	0
7	10	6	7	10	0
8	10	7	8	10	1
9	10	8	9	10	1
10	10	10	10	10	3
			20	10	5
			30	10	7
			40	10	10
Control	**10**	**0**	**Control**	**10**	**0**

For cypermethrin
Spearman Karber Trim : 10.00%
LC50 : 6 $mgkg^{-1}$
95% Lower confidence : 0.02 95%
95 % Upper confidence: 0.03 95 %

For Metabolites
Spearman Karber Trim: 10.00%
LC50 : 20$mgkg^{-1}$
Lower confidence : 0.03
Upper confidence : 0.04

Histopathological study of earthworm:





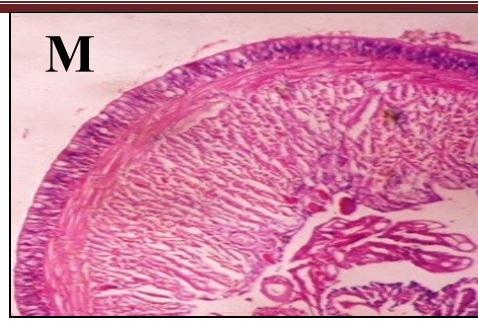


Fig. 2 C, P and M: Histology of earthworm intestine (C-control, P- Effect of cyeprmethrin pesticide, M- Effect of cypermethrin metabolites)

The toxicity of cypermethrin to the earthworms has also been measured previously by several workers. Inglesfield C. (1984) determined a 14-d LC50 of greater than 100 mg kg^{-1} and an acute (14-d) no observable effect of concentration of 100 mg kg^{-1} for alpha cypermethrin in artificial soil. The LC50 value of cypermethrin for *Eisenia fetida* was 26.11 Jg cm^{-2} (Roberts & Dorough, 1984).

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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