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Toxicological Study of Bifenthrin and its Metabolites on Earthworm (*Eisenia fetida*)

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ABSTRACT

Pyrethroids are used in agricultural to control pests on a variety of crops. Bifenthrin, a synthetic pyrethroid, is a broad spectrum insecticide. It acts mainly on the nervous system of vertebrates as well as invertebrates. It is susceptible to the biodegradation by some soil bacteria. The present paper deals with the toxicological studies of bifenthrin and its metabolites benzene 1,1(methylthio) ethylidine, resorcinol and monochloro trifluromethane that were produced by a newly isolated strain of *Paracoccus siganidrum* APGM1 on earthworms, which play an important role in the improvement of the soil fertility. 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 earthworms were more susceptible to bifenthrin than their metabolites. The LC_{50} value of bifenthrin and its metabolites was 6 ppm and 20 ppm respectively. The soil test showed that at 6 ppm concentration of bifenthrin, half the number of earthworms died after 14 days and with the increase in the concentration, mortality increased. At 10 ppm concentration, all the earthworms died. However, half the number of earthworms died after 14 days at the metabolite concentration of 20 ppm. The adverse effects of bifenthrin and very little effect of their metabolites on the morphological properties and structural integrity of the tissues were observed in histopathological studies. Thus, the metabolites of bifenthrin were less toxic to earthworms than the bifenthrin.

INTRODUCTION

The wide use and repeated application of synthetic pyrethroids have resulted in their constant occurrence in some environments. Usually, pyrethroids bind tightly with the soil components and therefore, they leach into the groundwater and form residues, which in turn is harmful to the ecosystems. Bifenthrin is mostly used in orchards, nurseries, homes and in agricultural crops such as corn (Ingham 2012). Because of their high toxicity to aquatic life, they have been registered as 'restricted use pesticides'. U.S. EPA has classified it as a category C pesticide as it is a possible human carcinogen (National Pesticide Information Centre 2015).

Earthworms play an important role in increasing the desirable properties of the soil. They constitute about 60 to 80% of the total animal biomass in soil (Olette et al. 2008, Jouquet et al. 2010). They are highly sensitive and susceptible to the agrochemicals as they lack hard cuticle around their body (Lanno et al. 2004, Nahmani et al. 2007). Decrease in earthworm population due to the use of pyrethroids has already been reported (Lukowicz & Krechniak 1992). Earthworms are also susceptible to the ecological changes, mostly those intrinsic to the soil. The earthworm behaviour can therefore imitate the soil contamination. Globally, earthworms are used as bioindicators for investigating chemical environmental pollution.

Many bacteria have the potential to degrade the pyrethroid pesticides including bifenthrin. 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. In the present study, bifenthrin and its metabolites such as benzene 1, 1(methylthio) ethylidine, resorcinol and monochloro trifluromethane produced by a newly isolated strain APGM1 of bacteria *Paracoccus siganidrum* was used to determine the toxicity of bifenthrin and its metabolites to earthworm.

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 the study.

48-hour contact filter paper test: Different concentrations of bifenthrin in the range of 1 to 10 ppm were prepared in distilled water. At the same time, different concentrations of metabolites in the range of 1 to 10 ppm and above obtained from the ethyl acetate extract of degradation medium were

prepared. The filter paper pieces were soaked with these different concentrations of the pesticides as well as metabolites and placed in a Petri dish. The earthworms were placed on the top of these filter papers. The experimental set was replicated three times for each of the concentration and a similar design was setup using distilled water as a control. The dishes were incubated in the dark at 20 ± 1°C for 48 hours and mortality was recorded at 12 hrs of time intervals.

14-Day soil test: The local natural soil was homogenized, air dried and sieved through 2 mm mesh. It was then mixed with different concentrations of the pesticide (1 to 10 ppm) and its metabolites. 600 g of pesticide mixed soil was taken in the plastic container of 3.5 litre capacity and 10 earthworms were placed on each container and allowed to borrow. The earthworms were fed with urine free dried and ground cattle manure (cow dung's) throughout the period of the experiment. This set up was repeated in three replicates for all the concentrations and controls were also prepared using distilled water. Mortality of earthworms was evaluated on a 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 was used to determine the LC_{50} value at 95% confidence level using SPSS.

Histopathological study of the earthworms: Live earthworms from each treatment including the control, metabolites and bifenthrin 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).

The histological study of the intestine of earthworm was performed by using routine paraffin method (Humason 1979). In this, the 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 Bovine fluid fixative immediately after autopsy. The fixation was carried out at room temperature for 24 h, 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°C 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.

RESULTS AND DISCUSSION

48-hour filter paper contact test (LC_{50} determination): The results of filter paper contact test are presented in Table 1. It shows that the bifentrin and its degraded metabolites varied in their contact toxicities. Earthworms were more susceptible to bifenthrin than their metabolites. The LC_{50} value of bifenthrin was 6 ppm while LC_{50} value of metabolites was 20 ppm. Hence, metabolites of bifenthrin degradation are less toxic to earthworm than bifenthrin. The contact filter paper test is reported to be an excellent screening technique to assess the relative toxicity of pesticides to the earthworms. It is one of the simpler, cheaper and quick methods. It is designed in such a way that the earthworms are exposed to the toxicant both by contact and in the aquatic phase (Edwards & Bohlen 1996).

14-day soil test (LC_{50} determination): The results of the soil test are as presented in Table 2. It shows that at 6 ppm concentration of bienthrin, half the number of earthworms died after 14 days and with the increase in the concentration, mortality increased. At 10 ppm concentration, all the earthworms died. The metabolites of degraded bifenthrin were also used to check its toxicity on earthworms where it was found that at concentration of 20 ppm of metabolites (LC_{50} value of 20ppm) half the number of earthworms died after 14 days. The soil test is a better representation of natural environment of earthworms, and the pesticides are mainly absorbed by gut in this method (De Silva & Van Gestel 2009, Udovic & Lestan 2010). Therefore, the soil test is more adequate when toxicity of pesticides to earthworms is to be evaluated (Wang et al. 2011).

Histopathological Study of Earthworm

Histopathological examination of intestinal sections of control earthworms showed normal structure of wall and intact nature of circular and longitudinal muscles (Fig. 2C). In the earthworms exposed to bifenthrin, disintegration of ectodermal layer as well as circular and longitudinal muscles was found. It may be due to the necrotic effect of bifenthrin at LC_{50} (6 ppm) for 48 h. There was total damage of body wall of the *Eisenia fetida* due to the internal and ectodermal tissue erosion (Fig. 2P). However, earthworms exposed to bifenthrin metabolites at LC_{50} (20 ppm) 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 bifenthrin and very little effect of me-

Table 1: LC_{50} determination the pesticide and its metabolites.

Concentration of Cypermethrin (ppm)	No. of exposed earthworms	Mortality	Concentration of metabolite (ppm)	No. of exposed earthworms	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 Distilled Water	10	0

For bifenthrin		For Metabolites	
Spearman Karber Trim	: 10.00%	Spearman Karber Trim	: 10.00%
LC_{50}	: 6 pmm	LC_{50}	: 20 ppm
95% Lower confidence	: 0.02	95% Lower confidence	: 0.03
95 % Upper confidence	: 0.03	95% Upper confidence	: 0.04

tabolites of bifenthrin on the morphological properties and structural integrity of the tissues. Many earlier reports also suggest the morphological and histopathological changes in earthworms on exposure to different toxic metals and organophosphate pesticides. The disintegrations of cuticular and ectodermal membranes were recorded by Amaral et al. (2006).

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, and 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. The toxic effects of

Fig. 1: Filter paper contact test with earthworms.

Table 2: LC_{50} determination.

Concentration of Cypermethrin in soil ($mg.kg^{-1}$)	No. of exposed earthworms	Mortality after 14^{th} day	Concentration of metabolites ($mg.kg^{-1}$)	No. of exposed earthworms	Mortality after 14^{th} day
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 (Distilled water)	10	0	Control (Distilled water)	10	0

For cypermethrin		For Metabolites	
Spearman Karber Trim	: 10.00%	Spearman Karber Trim	: 10.00%
LC_{50}	: 6 $mgkg^{-1}$	LC50	: 20$mgkg^{-1}$
95% Lower confidence	: 0.02	95% Lower confidence	: 0.03
95 % Upper confidence	: 0.03	95 % Upper confidence	: 0.04

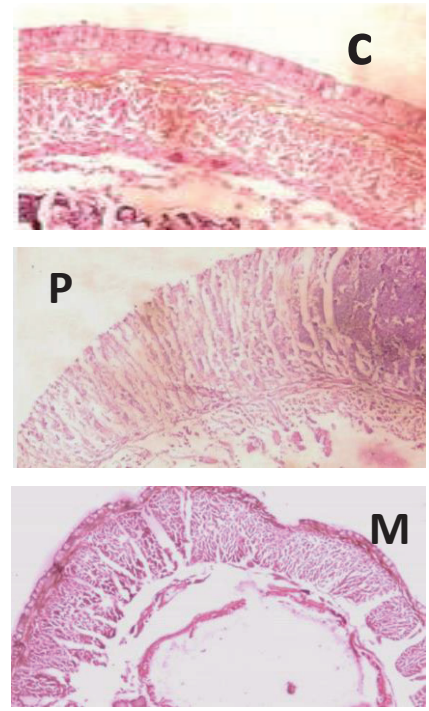

Fig. 2: C, P and M: Histology of earthworm intestine (C-control, P- Effect of bifenthrin pesticide, M-Effect of bifenthrin metabolites).

bifenthrin in soil on earthworms was also evaluated by Li et al. (2017) and reported that bifenthrin was moderately toxic in 72-h filter paper test and low toxic in 14-d soil test.

CONCLUSIONS

According to the regulation of environmental risk assessment for agricultural pesticides, the suggested standard of toxicity is 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. With this standard, the acute toxicity of bifenthrin on earthworm was found to be medium, while acute toxicity of degraded metabolites was found to be low.

REFERENCES

Amaral, A., Soto, M., Cunha, R., Marigomez, I. and Rodrigues, A. 2006. Bioavailability and cellular effects of metals on *Lumbricus terrestris* inhabiting volcanic soils. Environ. Pollut., 142: 103-108.

Edwards, C.A. and Bohlen, P.J., 1996. Effects of agricultural practices and chemicals on earthworms. In: Biology and Ecology of Earthworms, pp. 268-299, Chapman & Hall, London.

Gobi, M., Suman, J. and Ganesan, S.V. 2004. Sublethal toxicity of the herbicide butachlor on the earthworm *Perionyx sansibaricus* and its histological changes. Journal of Soils and Sediments, 5: 62-86.

Humason, G.L. 1979. Animal Tissue Techniques. 4th ed. W. H. Freeman & Co., San Francisco.

Ingham, P.E., McNeil, S.J. and Sunderland, M.R. 2012. Functional finishes for wool-ecoconsiderations. Advanced Materials Research, 441: 33-43.

Jouquet, P., Plumere, T., Thu, T.D., Rumpel, C., Duc, T.T. and Orange, D. 2010. The rehabilitation of tropical soils using compost and vermicompost is affected by the presence of endogeic earthworms. Appl. Soil Ecol., 46: 125-133.

Kidd, H. and James, D.R. (Eds.) 1991. The Agrochemicals Handbook, Third Edition. Royal Society of Chemistry Information Services. Cambridge, UK.

Lanno, R., Wells, J., Conder, J., Bradham, K. and Basta, N. 2004. The bioavailability of chemicals in soil for earthworms. Ecotoxicol. Environ. Saf., 57: 39-47.

Li, L., Yang, D., Song, Y., Shi, Y., Huang, B., Yan, J. and Dong, X. 2017. Effects of bifenthrin exposure in soil on whole-organism endpoints and biomarkers of earthworm *Eisenia feotida*. Chemosphere, 168: 41-48.

Lukowicz-Ratajczak, J. and Krechniak, J. 1992. Effects of deltamethrin on the immune system in mice. Environmental Research, 59: 467-475.

Nahmani, J., Hodson, M.E. and Black, S. 2007. Effects of metals on life cycle parameters of the earthworm *Eisenia fetida* exposed to field-contaminated, metal-polluted soils. Environ. Pollut., 149: 44-58.

Olette, R., Couderchet, M., Biagianti, S. and Eullaffroy, P. 2008. Toxicity and removal of pesticides by selected aquatic plants. Chemosphere, 70: 1414-1421.

Pokrzheyskii, A.D., Nico, M., Straaten, V. and Semenov, V.C. 2000. Agar as a medium for removing soil from earthworm guts. Soil Biology and Biochemistry, 32: 1315-1317.

Roberts Brian, L. and Dorough Wyman, H. 1984. Relative toxicities of chemicals to the earthworm *Eisenia fetida*. Environmental Toxicology and Chemistry, 3(1): 67-78.

Takahashi, N., Mikami, N., Matsuda, T. and Miyamoto, J. 1985. Photodegradation of the pyrethroid insecticide cypermethrin in water and on soil surface. J. Pesticide Sci., 10: 629-642.

Wang, B.Z., Ma, Y., Zhou, W., Zheng, J., Zhu, J., He, J. and Li, S. 2011. Biodegradation of synthetic pyrethroids by *Ochrobacterium tritici* strain pyd-1. World J. Microbiol Biotechnol., 27: 2315-2324.