Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 06, pp.33128-33132, June, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

EFFECT OF HERBICIDES ON DIGESTIVE ENZYME ACTIVITY OF EPIGEIC INDIGENOUS EARTHWORM *PERIONYX EXCAVATUS* (OLIGOCHAETA)

[1]Sanyal, S., [1,*]Chakravorty, P.P. and [2]Kaviraj, A.

[1]P.G Department of Zoology, Raja N. L. Khan Women's College, Midnapore, West Bengal, India
[2]Department of Zoology, University of Kalyani, Kalyani, West Bengal, India

ARTICLE INFO

Article History:
Received 07th March, 2016
Received in revised form
23rd April, 2016
Accepted 14th May, 2016
Published online 30th June, 2016

Key words:

Perionyx excavatus,
α-amylase,
Anacardium occidentale,
Pretilachlor,
Pendimethalin,
Natural Soil

ABSTRACT

Different levels of two herbicides Pendimethalin and Pretilachlor based on their recommended agricultural doses (RAD) were administered into the testboxes with a micropipette (Lofs-Holmin, 1983) with five numbers age synchronized specimens of *Perionyx excavatus* in each box. The 96 hrs acute toxicity tests showed that Pendimethalin with an LC_{50} value of 0.022mg/kg soil was more toxic than Pretilachlor, LC_{50} value 0.094 mg/kg soil. Open choice experiment was done on indigenous epigeic earthworm *Perionyx excavatus* with *Anacardium occidentale* (cashew), *Mangifera indica* (mango), *Shorea robusta* (shal), *Acacia auriculiformis* (Acacia) and *Eucalyptus citridora* (Eucalyptus), leaves to determine their food preference. In the feeding preference experiment the earthworms showed maximum preference for *Anacardium occidentale* (cashew) leaves. The activity of the digestive enzyme α-amylase was determined under laboratory conditions in natural garden soil by exposing the earthworms to sub-lethal doses of the two herbicides i.e., 25% and 50% of LC_{50} value, determined earlier. The activity of the enzyme, in Pendimethalin, grew significantly higher than the control value on the 15th day of the experiment In case on the 30th day of the experiment, the enzyme activity is decreased in both the sub-lethal doses i.e. 25% of LC_{50} and 50% of LC_{50} which are significantly lower than the control value. Pendimethalin was found more toxic than Pretilachlor but was less harmful to chronic exposure to the earthworms.

Citation: Sanyal, S., Chakravorty, P.P. and Kaviraj, A. 2016. "Effect of herbicides on digestive enzyme activity of epigeic indigenous earthworm perionyx excavatus (oligochaeta)", *International Journal of Current Research*, 8, (06), 33128-33132.

INTRODUCTION

Modern agricultural practice depends on agro-chemicals for enhancing productivity. There are 60,000 varieties of chemicals in use with several thousand being added annually (Maugh, 1978). Besides seeds, nutrients, water etc, use of insecticides including herbicides is indispensable. Alarming population growth throughout the globe necessitates more food and cash crops production results rapid growth of pesticide market (Ecobichon, 2001). Inspite of their benefits, increasing trend of herbicide application has deleterious effect on human environment and agro-ecosystem. These chemicals persist sometime in the environment (Primel *et al*., 2005). Inorganic chemicals used as herbicides dates back to 1896 (Brian, 1976). In India herbicides were used to control weeds as early as 1937 (Joshi, 1974). In the developed countries herbicides account for more than 70% of the total pesticides available in the market (Muthukaruppan, 2005). In India use of herbicides has gradually increased (Muthukaruppan, 2005).

****Corresponding author: Chakravorty, P.P.,***
P.G Department of Zoology, Raja N. L. Khan Women's College, Midnapore, West Bengal, India.

India now ranks 10th in the world in insecticide consumption (Hundel *et al*., 2006). However, pattern of pesticide consumption in India differ from rest of the world. The domestic demand of herbicides for 10% of total insecticides used in the country against 30% of global demand (Mathur, 1999). Soil organisms are important for their contribution to soil fertility and nutrient cycling (Longstaff *et al*., 1997) but herbicides adversely affect these beneficial (Eijsackers and Vande Bund, 1980; Mola *et al*., 1987; Kulshrestha and Singh, 1994; Sabatini *et al*., 1998; Hafez and Theimann, 2003). Herbicides application in long term can alter rates of organic matter decomposition and lead to nutrient loss (Hendrix and Parmelee, 1985). Herbicides which retain in soil in long run results soil quality degradation, because soil micro organisms play major role in maintaining soil quality (Gilman and Vardanis, 1974; Strenersen *et al*., 1974). Herbicides is able to alter community dynamics (Pereira *et al*., 2011), shortening of food chain as well as complete destruction of soil fauna (Hartley and West, 1969). Many herbicides also have lethal effect on birds, mammals etc. when comes in contact with these animals (Cookson and Nottingham, 1969; Ehrlic and Ehrlic, 1977).

Herbicides also affect different non-target organisms like earthworms (Yahia *et al*., 2003; Panda and Sahu, 2004; Yanhua *et al*., 2012). In the present study, experiments are carried out to evaluate the toxic effects of the sub-lethal doses of two herbicides on the α-amylase enzyme activity, total tissue carbohydrate content and cellulose content of the epigeic earthworm *Perionyx excavatus*. The herbicides used in the study are Pendimethalin and Pretilachlor.

MATERIALS AND METHODS

Collection and Culture of test specimens

Specimens of *Perionyx excavatus* were collected from the natural grassland soil that has never been used for any agricultural purpose and pest control. The specimens were brought to the laboratory and were cultured in large earthen pots. Finely grinded soil (collected from the same grasslands) and farmyard manure mixed in the ratio of 1:1 was used as the culture medium (Ismail, 1997). The culture pots were covered with fine meshed iron nets and kept inside BOD incubators at 28 ± 0.5ºC. An approximate level of 50% moisture was maintained by adding distilled water into the medium. Farmyard manure was added as feed every week during the entire period of culture (OECD 1984, 2004).

Table 1. Recommended Agricultural Doses of the two selected herbicides

Chemical Name	Trade Name	RAD*(mg/Kg)
Pendimethalin	Dhanutop	0.008
Pretilachlor	Racer	0.002

*RAD- Recommended Agricultural Dose

Studies were performed with age synchronized specimens (250–300 mg). Experiments were conducted in small inert polythene boxes (16 X 12 X 1 cm; total area, 192 cm2) containing soil, collected from grasslands, as the test medium. Soil samples were dried, grinded and sieved to get a particle size of 0.25 mm before filling in the experimental boxes. The moisture content of the soil was measured by Infrared Torsion balance moisture meter [Adair Dutt, Kolkata] (Joy and Chakravorty, 1991). Finally the experimental boxes were kept in an Environmental Chamber at a constant temperature of 28 ± 0.5°C and 60-65% relative humidity. The physiochemical parameters of both the soil media, viz, pH and Organic carbon Content were measured and the temperature and moisture content were kept constant (Table 2).

Table 2. Physical parameters of the test media

Natural Soil Parameters	Values
pH	7.17
Organic Carbon Content	0.86%
Moisture Content	61.2%

Experimental Procedures

Acute Toxicity Test

Different levels of the selected herbicides based on its recommended agricultural doses (RAD) (viz RAD, 1/2X-RAD, 2X-RAD and 3X-RAD) were administered into the test boxes with a micropipette (Lofs-Holmin, 1983). The amount of an herbicides required was determined from the total area of the experimental box and was converted intomg per kg soil taking into consideration the total amount of soil (200 g) contained in one box. The experiment was setup with three replicates for each level of the herbicide and control. The boxes were then left undisturbed for about 30 min for uniform spreading of the chemical in the soil medium. Ten numbers of age synchronized specimens of *Perionyx excavatus* were then transferred into the boxes. Observations were made every 24 h. Those individuals, who showed no apparent sign of life, even when poked with a needle, were considered dead and were removed. The total mortality obtained after 96 h of exposure were subjected to probit analysis by EPA probit analysis program, version 1.5(US EPA 2006) to determine LC_{50} value (Table 3) and 95% confidence limit of each insecticide. The entire experiment was repeated three times (Dasgupta *et al.,* 2010).

Determination of Feeding Preference of test organisms

Open choice experiment was done on epigeic earthworm *Perionyx excavatus* with five common tree species leaf litters viz., *Anacardium occidentale* (cashew), *Mangifera indica* (mango), *Shorea robusta* (shal), *Acacia auriculiformis* (Acacia) and *Eucalyptus citridora* (Eucalyptus), to study their food preference. The experiment was conducted in plastic trays containing five different randomly distributed leaf litter in pits in petri dishes inserted into a uniform layer sand bed (Maity and Joy, 1999a; 1999b). Twenty adult specimens of same size and age group were released in the centre of the plastic tray and they were to migrate among the litter types. Known amount of litter cuttings were used. Optimum moisture and temperature were maintained throughout the experimental period. The rate of migration and colonization of specimens were recorded by counting their number in each litter type at 15 days interval up to 90 days. Thus, cashew was selected as the source of food to be provided to the earthworms during the entire period of digestive enzyme estimation.

Estimation of Digestive Enzyme

A very important aspect of the laboratory study was the quantitative estimation of the digestive enzyme α-amylase (Sadasivam and Manickam, 2010) determined under laboratory conditions in natural garden soil (pH-7.17, organic carbon-0.86% moisture content-61.2%) by exposing the earthworms to sub-lethal doses of the heriicides, Pendimethalin and Pretilachlor, i.e., 25% and 50% of LC_{50} value. The specimen earthworms were kept inside inert polyethylene boxes of 192 cm^2 area each containing 200g of sieved garden soil along with 10 worms. Distilled water was added to maintain 60-70% moisture.

The earthworms were provided with finely cut cashew leaf litter as food during the entire experimental period on small a petri-dish inside each box into a uniform layer of soil. The experiment was set following the procedure of open choice experiment as described by Maity and Joy, 1999a; 1999b. The food was contaminated with fungicide in the treatment boxes. The whole set up was kept inside an Environmental chamber and the temperature (28±0.5°C) and humidity (67%) was maintained.

The determination of α-amylase activity was performed on 3rd, 7th, 15th and 30th day from the setting of the experiment. The test specimens were kept in starvation before setting of the experiment.

RESULTS

The 96 hrs acute toxicity tests showed that Pendimethalin with an LC_{50} value of 0.016 mg/kg soil was more toxic than Pretilachlor, LC_{50} value 0.052 mg/kg soil.

Table 3.

Pesticide Group	Technical Name	LC_{50} value(mg/Kg)
Herbicides	Pendimethalin	0.016
	Pretilachlor	0.052

In the feeding preference experiment the earthworms showed maximum preference for *Anacardium occidentale* (cashew) leaves. In case of Pendimethalin, the α amylase activity of the test specimens was higher in both the sublethal doses, 25% o LC_{50} and 50% of LC_{50}, viz, 0.080±0.001 mg of maltose/min/mg starch and 0.062±0.003 mg of maltose/min/mg starch respectively and 0.081±0.001 mg of maltose/min/mg starch and 0.080±0.001 mg of maltose/min/mg starch than that of the control values, viz, 0.041±0.004 mg of maltose/min/mg starch and 0.041±0.004 mg of maltose/min/mg starch on the 3rd and 7th day respectively after setting of the experiment.

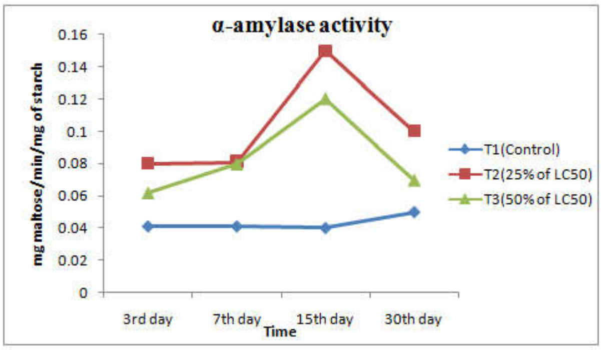

Fig1 α-amylase activity of *P. excavatus* exposed to Pendimethalin, T1(Control), T2 (25% of LC_{50}) and T3 (50% of LC_{50})

The activity of the enzyme grew significantly higher than the control value (0.040±0.001 mg of maltose/min/mg starch) on the 15th day of the experiment, viz., 0.150±0.005mg of maltose/min/mg starch and 0.120±0.004 mg of maltose/min/mg starch in both the sub-lethal doses i.e., 25% o LC_{50} and 50% of LC_{50} respectively. But on the 30th day of the experiment the activity of the enzyme decreased to 0.100±0.003 mg of maltose/min/mg starch and 0.070±0.001 mg of maltose/min/mg starch in both the sub-lethal doses i.e., 25% of LC_{50} and 50% of LC_{50} respectively, but the activity of the enzyme was still significantly higher than the control value i.e., 0.050±0.001 mg of maltose/min/mg starch (Fig A.1).

In case of Pretilachlor, α amylase activity of the test specimenson the 3rd day, in 25% of LC_{50} (0.100±0.002 mg of maltose/min/mg starch) is equal to that of the control value. But the enzyme activity in 50% of LC_{50} (0.084±0.003 mg of maltose/min/mg starch) is slightly less than that of the control value. On the 7th day of the experiment, the enzyme activity in the sub-lethal doses i.e. in 25% of LC_{50} the value is 0.113±0.004 mg of maltose/min/mg starch and in 50% of LC_{50} the value is 0.090±0.003 mg of maltose/min/mg starch, are significantly less than the control value i.e. 0.143±0.005 mg of maltose/min/mg starch. On the 15th day of the experiment, the value of enzyme activity in 25% of LC_{50} (0.120±0.003 mg of maltose/min/mg starch) is slightly less than that of the control value but the value of enzyme activity in 50% of LC_{50} (0.090±0.003 mg of maltose/min/mg starch) is much lower than that of the control value. Finally, on the 30th day of the experiment, the enzyme activity is decreased in both the sub-lethal doses i.e. 25% of LC_{50} and 50% of LC_{50} are 0.060±0.001 mg of maltose/min/mg starch and 0.043±0.002 mg of maltose/min/mg starch, which are significantly lower than the control value, 0.125±0.003 mg of maltose/min/mg starch (Fig A.2)

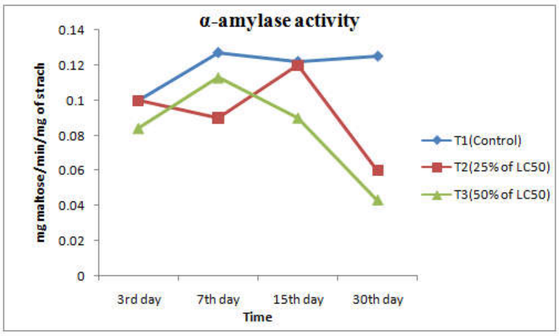

Fig. 2 α-amylase activity of *P. excavatus* exposed to Pretilachlor, T1(Control), T2 (25% of LC_{50}) and T3 (50% of LC_{50})

DISCUSSION

In case of Pendimethalin, on the 3rd day and the 7th day of the experiment α amylase activity of the earthworms significantly increased in both the sub-lethal doses compared to the control. This is probably because the test specimens were unable to sense the herbicide contamination in the food and consumed it, as a result of keeping them in starvation before setting of the experiment. But on the 15th day of the experiment following the similar trend, the amylase activity significantly increased further. This is due to the fact that the earthworms are not affected by the toxicity of the herbicide and as a result the palatability of the food increased. On the 30th day, the enzyme activity decreased in the sub-lethal doses compared to the 15thday of the experiment but not below the control value. This is probably because the herbicide is still persisting in the food but the toxicity is not affecting the earthworms so they are not avoiding the food. In contrast, in case of the herbicide Pretilachlor on the 7th, 15th and the 30th day of the experiment a significant decrease in the activity of the enzyme was observed. From the 7th day the test specimens could sense the contamination of the herbicide and avoiding the food decreasing α amylase activity in their gut.

Since, the activity of the enzyme is remaining significantly less than the control value up to the 30th day it can be concluded that the herbicide is persisting in the food and the earthworms are being prevented from consuming the food. The effect of 48h and 96h exposure to sub-lethal concentrations of $CdCl_2$, $HgCl_2$ and $K_2Cr_2O_7$ on the digestive enzyme activity of Daphnia magna was assessed. Both inhibition ($CdCl_2$ and $HgCl_2$) and increase ($K_2Cr_2O_7$) of the enzyme activities were noted after short-term (48h) exposure. No inhibition, however, was observed after prolonged exposure (96h) to $HgCl_2$ and $K_2Cr_2O_7$ and even an increase in activity was noted for $CdCl_2$ (De Coen and Janssen, 1997). Methanolic extract of Artemisia annua L., a weed collected around paddy fields in north of Iran, was investigated for its toxic effects on: feeding, growth, fecundity, fertility including the biochemical characteristics of elm leaf beetle Xanthogaleruca luteola, Mull. Twenty-four hours after treating 3rd instar larva with the extract the level ofα-amylasesignificantly changed.However, at 48 h the extract lost its potency (Shekari *et al*., 2008). Different other studieshave been done on the effects of pesticides on α-amylase activity on different groups of animals but studies on the effect of herbicides on the amylase activity of earthworms has not been reported so far.

Conclusion

From the above study it can be concluded that though Pendimethalin shows more toxicity upon the earthworms on short term exposure, it does not have harmful effect when long term exposure is done. So, Pendimethalin can be regarded as an ecologically safe herbicide. Whereas, Pretilachlor despite having low acute toxicity, compared to Pendimethalin, on the earthworms, has a hindering effect on the food uptake of the earthworms decreasing their gut α amylase activity. Thus, it can be concluded that Pretilachlor has toxic effects in the soil ecosystem and is harmful. Finally, it can be concluded that the enzyme α amylase can be used as a potential bio marker to detect pesticide pollution in agro ecosystems.

REFERENCES

Brian, R.C. 1976. The history and classification of herbicides, 1-54. In: Audus LJ (ed) Herbicides, Physiology, Biochemistry and Ecology. Academic Press, London, Pp 1- 608

Cookson, J. and Nottingham J.A. 1969. Survey of chemical and biological warfare.Monthly review press, New York, Pp 420

Das Gupta, R. Chakravorty, P.P. and Kaviraj, A. 2010. Studies on relative toxicities of six insecticides on epigeic earthworm, *Perionyx excavatus*. Bull Environ Contam Toxicol. 85, 83-86

De Coen, W.M. and Janssen, C.R. 1997. The use of biomarkers in *Daphnia magna* toxicity testing II. Digestive enzyme activity in *Daphnia magna* exposed to sublethal concentrations of cadmium, chromium and mercury. Chemosphere 35 (5) 1053–1067

Ecobichon, D.J. 2001. Pesticide use in developing countries. Toxicol 160, 27-33

Eijsackers, H. And van de Bund, C.F. 1980. Effects on soil fauna. In: Hance RJ (ed) Interactions between Herbicides and the soil. Academic Press, London, Pp 255-305

Gilman, A.P. and Vardanis, A. 1974. "Carbofuran: Comparative toxicity and metabolism in the worms *Lumbricus terrestris*and *Eisenia fetida*." J of Agric and Food Chem 22(4) 625-628

Hafez, H.F.H. and Thiemann, W.H.P. 2003. Persistence and biodegradation of Diazinone and Imidacloprid in soil.Proc XII Symp Pest Chem Congress Centre Universita Cattolica via Emilia 84, 35-42

Hartley, G.S and West, T.F. 1969. Chemical for pest control. Pregamon Press, Oxford, Pp 316

Hendrix, P.F. and Parmelee, R.W. 1985. Decomposition, nutrient loss and microarthropod densities in herbicide treated grass litter in a Georgia predmont agroecosystem. Sol Biol and Biochem.4, 421-428

Hundal, B.S., Singh, R., Sing, A. 2006. Pesticide marketing: The Indian scenario. The ICFAI J Manag Eco

Ismail, S.A. 1997. Vermicology- The Biology of Earthworms.Orient Longman, Chennai, India

Joshi, N.C. 1974. Manual of weed control. Research co Publications, Delhi, Pp 365

Joy, V.C. and Chakravorty, P.P. 1991. Impact of insecticides on nontarget microarthropod fauna in agricultural soil. Ecotoxicol Environ Safety 22, 8-16

Kulshrestha, G. and Singh, S.B. 1994. Effects of herbicides on soil environment. In: Prasad D, Gaur HS (ed) Soil Environment and Pesticides.Venus Publishing House, New Delhi, India, Pp 293-313

Longstaffm, B.C., Greenslade, P.J.M., Colloff, M., Reid, I., Hart, P. and Packer, I. 1997. The impact of soil tillage practice on soil fauna in the NSW wheat belt. Research Paper No 97. *Rural Industries Research and Development Corporation, Canberra*

Maity, S.K. and Joy, V.C. 1999a. Impact of antinutritional chemical compounds of leaf litter on detrivore soil arthropod fauna. Journal of Ecobiology 11(3) 193-202

Maity, S.K. and Joy, V.C. 1999b. Soil micro-arthropod feeding effect on non-nutrient chemicals of decomposing leaf litter. *Indian Journal of Environment And Ecoplanning* 2(3) 225-231

Mathur, S.C. 1999. Future of Indian Pesticide industry in next millennium. Pestic Infor 24(4) 9-23

Maugh, T.H. 1978. Science. 199, 162

Mola, L., Sabatini, M.A., Fratello, B. and Bertolani, R. 1987. Effects of atrazine on two species of collembola (Onychuridae) in laboratory tests. *Pedobiologia* 30, 145-149

Muthukaruppan, G., Janardan, S. and Vijayalakshmi, G.S. 2005. Sublethal toxicity of the herbicide butachlor on the earthworm *Perionyx sansibaricus* and its histological change. *J Sol and Sedmnt* 5(2) 82-86

OECD (Organization for Economic Co-Operation and Development). 1984. Guidelines for testing of chemicals No 207, Earthworm Acute Toxicity Test, OECD, Paris

OECD (Organization for Economic Co-Operation and Development). 2004. Guidelines for testing of chemicals No 222, Earthworm Reproduction Test. (Eiseniafetida/andrei), OECD, Paris

Pereira, J.L., da Silva, A.A., Picanco, M.C., de Barros, E.C. and Jakelaitis, A. 2011. Effects of herbicide and insecticide interaction on soil entomofauna under maize crop. Available via http:// Taylor & Francis online.com.Accessed 12.07.2012

Primel, E.G., Zenella, R., Kurz, M.H.S., Goncalves, F.F., Machado Sde, O. and Marchezan, E. 2005. Poluicão das águas.Porherbicidasutilizados no cultivo do arrozirrigadonaregião central do estado do riogrande do sul, Brasil: predicãoteóricaemonitoramento. QuÍmica Nova 28(4) 605-609

Sabatini, M.A., Rebecchi, L., Cappt, C., Guidi, A., Dinelli, C., Vicari, A., Bertolani, R. 1998. Side effects of the herbicide trisulfuron on Collembola under laboratory conditions. Chemosphere 37, 2963-2973

Shekari, M., Sendi, J.J., Etebari, K., Zibaee, A. and Shadparvar, A. 2008. Effects of *Artemisia annua* L. (Asteracea) on nutritional physiology and enzyme activities of elm leaf beetle, *Xanthogaleruca luteola* Mull. (Coleoptera: Chrysomellidae). Pesticide Biochemistry and Physiology 91(1) 66–74

Strenersen, J., Gilman, A.P. and Vardanis, A. 1974. "Carbofuran: its toxicity to and metabolism by earthworms (*Lumbricusterrestris*)." J of Agric and Food Chem 22(2) 342-347

Yahia, Y.M., Saad, M.M.I., Mohammed, T.A. and Yousery, M.A. 2003. Comparative Toxicity and biochemical responses of certain pesticides to the mature earthworm *Aporrectodea caliginose* under laboratory conditions. Environ Toxicol 18(5) 338-346