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Relative Toxicity Effects of Herbicide Metribuzin on the behavioural changes of earthworms *Lambito marutii* kept in different substrates

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Abstract

Earthworms have been studied as a readily available, easily maintainable and cheap test species for assessing chemical pollution. With the advent of the Green Revolution, there has been a quantum leap in the use of synthetic herbicides and pesticides throughout the world to sustain high yielding crop varieties. Continuous use of these synthetic chemicals leads to loss of soil fertility and soil organisms. Investigation of possible alternative substrates using locally available materials therefore is vital for performing such eco-toxicity tests, particularly in the tropics. The experiments were carried out to assess the relative acute toxicity effects of Herbicide Matribuzin on Earthworm (*Lambito mauritii*) to the suitability of Cow dung, Coir pith, Saw-dust and Tea dust waste for maintaining the soil health. It is important to note that the worms exposed to herbicide metribuzin mixed with different substrates experience the behavioural changes noted as initial symptoms. The mortality rate differences between I hr – LC_{50} and 120 hr – LC_{50} were calculated by the Finney's Probit Method (Finney, 1971). The earthworm biomass was found to be decreased with increasing herbicide concentration. The results suggest the substrate coir waste, saw dust and tea waste can be used as good manure to any crop which decreases the effects of herbicide metribuzin on earthworm *Lampito mauritii*.

Keywords: *Lampito mauritii*, Herbicide, Metribuzin, Eco-toxicity, Biological response, $LC_{50.}$

Introduction

The world population is expected to reach 9.1 billion by the year 2050 with the current annual growth rate of 1.2% (Cohen JE, et al, 2015). Increasing food productivity for food security is very essential in many tropical countries (FAO, 2005). Increasing agricultural activities for food security is very important. The increasing uses of agrochemicals such systems affects non-target organisms in soil and water (Reinecke SA, et al, 2007). Earthworms are common organisms in the soil and play an important role to improving the structure and fertility of the soil (M.D. Bartlett, et al 2010). They are considered not only composting agents and bio-fertilizers but also soil aerators, soil moisture retainers and biological agents. Earthworms can be used as bio-indicators to detect pesticide contamination in soils (Stenersen. J, et al, 1973). Since about ten year, earthworms have been widely used as model organisms to estimate environmental pollution because of their importance in the structure and the function of the ecosystems of soil

(M.J.G. Santos, et al, 2010 and A.N. Walker, etal, 2010). Earthworms are bio-indicators of the contamination of grounds and can be used to supply thresholds of security to watch degrees of contamination of soil and to examine the toxic effects of chemicals in laboratory conditions (S.Suthar, et al, 2008 and J.I. Lourenço, et al, 2011).

The soil not only acts as a substrate for organisms but also as a recipient medium for chemicals. Agrochemicals irrespective of their place or mode of application exert certain unwanted influence on this non-target organism, which in turn becomes undesirable victim. It is used as an efficient bio monitor for eco-toxicological studies, both as an indicator and test species. It is an excellent tool to detect soil pollutants (Bouche, 1988). The earthworm, *Eisenia foetida* (Savigny) is reported to be a suitable test species (Heimbach, 1988). Use of earthworm in toxicology studies is very common. The present study is aimed at to know the impact of different artificial substrates such as coir pith, tea waste, saw dust, and farm yard manure (used as good medium for vermiculture) on the toxicity of Herbicide Metribuzin to the earthworm, *Lampito mauritii*. This type studies were focused on growth, reproduction potential, avoidance behaviour, mortality and the impacts on earthworm in modern agriculture.

Materials and Methods

Collection and Maintenance of Earthworms:

The test organisms *L. mauritii* were collected from the paddy cultivating agricultural field in Kidarankondan Village, Tiruvarur, Tamil Nadu, India. They were maintained in the laboratory condition kept in large trays with a substrate medium, 50% farmyard manure and 50% soil (vol/vol) for two weeks at 28 ± 2^0 C with 50 – 60% moisture. The adolescent worms of 10 to 12 cm in length and 2 to 3 mm in width, with pink undifferentiated clitella were used for the present study.

Preparation of Different Substrates:

Dry soil was taken from the paddy cultivating agricultural field from where the earthworms collected for this study and powdered and used as a control. Cow dung (Substrate 1) was collected and dried also in shade place, Saw dust (Substrate 2), Dry coir pith (Substrate 3) and Tea dust waste (Substrate 4) were collected from the relevant Mills, Industry, Tea shops around Tiruvarur town. Each substrate prepared as a separate medium mixed with dry soil in the ratio 1:1 (vol/vol) and keeping the setup in moisture conditions whenever add needed water.

Chemicals:

A commercially available Carbomate Herbicide Metribuzin (- amino – 6 – tert – butyl - 4,5- dihydro - 3- methyltio -1, 2, 4- triazin -5-one 2 - Chloro - 2_, 6_- diethyl – N - (butoxymethyl) acetanilide) was purchased from local pesticide agency and used for carrying out the present study. Metribuzin is a broad spectrum, systematic, granular herbicide manufactured and supplied by Pesticides India Ltd. Udaipur, India. It is widely used for the control and eradicates the undesirable grasses in the paddy fields by the direct applications.

Acute toxicity test:

For assessing acute toxicity, the preparation of selected test medium of each substrate was taken and the test doses are expressed as mg active ingredient of herbicide metribuzin /kg dry substrates weight. The 10 earthworms were introduced into each test medium which is kept in polythene bag. The behaviour and the mortality rate of earthworms were observed after 1, 3, 6, 12, 24, 36, 48, 72, 96, 120 and 144 hours of exposure period. The Earthworms were considered dead if they did not respond to a gentle mechanical stimulus.

Determination of LC_{50} Values:

The median lethal concentrations and their 95% upper and lower confidence limits were determined by using graphic nomogram method of Licthfield and Wilcoxon. Lethal threshold concentration was obtained by adopting the procedures of Eaton.

Weight Gain or Loss:

To evaluate the feeding activity of earthworms, they were exposed to different substrates with non-lethal concentration of herbicide metribuzin for 120 hours and weight at 1, 2, 3, 6, 12, 24, 36, 48, 72, 96 and 120 hours. From the values obtained, percent weight gain or loss was calculated. Since earthworms show diurnal rhythms in their activity and toxic response, all the experiments were done during a particular period of the day.

Results

Earthworms are useful as test organisms to assess the toxicity of herbicidal contaminated soils, because of their behavioural changes occurred in biomass. The acute toxicity values and their lower and upper confidence limits of the earthworm, *Lampito mauritii* exposed to five different substrates mixed with herbicide metribuzin (mg /kg substrates) have been worked out for different time intervals. The effect of concentration and exposure time on biomass of earthworm resulted in the maximum reduction in weight loss at highest concentration of test chemical. The figure 1, 2, 3 and 4 shows the comparative toxic range of the substrate and the control. The toxic range between 1 hr-LC_{50} and 144 hrs – $LC_{50}$was 10.75 – 0.25, 09.00 – 3.00, 9.10 – 8.50, 8.70 – 6.60 and 8.65 – 7.35 mg active ingredient of herbicide metribuzin kg/dry substrate weight for the earthworm exposed to the substrates 1,2,3,4 and 5 respectively. While the constructing acute toxicity curves for the earthworm LC_{50} and different time periods, asymptote was reached in all case during 144 hours. Similar trend is also reported in other agrochemicals by a number of previous workers (R.Bakthavathsalam and P.Rajaraman, 2003, Bharathi and Subha Rao 1984) reported 96-h LC_{50} of Carbofuron, Phosphamidon, Monocrotophos and Dichlorvos on *Lampito mauritii* in artificial soil.

Fig. 1. Comparative toxic impact of herbicide metribuzin (mg/kg) concentration on *Lampito mauritii* in Control and Cow dung

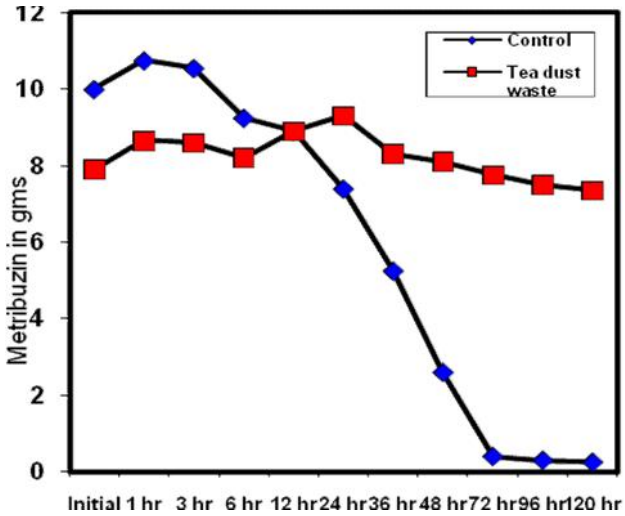


Fig. 2. Comparative toxic impact of herbicide metribuzin (mg/kg) concentration on *Lampito mauritii* in Control and Coir pith

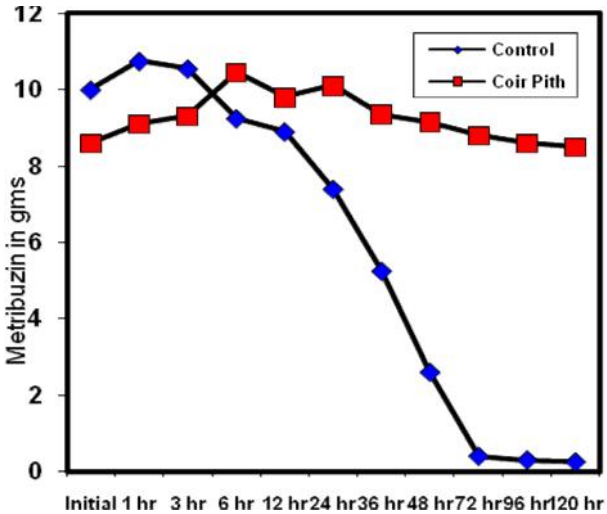


Fig. 3. Comparative toxic impact of herbicide metribuzin (mg/kg) concentration on *Lampito mauritii* in Control and Saw dust

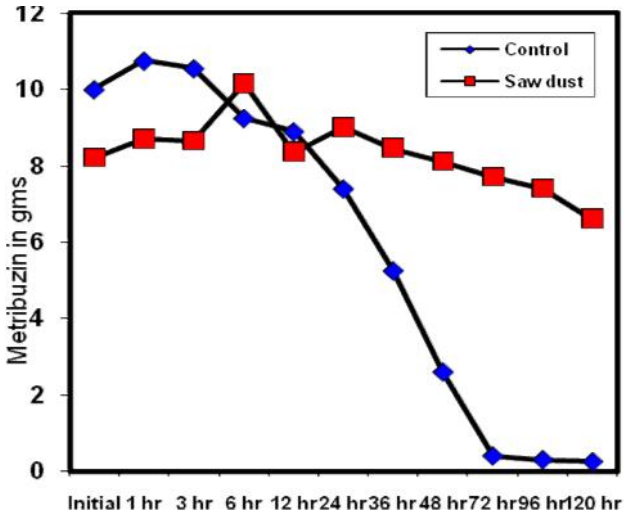
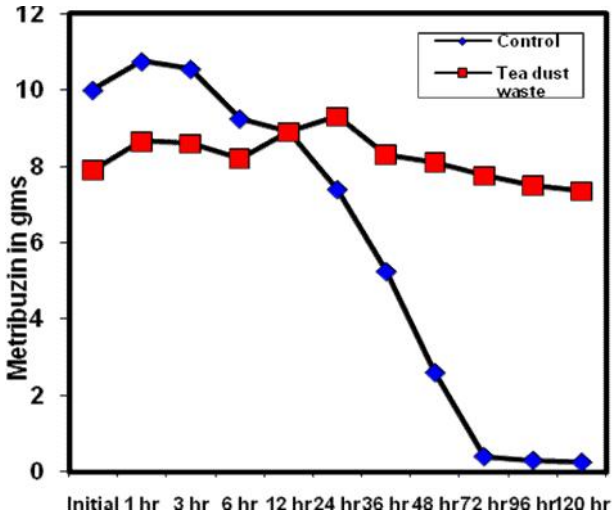


Fig. 4. Comparative toxic impact of herbicide metribuzin (mg/kg) concentration on *Lampito mauritii* in Control and Tea waste



Discussion

The remarkable quantities of pesticides are rejected into the environment, thus inducing a chronic contamination of an increasing number of ecosystems (A.Sarkar, etal 2006). This contamination causes a stress in bio-indicator organisms of pollution by distorting their physiological and biochemical process in different degrees. Acute toxicity of earthworm is an efficient tool in assessing ecological risks of contaminated soils (Lukkari *et al.*, 2005; Hemibach, 1985) and the end point is mortality (Karnak and Hamelink, 1982; Dean-Ross, 1983; Ellis *et al.*, 2007).

In this present experiment, Herbicide metribuzin showed no mortality at the recommended agricultural dose. The toxic range between 1 hr – LC_{50} and 120 6 hr – LC_{50} for the earthworm kept in substrate 1 (control) was found to be 10.75 – 0.25 mg active ingredient of herbicide metribuzin /kg dry substrate weight. The worms kept in substrate 2, (Cow Dung), the same toxic range was found to be 9.00 – 3.00 mg active ingredient of herbicide metribuzin /kg dry substrate weight. In the case of substrates 3 (Coir Pith) the similar toxic ranges was found to be 9.10 – 8.50 mg active ingredient of metribuzin / kg dry substrate

weight. The worms kept in substrate 4 (Saw Dust) and substrate 5 (Tea Dust Waste) the respective toxic range was found to be 8.7 – 6.6 and 8.65 – 7.35 mg active ingredient of herbicide metribuzin /kg dry substrate weight. In all the cases, asymptote was reached in the acute toxicity curve during 144 hours at the concentrations 0.25, 3.0, 8.5, 6.60 and 7.35 mg active ingredient of herbicide metribuzin/kg dry substrate weight due to the attainment of equilibrium in the insecticide medium.

It is important note that the worms exposed to herbicide metribuzin mixed with different substrates experience the following as initial symptoms: swelling in the segments of clitella and posterior region, oozing out of the mucous substances from the body surface, deep constriction at certain parts of the body, rolling of body surface, colour change and fragmentations of body segments due to autotomy. Marginal weight gain at 1 hour as revealed by swelling of body surface. But in subsequent hours of exposure the worms showed remarkable reduction in their body weights as revealed by oozing of mucous substances from the body surface. From these results, it is inferred that even the concentration now used as non-lethal, would cause drastic change in the body weight as revealed by low uptake of substrate and hence the concentration cannot be considered as safe for the worm. The results of this study indicate that Coir pith, Tea dust waste and Saw dust may be a good alternative artificial soil substrate to reduce the toxicity effect of Herbicide metribuzin on Earthworm *Lampito mauritii*.

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