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Effect of selected fungicides on cellulase activity of epigeic earthworm *Eisenia fetida* (Oligochaeta)

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Abstract
Eisenia fetida, as the test specimen was exposed to two selected fungicides, carbendazim and captan, commonly used by the farmers, in natural garden soil. According to the determined LC_{50} value of the two fungicides more toxic carbendazim was used for the cellulase enzyme activity assessment. Feeding preference experiment was carried out and they showed maximum preference for *Anacardium oxidentale* (Cashew) leaves. The test specimen was exposed to sub lethal doses of carbendazim, i.e. 25% of LC_{50} value and 50% of LC_{50} value, along with the control set. The enzyme activity measured on the 3rd, 7th, 15th and 30th day from the experiment. The enzyme activity was suppressed a little in between 7th and 15th day of the experiment. From the enzyme activity we can use it as a potential biomarker to detect pesticide pollution in agro ecosystem and can be further used in genotoxicity studies.

Keywords: Fungicide, *Eisenia fetida*, Carbendazim, Cellulase.

1. Introduction
Dependence on agro-chemicals for enhancing productivity is a great concern. There are 60,000 varieties of chemicals in use with several thousand being added annually [1]. Besides seeds, nutrients, water etc, use of pesticides including fungicides is indispensable. Alarming population growth throughout the globe necessitates more food and cash crops production results rapid growth of pesticide market [2]. In spite of their benefits, increasing trend of fungicide application has deleterious effect on human environment and agro-ecosystem.
Regular use of fungicides can potentially pose a risk to the environment, particularly if residues persist in the soil or migrate off-site and enter waterways (e.g. due to spray drift, run-off) [3-6] If this occurs it could lead to adverse impacts to the health of terrestrial and aquatic ecosystems. For instance, concerns have been raised over the long term use of copper-based fungicides, which can result in an accumulation of copper in the soil [7, 6]. This in turn can have adverse effects on soil organisms (e.g. earthworms, microorganisms) and potentially pose a risk to the long-term fertility of the soil [7, 6].
Extensive use of insecticides in agricultural field produces several deleterious effects on soil ecosystems. Insecticides produce inhibitory effect on the macrofaunal, mesofaunal and microfaunal population of the soil and disturb the equilibrium of soil organisms. Since earthworms constitute about 92% of the invertebrate biomass of the soil, researchers around the world have used earthworms as model organisms for soil toxicity testing. The inception, testing and standardization of the acute earthworm toxicity test by OECD (1984) [8] and EPA (1996) [9] have been the catalysts for the emergence of earthworms as one of the key organisms in environmental toxicology.
In the present study, two fungicides carbendazim and captam were used for acute toxicity test but only carbendazim used to evaluate the toxic effects of the sub-lethal doses on the cellulase enzyme activity, of the epigeic earthworm *Eisenia fetida.*

2. Materials and Methods
2.1 Period of study:
Total period of study was of four months, from the month of August to the month of November, 2015.

2.2 Fungicides used

Table 1: The fungicides used in the study with their respective RADs.

Chemical Name	Trade Name	RAD*(mg/kg)	Source of Procurement
Carbendazim	BAVISTIN	0.96	Rallis, TATA Enterprise (Local Dealer, Midnapore, West Bengal)
Captan	CAPTAF	4.80	BASF, Germany (Local Dealer,Midnapore, West Bengal)

*RAD- Recommended Agricultural Dose

2.3 Specimen used

Age synchronised clitellete *Eisenia fetida* each weighing 150-250mg were used for the test.

2.4 Instruments Used

i. Electronic Balance- Mettler Toledo (New Classic MS)
ii. Environmental Test Chamber- IIC-INSTIND
iii. Homogeniser- Remi Electrotechnik Ltd (Type RQP-127/A).
iv. Centrifuge- Remi Cooling Centrifuge (C-24BL).
v. Spectrophotometer- Systronics (UV-VIS Spectrophotometer 117)

3. Experimental Procedures:

The acute toxicity test of the two fungicides was performed for a period of 96 hours. The feeding preference of the test specimens was determined for 90 days at an interval of 15 days. Cellulase activity of the test specimen were studied on the 3rd day, 7th day, 15th day & 30th day from the day of setting of the experiment i.e. for a period of one month.

Studies were performed with age synchronized specimens (150-250 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] [10]. 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 the soil media, viz, pH and Organic carbon Content were measured and the temperature and moisture content were kept constant (Table 2).

Table 2: Physiochemical parameters of the natural soil used as medium in both the acute toxicity test and Enzyme activity estimation.

Natural soil parameters	Values
p^H	6.90
Organic Carbon Content	1.18%
Moisture	61.2%

3.1 Acute Toxicity Test:

Different levels of the carbendazim and captan based on their recommended agricultural doses (RAD) (viz RAD, 1/2X-RAD, 2X-RAD and 3X-RAD) were administered into the test boxes with a micropipette [11]. The amount of a fungicide required was determined from the total area of the experimental box and was converted into mg 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 fungicide and control. The boxes were then left undisturbed for about 30 min for uniform spreading of the chemical in the soil medium. Five numbers of age synchronized specimens of *Eisenia fetida* 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 [12].

Table 3: LC_{50} values of the two fungicides used in the Acute Toxicity study.

Chemical Name	Trade Name	LC_{50} Values
Carbendazim	Bavistin	5.38 mg/kg
Captan	Captaf	10.41 mg/kg

3.2 Determination of Feeding Preference of test organisms:

Open choice experiment was done on epigeic earthworm *Eisenia fetida* 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 [13] [14]. Fifty 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.

3.3 Estimation of Digestive Enzyme

A very important aspect of the laboratory study was the quantitative estimation of the digestive enzyme cellulase [15] determined under laboratory conditions in natural garden soil (pH-6.90, organic carbon-1.18% moisture content-61.2%) by exposing the earthworms to sub-lethal dose of the fungicide, carbendazim, 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 250g of sieved garden soil along with 15 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 a small 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 cellulase 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. One way ANOVA has been done using SPSS ver.16.0

4. Results

The 96 hrs acute toxicity tests showed that Carbendazim with an LC_{50} value of 5.38 mg/kg soil was more toxic than Captan, LC_{50} value 10.41 mg/kg soil. The LC_{50} value of carbendazim is about five times higher than its RAD and in case of captan it is about two times higher than its RAD.

In the feeding preference experiment the earthworms showed maximum preference for *Anacardium occidentale* (cashew) leaves followed by *Mangifera indica* (mango), *Shorea robusta* (shal), *Acacia auriculiformis* (Acacia) and *Eucalyptus citridora* (Eucalyptus) (Fig A).

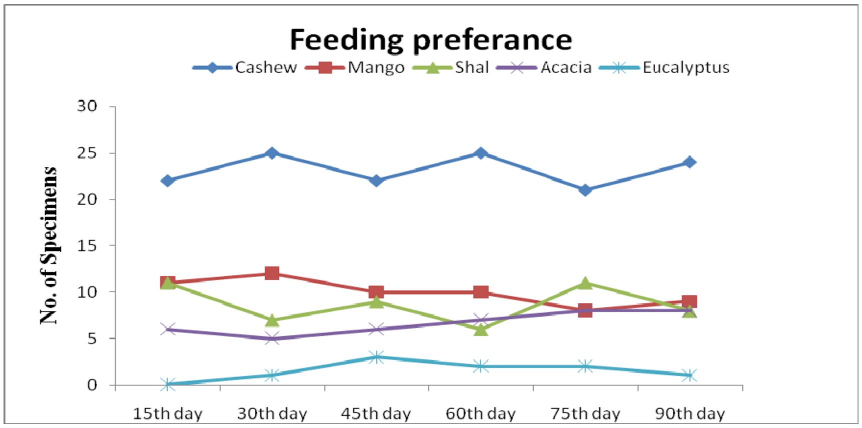

Fig: A: Feeding preference of *Eisenia fetida* in the leaf litter of five tree species.

Due to more toxic LC_{50} value of carbendazim determined by the acute toxicity test, the chronic toxicity test was carried forward with only carbendazim. In this experiment the cellulase enzyme activity in response to the carbendazim, of the test specimen was higher in both the sub lethal doses, 25% LC_{50} and 50% LC_{50} viz, 1.59±0.50 mg of glucose/min./mg protein and 1.25±0.45 mg of glucose/min./mg protein respectively and 1.50±0.50 mg of glucose/min./mg of protein and 1.75±0.55 mg of glucose/min./mg protein respectively than that of the control values viz, 1.09±0.40 mg of glucose/min./mg protein and 1.29±0.48 mg of glucose/min./mg protein on the 3rd and 7th day respectively after setting of the experiment. The activity of the enzyme diminished significantly than the control value (2.25±0.85 mg of glucose/min./mg protein) on the 15th day of the experiment viz, 1.58±0.60 mg of glucose/min./mg protein in both the sub lethal doses i.e. 25% of LC_{50} and 50% of LC_{50} value respectively. But on the 30th day of the experiment the activity of the enzyme increased to 3.05±1.30 mg of glucose/min./mg protein and 3.05±1.35 mg of glucose/min./mg protein in both the sub lethal doses i.e. 25% of LC_{50} and 50% of LC_{50} respectively which are slightly higher than the control value i.e. 2.75±1.15 mg of glucose /min./mg protein (Fig B).

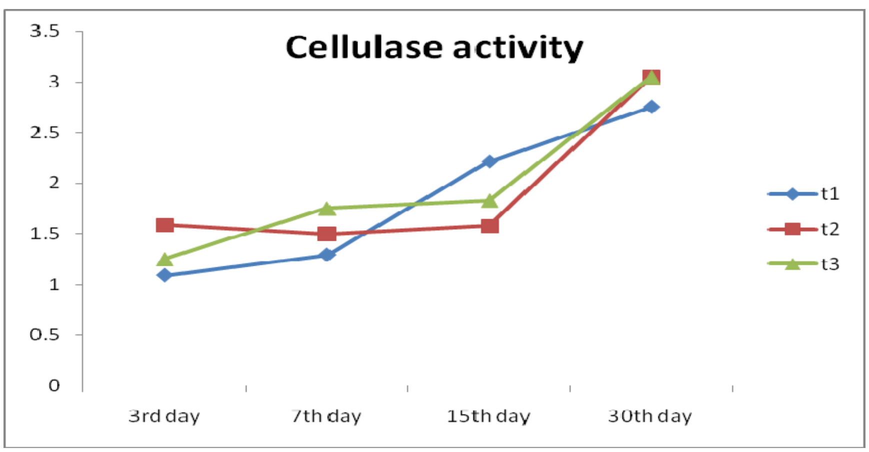

Fig B: Cellulase activity of *Eisenia fetida*, T1(Control), T2 (25% of LC_{50}) and T3 (50% of LC_{50}). Values of the enzyme are expressed in least significant difference, p<0.05 probability value.

5. Discussion

The LC_{50} value of carbendazim is higher than its RAD which indicates that this fungicide is ecologically safe in respect of short term (96 hours) acute toxicity. Studies of acute risk on *Eisenia fetida* after application of carbendazim in vineyards shows a LC_{50} value of 5.7mg/kg [16].

Maximum colonization in Cashew and Mango with higher rates of degradation of these leaf litters can again be related to their lower antinutrient contents, viz polyphenol and tannin leading to higher palatability [17-20].

On the 3rd and 7th day of the experiment, cellulase activity of the earthworms somewhat significantly increased in both the sub lethal doses as compared to the control. This is probably because of the test specimen were unable to sense the fungicide contamination in the food and consumed it, as a result of keeping them in starvation before setting of the experiment. On the 15th day there was a little increase in the cellulase activity. This is because of that the earthworms were little bit affected by the fungicide but the enzyme activity didn't increase in the same rate as observed between 3rd and 7th day in sub lethal doses but in control the enzyme activity increased. On the 30th day the enzyme activity increased further in sub lethal doses and also in control compared to the 15th day's result of the experiment. Probable cause of this increase in cellulase activity is that the fungicide is been degraded in food or the earthworms after sensing the fungicide become resistant to it. As the enzyme activity increased it can be said that the earthworms are not avoiding the food and restoring their enzyme activity to normal value. Studies on the effect of carbendazim on the cellulase activity of *Eisenia fetida* has not been reported so far.

6. Conclusion

From the above study it can be concluded that carbendazim shows less toxicity upon the earthworm after a certain period from the initial date of exposure, it does not have harmful effect when long term exposure is performed. In this regard carbendazim can be treated as an ecologically safe fungicide. Last of all, it can be concluded that the enzyme cellulase can be used as a potential biomarker to detect pesticide pollution in agro ecosystem.

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8. References

1. Maugh TH, Science, 1978; 199,162.
2. Ecobichon DJ. Pesticide use in developing countries. Toxicol. 2001; 160:27-33.
3. Kookana RS, Baskaran S, Naidu R. Pesticide fate and behaviour in Australian Soils in relation to contamination and management of soil and water: a review. Australian Journal of Soil Research. 1998; 36 (5):715–764.
4. Wightwick A, Allinson G. Pesticide residues in Victorian waterways: A review. Australasian Journal of Ecotoxicology. 2007; 13:91–112.
5. Kibria G, Yousuf Haroon AK, Nugegoda D, Rose G. *Climate change and chemicals. Environmental and biological aspects*, New India Publishing Agency, Pitam Pura, New Delhi, 2010, 460.
6. Komarek M, Cadkova E, Chrastny V, Bordas F, Bollinger JC. Contamination of vineyard soils with fungicides: A review of environmental and toxicological aspects. Environment International, 2010; 36:138–151.
7. Wightwick A, Mollah M, Partington D, Allinson G. Copper fungicide residues in Australian vineyard soils. Journal of Agricultural & Food Chemistry. 2008; 56: 2457–2464.
8. OECD (Organization for Economic Co-Operation and Development), Guidelines for testing of chemicals No 207, Earthworm Acute Toxicity Test, OECD 1984, Paris, Fance.
9. URL 1 EPA 1996. http://www.epa.gov/nerleerd/stat2.htm accessed 2 Nov 2009
10. Joy VC, Chakravorty PP. Impact of insecticides on non target micro arthropod fauna in agricultural soil. Eco toxicol. Environ. Safety 1991; 22:8-16.
11. Bostrom U, Lofs-holmin A. Testing Side effects of pesticides On Soilfauna Critical Literature Review, Report 12, Swedish Univ. Agri. Sci., and Uppsala. 1982, 70.
12. Dasgupta R, Chakravorty PP, Kaviraj A. Studies on relative toxicities of six insecticides on epigeic earthworm, Perionyx excavates. Bull. Environ. Contam. Toxicol. 2010; 85:83-86.
13. Maity SK, Joy VC. Impact of antinutritional chemical compounds of leaf litter on detrivore soil arthropod fauna. Journal of Ecobiology. 1999a; 11(3):193-202.
14. Maity SK, Joy VC. Soil micro arthropod feeding effect on nonnutrient chemicals of decomposing leaf litter. Indian Journal of Environment and Ecoplanning. 1999b; 2(3):225-231.
15. Sadasivam S, Manickam A. Biochemical Methods. 3rd, New Age International (P) Ltd. Publishers, New Delhi, 2008, 116-117.
16. URL 2 www.inchem.org/documents/jmpr/jmpmono/v95pr19.htm
17. Hendriksen NB. Leaf litter selection by detritivore and geophagus earthworms. Biology and Fertility of Soils 1990; 10:17-21.
18. Hobbie SE, Reich PB, Oleksyn J, Ogdachi M, Zytkowiak R, Hale C, *et al.* Tree species effects on decomposition and forest dynamics in a common garden. Ecology, 2006; 87(9): 2288-97.
19. Patricio MS, Nunes LF, and Pereira EL. Litter fall and litter decomposition in chestnut high forest stand in northern Portugal. Forest Sys. 2012; 21(2):259-271.
20. Johansson MB, Berg B, Meentenmeyer V. Litter mass loss rates in late stages of decomposition a climatic transect of pine forest. Long term decomposition in a Scots pine forest. IX. Can. J. Bot. 1995; 73:1509-1521.