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Imidachloprid and chloropyrifos pesticides impact on some anti-oxidant enzymes of earthworm, *Eisenia fetida* in semi-arid tropical regions of Medak District

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Abstract

The present study found that worms, *Eisenia fetida* exposed to imidachloprid and chloropyrifos pesticides showed elevated levels of Superoxide Dismutase (SOD) and catalase, which prevent oxidative damage. Elevated levels of SOD activity levels were 36.08, 31.24 were observed in worms exposed to imidachloprid and chloropyrifos respectively, it was be 28.74 units/mg proteins in the control Catalase “enzyme activity in the control worm”, *E. fetida*, was 25.71 units/mg proteins; 31.42 and 28.91 from pesticide exposed worms. Glutathione peroxidase enzyme activities of 13.24 and 11.51 µmoles of formazan liberated/mg protein/hour, while the control worm had enzyme activities of 6.9 µmoles of formazan liberated/mg protein/hour.

Keywords: *Eisenia fetida*, imidachloprid, chloropyrifos, superoxide dismutase, catalase and Glutathione peroxidase

Introduction

The soil is a complex ecosystem having different organisms, humus, organic matter, enzymes, minerals, and nutrients. Earthworms are used as a biological agent, aerator, moisture retainer, plough, and crusher in addition to being biofertilizers and composting agents (Eguchi *et al.*, 1995) ^[1]. According to Dash and Senapathi (1986) ^[2], earthworms are so crucial that vermin casting has significantly increased agricultural yields while using relatively little pesticide and virtually no chemical fertilizer. Vishwanathan, 1997; 1993) ^[3]. An ecological imbalance may result from pesticide and heavy metal toxicity to earthworms' natural habitat (Laird and Kroger, 1981) ^[4].

Earthworms have an "antioxidant defense system" that guards them against lipid peroxidation under adverse circumstances. Various “important enzymatic scavengers of the Superoxide (O₂⁻) and Hydroxide (OH⁻)” ions exist,” such as Glutathione Peroxidase (GPx), Catalase (CAT), and Superoxide Dismutase (SOD)”. By blocking the production of hydroxyl free radicals, these enzymes function as scavengers, protecting the components of cells from damage caused by oxidation (Scott *et al.*, 1991) ^[5].

In agro-ecosystems, earthworms are important biotic resources with enormous potential. Earthworms are soil engineers. Earthworms serve as an ecosystem's secondary decomposer “(Eisenhauer & Eisenhauer, 2020) ^[6]. For an ecosystem to be sustainable, its biodiversity must be well-balanced and flourishing (Prakash and Srivastava, 2019)” ^[7].

Earthworms can recycle agricultural waste, which addresses many of our concerning problems with solid waste management and water contamination (Gupta *et al.*, 2019) ^[8]. Their coelomic cavity is filled with fluid and contains a huge number of coelomocytes, plasma, and a watery matrix. These cells are crucial to the development of innate immunity. The coelomic fluid carries out physiological functions and keeps the moisture content stable (Griffith *et al.*, 2019) ^[9].

Pesticides can have an impact on the Earthworms and other non-target soil species seriously “harm the ecosystem (Reinecke and Reinecke, 2007)” ^[10]. There may be a chance that these pesticides will reach higher trophic levels because” amphibians (Lescure, 1966) ^[11], reptiles (Catling and Freedman, 1980)” ^[12], “birds (Harlin, 1977) ^[13], and mammals”.

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Eisenia fetida extract contains” antioxidant enzymes such as glutathione peroxidase”, catalase, and sodium dismutase. Antioxidants prevent oxidation, preserving metabolic processes. Free radicals can be produced by an oxidation reaction. These free radicals can damage cells by initiating chain reactions.

Materials and Methods

The goal of this study was to determine imidachloprid and chlorpyrifos impact on antioxidant enzymes such as Glutathione Peroxidase (GPx), Sodium Dismutase (SOD), Catalase (CAT), and Acetyl Cholinesterase (AChE) from *Eisenia fetida*. They stop biological tissues from oxidizing and scavenge free radicals. Other variables, like as oxygen availability and nutritional state, also affect the catalytic activity [1]. Prior to testing, these worms were housed in feed boxes with four-inch layers of clean red soil at the bottom (base soil), a thin layer of leaves, sixteen inches of meshed cow dung mixed with soft soil in a 1:1 ratio, and a thin layer of dried grass on top (growth medium) for seven days in a lab setting. The feed boxes were covered with wet gunny bags.

Preparing the sample

Making an enzyme extract

To allow their gut contents to be depurated, earthworms were kept in petri dishes on moist filter paper for a whole day. 50 mM phosphate buffer (pH 7.2) for SOD, “50 mM phosphate buffer (pH 7.4) for Catalase, and” 100 mM phosphate buffer (pH 6.0) for POD were used to homogenize 1 gm of gut-cleared earthworm in a pre-chilled mortar and pestle (Plate 2). The homogenate was centrifuged for 10 minutes at 4 °C at 10,000 rpm, and the enzyme activity assay was performed using the supernatant. Super-Oxide Dismutase Assay: “2.5 ml of phosphate buffer, 0.1 ml EDTA, 0.1 ml methionine, 0.1 ml NBT, 0.1 ml riboflavin, and 0.1 ml enzyme extract” were all included in the 3 ml assay mixture. After adding riboflavin last, the tubes were shook and exposed to 4000 lx fluorescent lights. After letting the reaction run for twenty minutes, the lights were turned out and a black cloth was placed over the tubes. The reaction mixture's absorbance was then measured at 560 nm.” The amount of enzyme needed to produce a 50% inhibition of the NBT photo-reduction rate was known as one unit of SOD activity (U), and” the results were expressed as U mg⁻¹ protein.

$$\text{Percent inhibition} = \frac{V - v}{V} \times 100$$

“Where,

V= Rate of assay reaction in absence of SOD

v= Rate of assay reaction in presence of SOD”

Catalase assay: 50 µl of enzyme extract and 0.55 ml of phosphate buffer were added to 0.4 ml of H₂O₂. After

carefully mixing the concoction, it was incubated for one minute 3.0 ml of potassium dichromate: acetic acid in a 1:3 ratio was then added to it following incubation. The control tube, which contained 0.6 ml of buffer and 0.4 ml of H₂O₂ devoid of enzyme, was placed in a boiling water bath for 10 minutes. After cooling, the absorbance was measured at 570 nm using acetic acid and dichromate as a blank. The quantity of H₂O₂ can be computed by deducting the absorbance of the samples from that of the control. The quantity of enzyme needed to consume one µmol of H₂O₂ U mg⁻¹ protein is known as one CAT enzyme unit.

Glutathione peroxidase assay

2.15 milliliters of phosphate buffer were pipetted into a cuvette. 0.1 ml of enzyme extract and 0.6 ml of guaiacol (0.01%) were added to it. After that, 25 µl of H₂O₂ was added. After thoroughly mixing the solution, 100% transmission at 470 nm was adjusted. Absorbance increased for three minutes at 15-second intervals. The quantity of enzyme needed to oxidize one n-mole of guaiacol was known as one activity unit of POD, and the result was given as µ mg⁻¹protein.

Nitro Blue Tetrazolium (NBT) (750 µM),”Ethylene Diamine Tetra Acetate (EDTA) (100 µM), methionine (130 µM), riboflavin (20 µM), phosphate buffer (50 mM for SOD [pH 7.2], CAT [pH 7.4], and” 100 mM for POD [pH 6.0]), “peroxide (H₂O₂) (0.2 M for CAT and 30% for POD), guaiacol (0.01%), 5% potassium dichromate, and glacial acetic acid.

The data obtained was “presented in Table 1 and Figure”1. Superoxide dismutase transforms super oxides into peroxides (O₂ to H₂O₂) shortly after reactive oxygen species (ROS) are produced. Worms treated to imidachloprid and chlorpyrifos showed elevated SOD activity levels of 36.08 and 31.24, respectively. The control worm, *E. fetida*, had superoxide dismutase activity of 28.74 units/mg proteins. There was a statistically significant increase of 8.69% in superoxide dismutase activity and a 25% increase in superoxide dismutase rate ($p < 0.05$).

Catalase hydrolyzes H₂O₂ and prevents oxidative damage at the cellular level.” The current study found that elevated” SOD activity in turn induces enhanced Catalase activity””. It was discovered that “the enzyme activity in the control worm, *E. fetida*”, was 25.71 units/mg proteins; 31.42 and 28.91 from the treated worms. A 12.44% and 22.20% increase in catalase activity was observed for imidachloprid and chlorpyrifos respectively and it was statistically significant ($p < 0.01$).

Glutathione peroxidase” protects the biological system from oxidative stress by converting H₂O₂ into water. The worms exposed to imidachloprid and chlorpyrifos had enzyme activities of 13.24 and 11.51” µmoles of formazan liberated/mg protein/hour”, while the control worm had enzyme activities of 6.9 “µmoles of formazan liberated/mg protein/hour”.

Table 1: Anti-oxidant activity of *Eisenia fetida* (All values in this table represent the mean ± SD (n=3))

Enzyme	Control	Imidachloprid	Chlorpyrifos
AChE	0.015±0.05	0.034±0.04	0.021±0.02
Catalase (µ /mg)	25.71±0.64	31.42±0.64	28.91±0.43
Superoxide Dismutase (µ/mg)	28.74±0.35	36.08±0.21	31.24±0.24
Glutathione peroxidase (mg/100 gm)	6.9±0.64	13.24±0.24	11.51±0.18

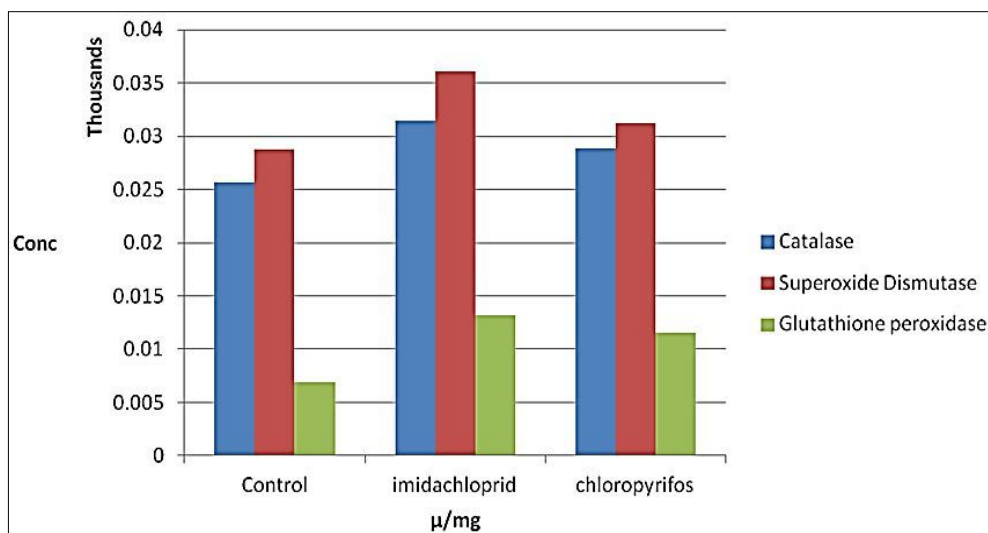


Fig 1: Anti-oxidant activity of *Eisenia fetida*

A 91.88% and 66.81% increase in catalase activity was observed for imidachloprid and chloropyrifos respectively and it was statistically significant ($p < 0.01$).

It has long been known that earthworms can adapt to stressful situations." Physical and chemical characteristics" of soil, including as "pH, texture, organic matter content, and mineral availability, affect survival, growth, and reproduction" [Maity 15]. *Eisenia fetida*'s possible detoxifying capacity is allowed it to survive. The current study's findings showed a rise in the levels of antioxidant enzyme expression. The imidachloprid and chloropyrifos increased SOD and catalase levels sandalio [16]. These antioxidant enzymes scavenge Reactive Oxygen Species (ROS) and free radicals".

Conclusions

The study found that worms exposed to imidachloprid and chloropyrifos showed elevated levels of Superoxide Dismutase (SOD) and catalase, which help prevent oxidative damage. Elevated SOD activity increased catalase activity, while glutathione peroxidase activity increased. Earthworms can adapt to stressful situations, and the physical and chemical characteristics of soil affect survival, growth, and reproduction. The study also found that soil-specific antioxidant enzyme expression increased, allowing *Eisenia fetida* to survive. These enzymes help scavenge reactive oxygen species and free radicals.

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