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A study on the anti-oxidant enzyme levels in *Eisenia fetida* from BT cotton fields of Medak, Telangana

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Abstract
The present study was conducted to know the antioxidant enzyme levels in *Eisenia fetida*. The levels of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), and Glutathione peroxidase were determined. The SOD, CAT and glutathione peroxidase activities significantly ($p< 0.05$) increased. This is due to the effect of Cry protein present in Bt cotton. The present results suggest that the elevated levels of anti-oxidant enzymes are in response to the stress and they protect from oxidative damage by scavenging free radicals. Superoxide dismutase activities in the control worm *E. fetida* were found to be 29.93 units/mg proteins 32.08 in worms from Bt crop field. Catalase activity in the control worm *E. fetida* were found to be 22.34 units/mg proteins 27.41 worms from Bt crop field. Glutathione peroxidase were found to be 7.8 µmoles of formazan liberated/mg protein/hour mg; and 9.1 µmoles of formazan liberated/mg protein/hour in worms from non Bt and Bt crop fields respectively.

Keywords: Anti-oxidative enzymes, super-oxide dismutase, catalase, glutathione peroxidase, free radicals

Introduction
Earthworms are present in almost all ecosystems around the globe. It lives in soil, feeds on litter and forms no burrows. Earthworms are saprophytic organisms. They live in an environment replete with microorganisms. They are soil dwelling, effect pore size, enhance the nutrients. They increase aeration, nutrient availability to the plants, root development, infiltration of the water and water holding capacity. They reduce surface runoff. They are popularly called as Golden fertilizers of the nature. *Eisenia fetida* is a member of Oligochaeta and is an epigeic worm.

Eisenia fetida are called as Red worms, they exhibit short life cycle, complete hatching in 3-4 weeks attain maturity within 7 to 8 weeks. Since they are detritus, they feed on plant residues and excreta aggregate to form Earthworm castings. They are influenced by climatic conditions, soil characteristics, tillage, use of pesticides and fertilisers and other management practices. Numerous reports are available on the role of earthworms in enriching and maintenance of soil health. Since earthworms are located at the base of the food chain, it is important to know the impact of external factors on earthworm and further on the food chain.

The present study has been undertaken to know the impact of Cry Protein (Bt Cotton) on the anti-oxidant enzyme like Sodium dismutase (SOD), Catalase and glutathione peroxidase (GPX) activity. The catalytic activity is also influenced by other factors like nutritional status and oxygen availability [1]. Antioxidant enzymes Sodium dismutase, Catalase and glutathione peroxidase were studied from *Eisenia fetida*. They scavenge free radicals and prevent oxidation of biological tissues.

Eisenia fetida is used as a standard test practice in toxic studies [2]. Organisms usually produce reactive oxygen species [3]. In the present investigation it has been planned to study the impact of Cry protein on superoxide dismutase SOD, glutathione peroxidase (GPX) and Catalase under normal conditions and under Bt cotton (Cry) stress in the natural crop fields. Coelomic fluid contains plasma and coelomocytes that exhibits immune response [4].

Nutrition, growth, development, survival and reproduction are affected by environmental factors [5].There are considered as ecological indicators by Organization for Economic Cooperation and Development (OECD) [6] [7].

Sodium dismutase, Catalase and glutathione peroxidase were reported from *Eisenia fetida* [8].

Material and Methods

The study was conducted during 2020 in BT and non BT Cotton crop fields at Chandanagar, Medak district of Telangana, India to know the impact of Cry protein on the anti-oxidant enzymes. The study site is located between 17.48 latitude and 78.31 longitudes. The annual rainfall is around 1322.4 mm in the year 2020, it is a semi-arid zone. Telangana receives rain from southwest monsoon. Annual mean maximum temperature of the district is 32.44 °C and minimum is 24.31 °C. The mean minimum temperature is recorded during December (17 °C) and maximum in May (42 °C). Superoxide dismutase activity was determined [9]. Catalase and Glutathione peroxidase activities were determined [10].

Results and Discussion

The obtained results were presented in table 1 and figure 1. Superoxide dismutase acts soon after the generation of Reactive Oxygen Species (ROS), it converts super oxides into peroxides (O^{-2} to H_2O_2). Elevated levels of SOD activity (32.08) was observed in worms from Bt crop fields (29.93) when compared to worms from non-Bt crop fields. Superoxide dismutase activities in the control worm *E fetida* were found to be 29.93 units/mg proteins 32.08 in worms from Bt crop field. Superoxide dismutase activities increased by 7.03% and increased rate of superoxide dismutase was statistically significant at (p<0.05).

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

Enzyme	*E. fetida* from Bt crop	*E. fetida* from non Bt crop
Catalase (U/mg)	27.41±0.17	22.34±0.18
Superoxide Dismutase (U/mg)	32.08±0.19	29.93±0.84
Glutathione peroxidase (mg/100 gm.)	9.1±0.14	7.8±0.21

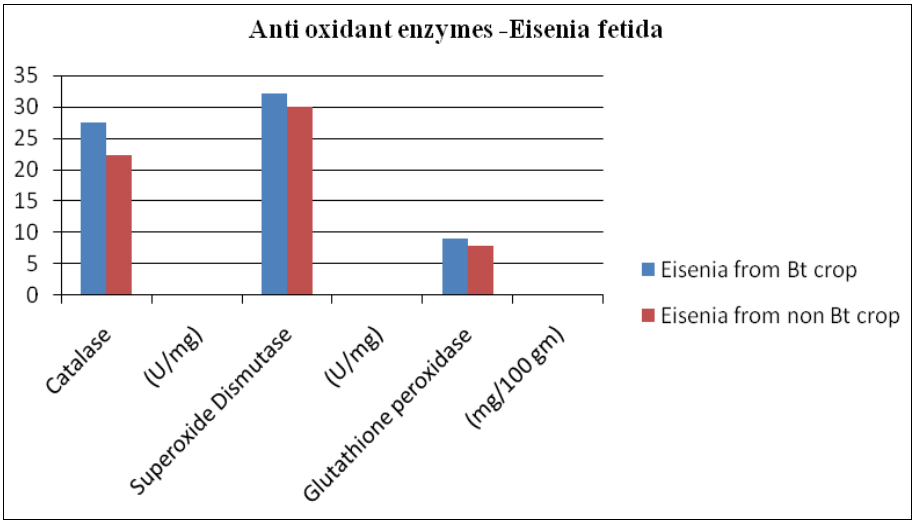

Fig 1: Anti-oxidant activity of Eisenia

Catalase hydrolyses H_2O_2 and averts oxidative damage at cellular level, increased SOD activity in turn stimulates elevation in Catalase activity and it was observed in present study. Enzyme activity in the control worm *E. fetida* were found to be 22.34 units/mg proteins 27.41 worms from Bt crop field. The catalase activity, increased by 20.38% and it was statistically significant at (p<0.01).

Glutathione peroxidase metabolises H_2O_2 into water, protect the cellular system under oxidative stress. The enzyme activities in the control worm were found to be 7.8 µmoles of formazan liberated/mg protein/hour mg; and 9.1 µmoles of formazan liberated/mg protein/hour in worms from Bt crop field. The Glutathione peroxidase activity, increased by 14.11and it was statistically significant at (p<0.01).

Earth worms are long been known for their adaptation in response to the stress conditions. Survival, growth, reproduction is influenced by physical and chemical factors of soil like pH, texture, organic matter content and mineral availability [11]. The successful survival of *Eisenia fetida* is due to its potential detoxification capability. The results of present study revealed a slight increase in expression of antioxidant enzyme levels. The elevated levels of SOD and catalase are due to the effect of Cry protein [12]. These antioxidant enzymes are scavenge the free radicals and reactive oxygen species (ROS).

Conclusion

The present study has undertaken to evaluate the impact of Cry protein on the *Eisenia fetida*. Antioxidant enzymes are used as potential biomarkers. The present study shows the ability of *Eisenia fetida* to survive under cry protein stress. The levels of these enzymes are more when compared to enzyme levels of the Earthworms in the non Bt crop fields.

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