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## Effect on superoxide dismutase activity in earthworm *Eisenia fetida* on direct exposure to neonicotinoid insecticide imidacloprid

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### Abstract

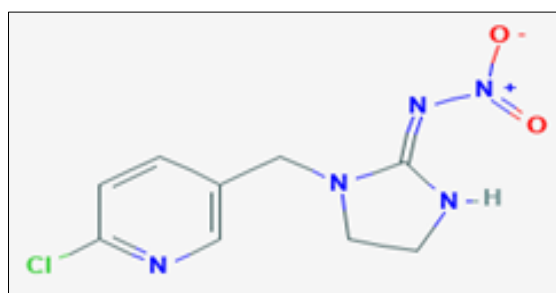
Imidacloprid is a systemic insecticide that acts as an insect neurotoxin and belongs to class of chemicals called the neonicotinoides which act on central nervous system of insects. Three concentration of imidacloprid (0.134 µl, 0.195 µl and 0.285 µl) have been used during the experiment for evaluation of enzymatic activity. The increase in superoxide dismutase (SOD) activity suggests that SOD has the capacity to scavenge reactive oxygen species (ROS) under the stress at lower concentration of imidacloprid and it also indicated that the activity of the superoxide dismutase had shown different responses to toxicity of various concentrations of imidacloprid for different exposure periods in earthworm *Eisenia fetida*. Imidacloprid at concentrations of 0.195 µl and 0.285µl had shown the SOD activities of 6.773 and 7.263 U/mg protein respectively at 48 hr of exposure, while a concentration of 0.285µl has showed an enzyme activity of 6.835 U/mg protein at 24 hr of exposure. The study revealed that the activity of antioxidant enzyme of earthworms is altered due to stress produced by neonicotinoid insecticide. The statistical data of pesticide is highly significant with respect to treatment and time of exposure.

**Keywords:** neonicotinoid, imidacloprid, superoxide dismutase and *Eisenia fetida*

### Introduction

Imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin- 2-ylideneamine] (Figure 1) has been used extensively to manage sucking insects, soil insects, termites and chewing insects in agriculture worldwide (Sur and Stork 2003; Laycock *et al.*, 2012) [13, 8].

The chemical works by interfering with the transmission of stimuli in the insect nervous system. Specifically, it causes a blockage of the nicotinic neuronal pathway by blocking nicotinic acetylcholine receptors and stops acetylcholine from transmitting impulses between nerves, resulting insect's paralysis and eventual death. It is effective on contact or through stomach action and imidacloprid is the one of the most widely used pesticide in the world (Yamamoto and Izuru, 1999) [14]. Bayer crop science has been producing this chemical in different forms for wide range of applications. The formulations are intended to apply by injection to soil, trees, granular forms for broad cast, direct soil application and foliar sprays. It is very effective systemic insecticide against the sucking insect on major food crops. Imidacloprid was most extensively used on cotton to kill sucking pests at early stages of the crop development. This chemical also found its use to control domestic pests likes, houseflies, termites and so forth (Gervais, *et al.*, 2010).



**Fig 1:** Chemical structure of imidacloprid

Neonicotinoids insecticides such as imidacloprid, acetamiprid, nitenpyram, clothianidin and thiacloprid act as agonists on nicotinic acetylcholine receptors by showing different toxicity to different organisms. The results of the Qingming *et al.*, (2014) [11] studies showed that the activity of superoxide dismutase (SOD) was significantly increased at concentration of 0.66 and 2 mgkg<sup>-1</sup> imidacloprid over the 14 days of exposure. This phenomenon indicated that two doses of imidacloprid could induce the formation of O<sup>2-</sup> and then stimulate the biosynthesis of SOD in earthworm to protect the cells from oxidative damage.

### Materials and Methods

- Preparation of earthworm tissue homogenates by method of Jeyanthi *et al.*, (2016) [6].
- (B) The protein content was estimated in each earthworm sample using the method of Lowry *et al.* (1951) [10].
- Estimation of superoxide dismutase activity (SOD)

Three concentration of imidacloprid (0.134 µl, 0.195 µl and 0.285 µl) have been used during the experiment for evaluation of enzymatic activity. Superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) adopting the method of Giannopolitis and Ries, (1977) [5]. The reaction mixture (3.0 ml) contained 2.5 ml of 60 mM Tris-HCl (pH 7.8), 0.1 ml each of 420 Mm/L-methionine, 1.80 mM NBT, 90 µM riboflavin, 3.0 mM EDTA and enzyme extract. Riboflavin was added at the end. The tubes were shaken properly and placed 30 cm below light source consisting of three 20W fluorescent lamps (Phillips, India).

The reaction was started by switching on the light and terminated after 40 min of incubation by switching off the light. After terminating the reaction, the tubes were covered with black cloth to protect them from light. A non-irradiated reaction mixture that did not develop colour served as the control. The reaction mixture without enzyme extract developed maximum colour and its absorbance decreased with the addition of enzyme. The absorbance was recorded at 560 nm. One enzyme unit was defined as the amount of enzyme that inhibits the nitro blue tetrazolium photoreduction by 50 percent inhibition was calculated by following formula

stated by Asada *et al.*, (1974) [1].

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

Where,

V = Rate of assay reaction in absence of SOD.

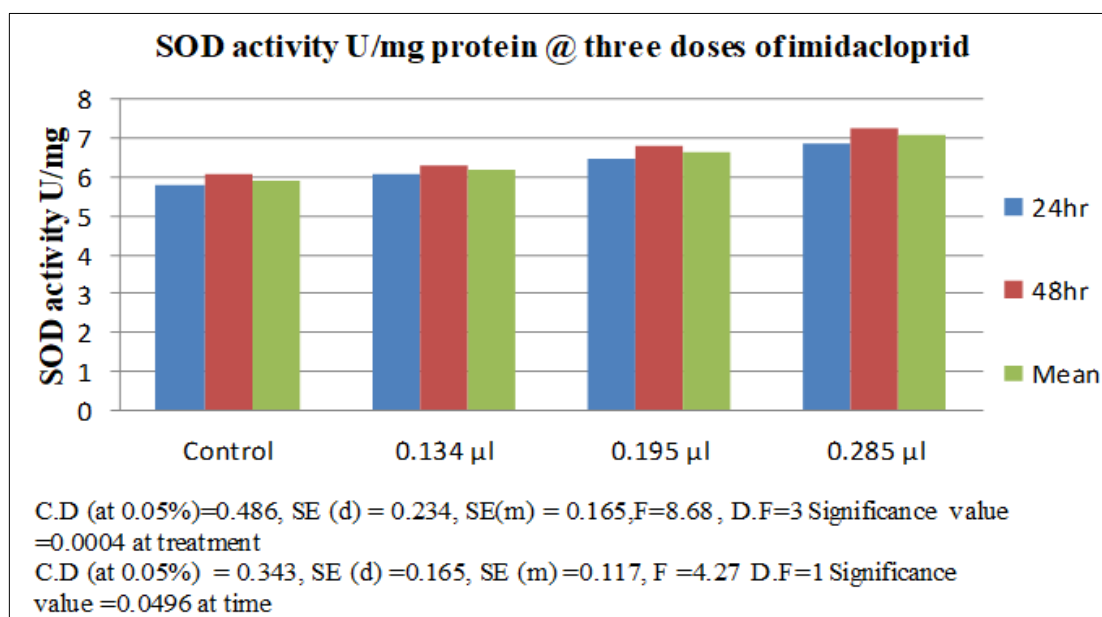
v = Rate of assay reaction in presence of SOD.

### Statistical Analysis

The standard statistical tools were used for analysis of data recorded in experiment. The experimental design for lab study was completely randomized block with four replicates. A critical difference (CD) was calculated between the treatments by CRD (*in vitro*), accordingly, using software OPSTAT, developed at the Computer Center, College of Basic Science and Humanities, CCS Haryana Agriculture University, Hisar, Haryana.

### Results

The study revealed that the activity of antioxidant enzyme of earthworms is altered due to stress produced by neonicotinoid insecticide. So the activity of important antioxidant defense enzyme *viz.* Superoxide Dismutase (SOD) was analyzed in *E. fetida* at various concentrations of imidacloprid. The results of SOD activity revealed that it has completely depended on time and concentrations of pesticide. Three doses of imidacloprid have been used during the experiment for identification of enzymatic activity. The SOD activity of 5.783 and 6.068 U/mg protein in control have been found at 24 hr and 48 hr of exposure respectively. It was observed that the activities of the enzyme have increased with an increase of imidacloprid concentration at 48 hr. From fig. 1 it has been apparent that increasing trend has been observed with imidacloprid. Imidacloprid at concentrations of 0.195 µl and 0.285 µl had shown the SOD activities of 6.773 and 7.263 U/mg protein respectively at 48 hr of exposure, while a concentration of 0.285 µl of imidacloprid has showed an enzyme activity of 6.835 U/mg protein at 24 hr of exposure. The statistical data of pesticides are highly significant with respect to treatment and time of exposure.



**Fig 1:** Effect of imidacloprid on SOD activity in *Eisenia fetida*

## Discussion

Earthworms are important bio-indicators for ecological factor, key part of food web and are non-target species affected by insecticides as a consequence of reach of these chemicals into soil and water bodies and eventually affect the ecosystems.

Zhang (2014) [15] studied and reported that the effect of imidacloprid on antioxidant defence system (SOD, CAT and POD) in *E. fetida* at 1<sup>st</sup>, 7<sup>th</sup> and 14<sup>th</sup> days, showed no significant changes with regard to SOD activity compared with the control when low doses were used where as significant increase in SOD activity was observed with high doses under prolonged exposure. SOD dismutates superoxide to hydrogen peroxide which will be subsequently detoxified by CAT and POD enzymes (Sun *et al.*, 2007; Liu *et al.*, 2010) [12, 9].

In the present study, the activity of the enzyme fluctuates throughout the exposure period. The large amount of free radicals usually produced within the body of an organism due to entry of pollutants into the body of earthworms from ecological environment that stimulate the organism and begins self defence to reduce ROS damage. The antioxidant detoxification enzyme system rapidly starts function and increases the activities of SOD enzyme to remove the free radicals. If the ROS production is higher than the scavenging pace of an organism in the body, the free radicals will accumulate and reduce body's ability to withstand to toxic radicals invading inside and cause cell membrane damaged by lipid peroxidation, increasing malonaldehyde (MDA) content significantly (Bing *et al.*, 2018) [2].

Similarly Qingming *et al.*, (2014) [11] showed that the activity of SOD was significantly increased at 0.66 and 2 mg kg<sup>-1</sup> imidacloprid over fourteen days of exposure time and noticed the activity of SOD was the most sensitive to imidacloprid. It indicates that imidacloprid could induce the formation of superoxide and stimulate the biosynthesis of SOD in earthworm to protect the cells from oxidant damage. The increase in SOD activity also suggests that the enzyme had the capacity to scavenge ROS under stress at lower concentration of imidacloprid. It also indicated that the activity of the SOD had different responses to the toxicity of different concentrations of imidacloprid for different exposure periods. Oxidative stress plays an important role in the toxicity of imidacloprid and induces destructive reactive oxygen species. Accumulation of ROS particularly hydrogen peroxide and superoxide radicals damage cellular components including DNA, proteins and lipids (Cooke *et al.*, 2003) [3].

Bing *et al.*, (2018) [2] also reported similar results on SOD and CAT activities exposed to acetamiprid and postulated that increase in enzyme activities are dose and time of exposure dependent. According to study of Koivula *et al.*, (2011) [7] the SOD was the major remover of oxygen free radical and only effective enzyme that can catalyze the dismutation reaction of superoxide to hydrogen peroxide that can be further detoxified by CAT.

## Conclusion

The results of SOD activity revealed that their activities were completely depending on time and concentrations of pesticide. With increased concentration of imidacloprid increased the activities of enzyme at 48hr exposure.

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