Environment and Ecology 39 (2) : 370—374, April—June 2021
ISSN 0970-0420

Effect of Neonicotinoid Insecticides Imidacloprid and Acetamiprid on Catalase Activity in Earthworm *Eisenia fetida*

Parveen Gill, Dommapati Sudhakara Rao, R. K. Gupta, Tejpal Dahiya, Deepika Lather, Naresh Kumar

Received 23 April 2021, Accepted 23 May 2021, Published on 10 June 2021

ABSTRACT

Neonicotinoid insecticides are the most important agricultural chemicals applied to crops and are considered as the world's largest selling insecticides form many years. They account for one-fourth of the entire insecticide market because of their excellent insecticidal activity. Imidacloprid and acetamiprid are the predominantly used neonicotinoide insecticides with harmful effect on antioxidant enzyme of earthworms of *Eisenia fetida.* Various concentrations of imidacloprid and acetamiprid were applied directly on earthworms and catalase activities have been studied to find out the effect. Catalase (CAT) activities in earthworms in control were 20.33 U/mg and 20.83 U/mg at 24 h and 48 h of exposure respectively. Acetamiprid at concentrations of 0.165 and 0.188µg have resulted the CAT activities of 22.83 and 24.00 U/mg proteins respectively at 48 h of exposure. At 24 h of exposure time the CAT activities were found to be 23.16 U/mg proteins at 0.188µg of acetamiprid concentration and effect of imidacloprid on catalase activity in earthworms at 48 h of exposure time have studied and found that the CAT activities were 23.40 U/mg proteins at 0.195 µl and 24.66 U/mg proteins at 0.285µl concentrations respectively. At 24h exposure to imidacloprid the CAT activity was 23.26 U/mg proteins at 0.285µl concentration. The study revealed that CAT activities are affected by both insecticides.

Keywords Neonicotinoide, Imidacloprid, Acetamiprid, CAT activity, *Eisenia fetida.*

INTRODUCTION

The soil is arguably the most diverse habitat within terrestrial ecosystems. In fact, survey data from several studies revealed that a quarter of invertebrate and vertebrate species inhabit the soil and it has recently been estimated that the biodiversity of soil animals comprises 23% of the biodiversity of all described species (Brown *et al.* 2000). Therefore, soil biodiversity is an essential component of the general biodiversity concept and nowadays it is recognized that soil organisms are responsible for the provision of many ecosystem services necessary for human wellbeing.

Parveen Gill*, R.K.Gupta, Tejpal Dahiya
Department of Zoology,
Dommapati Sudhakara Rao
Department of Chemistry and Biochemistry
Deepika Lather
Department of Veterinary Pathology
Naresh Kumar
Department of Agricultural Meteorology
Chaudhary Charan Singh Haryana Agricultural University,
Hisar, Haryana 125004, India.
Email: parveengill135@gmail.com
*Corresponding author

Pesticides have been used for crop protection by preventing range of pests. Some pesticides are even used in control of house hold pests to prevent dengue, malaria and other insect born diseases. Pesticides include insecticides, insect repellents, fungicides and weedicides are extensively used in Indian agriculture. Since the uses of pesticides have started the major increase in food production in our country due to prevention of loses caused by various pests on crops.

The pesticides target various vital metabolic systems in the organism and affect their function and cause death. Various enzymes are also prime targets of pesticides. They directly help agriculture but at the same time pose threat to environment causing range of biohazards and because of their toxicity they, as well pose danger to human being. The applied pesticide will normally decompose in the soil over time and their longevity in the soil will depend on the type of chemical used in formulations. Upon decomposition of these pesticides they will release nutrients like carbon, nitrogen, phosphorus and sulfur. The released nutrients may lose through water flow and drift. Undecomposed chemical pesticides often enter into food chain cause serious health effects to human (Anne *et al*. 2010). Neonicotinoids are now the most prominent class of insecticides and are being used globally as selective agonists of insect nicotinic acetylcholine receptors (Jeschke *et al.* 2011, Szczepaniec *et al.* 2013). Besides their use in agriculture in various formulations such as granules or foliar sprays, they even found their way to control house hold insects likes termites, cockroaches. They are also been used in veterinary medicine to control ectoparasites (Jeschke *et al.* 2011, Goulson 2013). Imidacloprid is a systemic insecticide that acts as an insect neurotoxin and belongs to a class of chemicals called the neonicotinoids which act on the central_ nervous_ system of insects. Acetamiprid is the second-generation of neonicotinoid insecticides and Japan commercialized it by Nippon Soda in 1995 and is used as foliar applications while direct soil uses are restricted (Elbert *et al.* 2008). Earthworms are considered the most significant soil invertebrates in most terrestrial ecosystems worldwide (Rombke *et al.* 2005). They are called 'ecosystem engineers' because of their ability to create or modify the soil habitat through various activities such as burrowing, casting, breakdown of organic matter, seed dispersal, ingestion of soil particles and symbiotic interactions with soil microbes (Cole *et al.* 2006, Gill *et al.* 2019, Jouquet *et al.* 2006, Rombke *et al.* 2005).

MATERIALS AND METHODS

(A) Preparation of earthworm tissue homogenates by method of Jeyanthi *et al.* (2016).
(B) The protein content was estimated in each earthworm sample using the method of Lowry *et al.* (1951).
(C) Catalase activity was determined by slightly modified method of Xu *et al.* (1997).

To 1 ml of sodium potassium phosphate buffer (0.05 M, pH 7.4), added 1.25 ml of H_2O_2 (0.066M) and 250 µl of enzyme extract. Blank was prepared by adding 1ml assay buffer to 1.25 ml of H_2O_2 (0.066M) without enzyme extract along with the samples. One unit of enzyme activity was equal to the amount of enzyme consumed after decreasing half of H_2O_2 over 100 sec at 25° C. Subtract the absorbance of samples from that of blank and calculate the amount of H_2O_2 from the standard curve.

Statistical analysis

The standard statistical tools were used for analysis of data recorded in experiments. 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 catalase activities in earthworm's *Eisenia fetida*, which are exposed to three concentrations of imidacloprid and acetamiprid with regard to exposure time, have been studied and the obtained results are presented in Fig. 1 and 2 from these observations it was clear that CAT activity of earthworms has depend on concentration of pesticides and time of exposure.

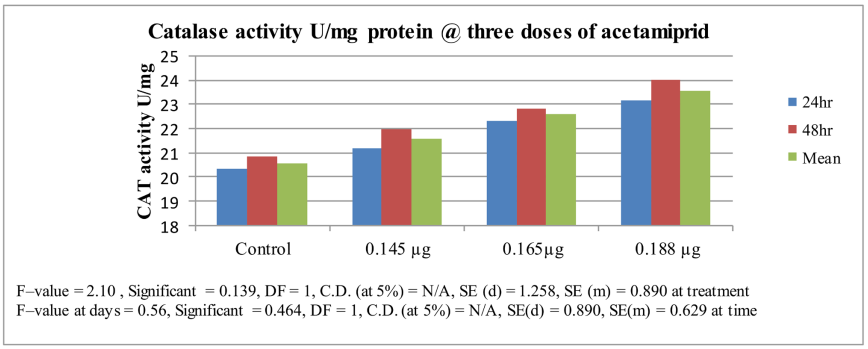

Fig. 1. Effect of acetamiprid on CAT activity in *Eisenia fetida.*

From results given in Fig. 1 showed that the CAT activities in *Eisenia fetida* in control were 20.33 U/mg and 20.83 U/mg at 24 h and 48 h of exposure respectively. Acetamiprid at concentrations of 0.165 and 0.188µg have resulted the CAT activities of 22.83 and 24.00 U/mg proteins respectively at 48h of exposure. At 24 h of exposure time the CAT activity was found to be 23.16 U/mg proteins at 0.188µg of acetamiprid concentration.

From experimental results presented in Fig. 2, it is revealed that the CAT activities in *Eisenia fetida* in control were 20.50 U/mg and 20.90 U/mg at 24 h and 48 h of exposure respectively. Effect of imidacloprid on catalase activity in earthworms at 48 h of exposure time have studied and found that the CAT activity was 23.40 U/mg proteins at 0.195 µl and 24.66 U/mg proteins at 0.285µl concentrations respectively whereas at 24 h exposure of imidacloprid the CAT activity was 23.26 U/mg protein at 0.285µl concentration. Statistically data of both pesticides are significant with regard to treatment and time of exposure.

DISCUSSION

Earthworms have a key role in agriculture because of its excreta providing quickly available plant nutrients like Nitrogen, Phosphorus and Potassium. It burrows in the soil and increase its porosity but they are directly or indirectly affected by pollutants as a result of indiscriminate use of pesticides for better crop yield. 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. Fish is the major species affected by these pollutants in water bodies and/or indirectly affected by consuming intoxicated earthworms upon eating in aqua systems.

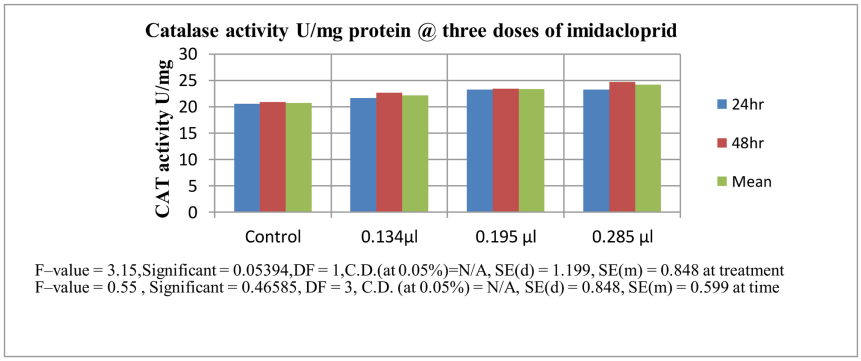

Fig. 2. Effect of imidacloprid on CAT activity in *Eisenia fetida.*

The present study revealed the activities of antioxidant enzymes in *E. fetida* were changed as a result of stress produced by acetamiprid and imidacloprid insecticides up on exposure. Reactive oxygen species (ROS) contain hydrogen peroxide (H_2O_2), superoxide radical (O_2-) and hydroxyl radicals (OH-) engage in destruction of cellular components and affect physiological and biochemical functions of an organism (Liu *et al.* 2017). CAT is a significant antioxidant enzyme which exists in Cytosol, mitochondria and peroxisomes that act in the degradation of hydrogen peroxide to water and oxygen (Satyro *et al.* 2017 Zhang *et al*. 2013). CAT enzyme decomposes hydrogen peroxide to stop the oxidative damage at cellular levels (Valavanidis *et al.* 2006, Hyrsl *et al.* 2007). From present observations it was clear that CAT activity of earthworms was depend on doses of insecticides and exposure time and similar increasing trend of CAT activities were observed by Enrico *et al.* (2019) after 24 h exposure to highest acetamiprid dose (1.6×10^{-3} µg/cm^2) whereas at 48 h exposure significant increase was observed at 1.6×10^{-4}, 8.3×10^{-4} and 1.6×10^{-3} µg/cm^2 and on 30th and 45th days as well as with various concentrations of acetamiprid (0.001,0.01, 0.05 and 0.1 mg /kg , 1.6×10^{-4}, 8.3×10^{-4}, 1.6×10^{-3} µgcm^{-2}) on earthworms of *E. andrei.* Normally after acetamiprid exposure to earthworms, the CAT activity involved in an activation and recovery process to control levels throughout.

According to study of Zhang *et al.* (2014) acetamiprid and imidacloprid were able to induce earthworm's antioxidant defense system in early exposure stages. Increasing trend of CAT activities was also reported by Liu *et al.* (2017) with exposure period of seven days to neonicotinoid clothianidin. Similar result was also revealed by the study of Bing *et al.* (2018). The study of Yanning *et al.* (2017) also showed the similar trend of SOD and CAT with imidacloprid doses of 0.3 and 1.0 mg /kg at 7th, 14th, 35th, 42nd and 56th day on *E. fetida.*

CONCLUSION

Three doses of imidacloprid as well as acetamiprid have been used during the experiment for identification of catalase enzymatic activity in earthworm *E. fetida*. The results of CAT activity revealed that their activities were completely depending on time and concentrations of pesticides. With increased concentration of imidacloprid and acetamiprid increased the activities of enzyme at 48h exposure.

ACKNOWLEDGEMENT

The authors are acknowledging the support received from CCS Haryana Agricultural University, Hisar, Haryana and Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS) Hisar.

REFERENCES

Anne Turbe, Arianna de Toni, Patricia Benito, Patrick Lavelle, Perrine Lavelle (2010) Soil biodiversity: Functions, threats and tools for policy makers. Ff bio emco-00560420.

Bing Li, Xiaoming Xia, Jinhua Wang, Lusheng Zhu, Jun Wang, Guangchi Wang (2018) Evaluation of acetamiprid-induced genotoxic and oxidative responses in *Eisenia fetida. Ecotoxicol Environm Safety* 161 (2018) : 610—615.

Brown GG, Barois I, Lavelle P (2000) Regulation of soil organic matter dynamics and microbial activity in the drilosphere and the role of interactions with other edaphic functional domains. *Eur J Soil Biol* 36(3-4) : 177—198.

Cole L, Bradford MA, Shaw PJA, Bardgett RD (2006) The abundance, richness and functional role of soil meso- and macrofauna in temperate grassland—A case study. *Appl Soil Ecol* 33(2) : 186—198.

Elbert A, Haas M, Springer B, Thierlert W, Nauen R (2008) Applied aspects of neonicotinoid uses in crop protection. *Pest Manag Sci* 64 : 1099—1105.

Enrico Mendes Saggioroa, Danielli Gundes do Espírito Santo, Sidney Fernandes Sales Júnior, Rachel Ann Hauser-Davis, Fabio Veríssimo Correia (2019) Lethal and sublethal effects of acetamiprid on *Eisenia andrei* : Behavior, reproduction, cytotoxicity and oxidative stress. *Ecotoxicol Environm Safety* 183 (2019) : 109—572.

Goulson D (2013) Review : An overview of the environmental risks posed by neonicotinoid insecticides. Kleijn D ed. *J Appl Ecol* 50(4) : 977—987.

Hyrsl P, Buyukguzel E. Buyukguzel K (2007) The effects of boric acid-induced oxidative stress on antioxidant enzymes and survivorship in *Galleria mellonella. Arch Insect Biochem Physiol* 66 : 23—31.

Jeschke P, Nauen R, Schindler M, Elbert A (2011) Overview of the status and global strategy for neonicotinoids. *J Agric Food Chem* 59 : 2897—2908.

Jeyanthi V, Paul JAJ, Selvi BK, Karmegam N (2016) Com parative study of biochemical responses in three species of earthworms exposed to pesticide and metal contaminated soil. *Environ Process* 3 : 167—178.

Jouquet P, Dauber J, Lagerlöf J, Lavelle P, Lepage M (2006) Soil invertebrates as ecosystem engineers : Intended and accidental effects on soil and feedback loops. *Appl Soil Ecol* 32(2) : 153—164.

Liu T, Wang X, You X, Chen D, Li Y, Wang F (2017) Oxidative stress and gene expression of earthworm (*Eisenia fetida*) to clothianidin. *Ecotoxicol Environ Saf* 142 : 489—496. https://doi.org/10.1016/j.ecoenv.2017.04.012.

Lowry OH, Rosebrough N , Farr AL, Randall R (1951) Protein measurement with the folin phenol reagent. *J Biol Chem* 193 : 265—267.

Gill P, Singh D, Gupta RK, Urmila, Lata H (2019) Comparative Chemical Evaluation of Vermicompost Produced by Using Different OrganicWastes.© *Springer Nature Sin gapore Pte Ltd.* 2019 S. K. Ghosh (ed) *Waste Valorisation and Recycling*, https://doi.org/10.1007/978-981-13-2784-1-20.

Rombke J, Jansch S, Didden W (2005) The use of earthworms in ecological soil classification and assessment concepts. *Ecotoxicol Environm Safety* 62 : 249—265.

Satyro S, Saggioro EM, Veríssimo F, Buss DF, de Paiva Magalhães D, Oliveira A (2017) Triclocarban: UV photolysis, wastewater disinfection and ecotoxicity assessment using molecular biomarkers. *Environ. Sci.* Pollut. Res. 24. https://doi.org/10. 1007/s11356-017-9165-4.

Szczepaniec A, Raupp MJ, Parker RD, Kerns D, Eubanks MD (2013) Neonicotinoid insecticides alter induced defenses and increase susceptibility to spider mites in distantly related crop plants. *PloS one, e62620* 8(5) : 1—11.

Valavanidis A, Vlahogianni T, Dassenakis M, Scoullos M (2006) Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicol Environ Saf* 64 (2) : 178—189.

Xu JB, Yuan XF, Lang PZ (1997) Determination of catalase activity and catalase inhibition by ultraviolet spectrophotometry. *Environ Chem* 16 : 73—76.

Yanning Zhang, Lan Zhang, Lei Feng, Liangang Mao, Hongyun Jiang (2017) Oxidative stress of imidaclothiz on earthworm *Eisenia fetida*. *Compara Biochem Physiol Part C* 191 (2017) : 1—6.

Zhang Q, Zhang B, Wang C (2014) Ecotoxicological effects on the earthworm *Eisenia fetida* following exposure to soil contaminated with imidacloprid. *Environ Sci Pollut Res* 21: 12345–12353. https://doi.org/10.1007/s11356-014-3178-z.

Zhang Q, Zhu L, Wang J, Xie H, Wang J, Han Y, Yang J (2013) Oxidative stress and lipid peroxidation in the earthworm *Eisenia fetida* induced by low doses of fomesafen. *Environ Sci Pollut Res* 20 : 201–208. https://doi.org/10.1007/s11356- 012-0962-5.