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Short Communication

Acetylcholinesterase and Glutathione-S-Transferase as Biomarkers for Imidacloprid Toxicity in Earthworm *Eudrilus eugeniae* and *Metaphire posthuma*

Harpreet Kaur* and S.S. Hundal

Department of Zoology, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana.

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HK conducted the research work, data analysis and wrote the manuscript. SSH provided necessary guidance for the research work, data analysis, correcting and editing the manuscript.

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Eudrilus eugeniae, Metaphire posthuma, Imidacloprid, AChE and GST, Acetylcholinesterase, Glutathione-S-Transferase

ABSTRACT

The present study investigated the effect of imidacloprid on the AChE and GST activity in earthworm *Eudrilus eugeniae* and *Metaphire posthuma*. *E. eugeniae* and *M. posthuma* were exposed to soil spiked with different concentrations (0.3, 0.6 and 1.0 mg/kg dry soil) of imidacloprid under laboratory conditions. The activity of AChE and GST have been reported as potential biomarkers to assess toxicity levels, hence the activity of these enzymes were assessed to study imidacloprid toxicity. Inhibition of AChE activity was observed in both the earthworm species in all the doses of imidacloprid, indicating neurotoxicity. There was an initial increase in the GST activity followed by its decrease with the duration of exposure to imidacloprid. Imidacloprid is highly toxic to earthworm inducing physiological which may cause catabolism of enzymes. Earthworm *M. posthuma* was observed to be more susceptible as compared to *E. eugeniae*. The current study signifies that the irrational use of such insecticides could pose high threat to non-target organisms, for example earthworms which play a key role in soil ecosystem productivity.

Modern agricultural practices directly depend on insecticides for ensuring high yields to meet the increasing demands of exponentially growing population. Insecticides are used in management of crop against pest insects, resulting in better food production but they are purchased and used irrationally in high amount leading to soil contamination (Rombke *et al*., 2005; Bansiwal and Rai, 2014; Tiwari *et al*., 2016). Earthworms are considered to be one of the most significant megafauna in regulating the soil structure and they act as the main driving element for the formation of soil organic matter (Le Bayon *et al*., 2017). They are susceptible to diverse impacts on soil as they are continuously in direct contact with the contaminant through their skin and alimentary surfaces (Udovic *et al*., 2007). Hence, to determine insecticide toxicity earthworms can be used as sentinel species (Rodriguez-Castellanus and Sanchez-Hernandez, 2007). In response to contamination an organism shows biological alterations in morphological, physiological, biochemical, cellular and behavioural aspect (Depledge, 1994; Lagadic *et al*., 2000). To assess sublethal effects of contaminants on an organism, biomarkers perform an essential role in ecological risk assessment (Rodriguez-Castellanus and Sanchez-Hernandez, 2007). The evaluation of different enzymes as biomarkers to study insecticide toxicity is appropriate as the enzymes perform important role in nerve transmission and homeostatis (Tiwari *et al*., 2016; Sanchez- Hernandez, 2006). AChE is a major enzyme in central nervous system of earthworm. It acts as a neurotransmitter and plays role in transmission of nerve impulses by catalyzing the hydrolysis of AChE to choline and acetate. Imidacloprid selectively act on nAChRs (nicotinic acetylcholine receptors), a family of ligand-gated ion channels located in the CNS of insects, responsible for neurotransmission and wide range of universal and translaminar effects (Simon-Delso *et al*., 2015). Glutathione S-tranferase (GST) family consists of detoxifying enzymes that play significant role in phase II detoxification process, neutralizing and biotransforming the toxicants in the earthworm's body (Hayers *et al*., 2005). In the present study we evaluated the effect of neonicotinoid insecticide imidacloprid on

* Corresponding author: harpreetimkaur@gmail.com
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an indigenous species *Metaphire posthuma* and an exotic species *Eudrilus eugeniae* in laboratory conditions. Imidacloprid (Bayer CropScience, CAS# 138261-41-3), a neonicotinoid, is an important constituent of various commonly used pesticides and is comparatively constant in soils (García-Chao *et al*., 2010). Neonicotinoids being systematic in nature are not only absorbed by the plants but also have the ability to translocate to all parts of the organism inhabiting and feeding on crop plant; hence used against broad spectrum for pest insects (Simon-Delso *et al*., 2015).

Materials and methods

The soil comprised of 20% kaolin clay, 70% quartz sand, 10% sphagnum peat to which was added calcium carbonate to adjust the neutral pH of soil (OECD, 1984). Earthworms *Eudrilus eugeniae* and *Metaphire posthuma* were acclimatized for one week and then introduced into artificial soil spiked with different concentrations of imidacloprid. Different concentrations (0.3, 0.6 and 1.0 mg/kg) of imidacloprid were prepared to test the toxicological assay as these values are the environmental predicted values. The experiment was performed in triplicate. Out of the three trays, one tray was set as control. The earthworms were picked on day 0, 7th, 14th, 21st and 28th day for the assay. The earthworms were washed with distilled water, homogenized in 0.02M phosphate buffer (pH 7.5) and centrifuged at 3000 rpm for 10 minutes. The supernatant of homogenate was used for the study. To determine AChE activity and GST activity Ellman *et al*., (1961) and Habig *et al*. (1974) methodology was followed. Lowry *et al*. (1951) method was adapted to evaluate the total protein content. The AChE activity was expressed as nmol acetylthiocholine hydrolysed/min/mg protein. The GST activity was expressed as nmol of GST-CDNB conjugate formed/min/mg protein.

The data of all the parameters were expressed as the mean ± standard error of the (SEM). Two-way analysis of variance (ANOVA) with post hoc test (DMRT) was used to evaluate various treatments with control using SPSS software (standard version 23.0) at "p" value of 0.05.

Results and discussion

The AChE activity decreased in earthworm, *E. eugeniae* and *M. posthuma* (Table I) after exposure to imidacloprid. Imidacloprid concentration (0.3, 0.6 and 1.0 mg/kg dry soil) resulted in decline in AChE activity significantly ($p<0.05$) in both the earthworm species after 7 days and the trend continued till 4 weeks of exposure period. The AChE activity inhibition was more in high dose (1.0 mg/kg dry soil) as compared to level of the agrochemical (0.3 and 0.6mg/kg dry soil). Non significant increase in AChE activity was observed in control. The GST activity decreased non significantly in *E. eugeniae* and *M. posthuma* (Table I) in control. In doses (0.3, 0.6 and 1.0 mg/kg dry soil) GST activity increased on 7th day and then significantly ($p<0.05$) decreased till 28th day of exposure period in both the earthworms. Significant ($p<0.05$) difference was observed in the mean AChE and GST activity of earthworms as compared to control. Inhibition of AChE and GST activity was more in *M. posthuma* as compared to *E. eugeniae*. The different dosages of exposure revealed a major change in the enzyme activity, which are strong pointers to the toxicity.

Oxidative stress is induced by insecticides either by alterations in antioxidant defense mechanism or by overproduction of free radicals (Abdollahi *et al*., 2004). Antioxidant enzymes protect the cells against reactive oxygen species (ROS). In toxicity of various insecticides oxidative stress plays a significant role (Ranjbar *et al*., 2002). The toxicity is due to oxidative stress and consequent production of ROS resulting in cell damage and death. Qi *et al*. (2018) reported inhibition in AChE activity after exposure to cycoxaprid, neonicotinoid insecticide in *Eisenia fetida.* Wang *et al*. (2015) calculated the lowest value of imidacloprid to cause disturbance in AChE activity in earthworm *E. fetida* i.e., 0.1 mg/kg. Acetylcholine is secreted from the neurosecretory cells. It is a neurotransmitter which plays a significant role in transmission of nerve impulse through the synaptic cleft in nerve cells. The imidacloprid binds to the active site of cholinesterase enzyme which inhibits the breakdown of acetylcholine in the cleft region of neuron. This leads to accumulation of acetlycholine in synaptic space which results overstimulation of nerve cells and neurotoxicity. In the present study it was observed that period of exposure to imidacloprid significantly changed the effects on earthworm *E. eugeniae* and *M. posthuma*. There was an initial increase in the GST activity followed by its decrease with the duration of exposure to mixture of 6 insecticides in earthworm *Aporrectodea caliginosa nocturna* (Schreck *et al*., 2008). The possible explanation given by Cossu *et al*. (1997) is that the initial introduction to insecticide can result in induction of antioxidant enzymes which allows the organism to detoxify its body against the ROS but as the level of toxicity increases it leads to its inhibition. The duration of exposure time to imidacloprid resulted in increased toxicity which led to decrease in GST activity. Similar results were reported by Wang *et al*., (2019) in GST activity after exposure to imidacloprid in *E. fetida*. Wang *et al*. (2016) observed that GST activity was significantly induced in earthworm *E. fetida* after exposure to imidacloprid. GST activity was inhibited in *E. fetida* after exposure to thiacloprid (Feng *et al*., 2015).

Table I. Effect of imidacloprid on the specific activities of acetylcholinesterase (AChE) and glutathione-S-transferase (GST) activity (nmol/min/mg protein) in earthworm *Eudrilus eugeniae* and *Metaphire posthuma.*

Treatments	Days				
	0	**7th**	**14th**	**21st**	**28th**
AChE activity in *E. eugeniae*					
Control	170.57±7.36^{a1}	172.15±6.11^{a3}	174.18±6.14^{a4}	176.46±6.03^{a4}	174.40±11.60^{a4}
T1	170.33±3.46^{a1}	167.15±4.37^{ab12}	164.37±4.23^{ab3}	158.89±6.07^{b3}	158.44±3.90^{b3}
T2	165.51±4.98^{a1}	162.47±2.67^{a2}	153.31±3.05^{b2}	142.37±4.10^{c2}	139.50±3.37^{c2}
T3	166.48±6.14^{a1}	149.35±4.22^{b1}	130.17±4.45^{c1}	113.19±5.12^{d1}	93.08±4.89^{e1}
AChE activity in *M. posthuma*					
Control	168.46±5.95^{a1}	171.01±6.87^{a2}	172.10±5.95^{a3}	173.06±6.07^{a3}	173.99±5.93^{a3}
T1	171.76±2.88^{a1}	168.10±3.14^{a2}	163.84±4.64^{ab23}	158.68±4.18^{cb2}	152.49±6.10^{c2}
T2	173.74±5.03^{a1}	170.45±1.74^{a2}	159.21±3.87^{b2}	156.06±7.26^{b2}	143.44±4.72^{c2}
T3	169.87±5.08^{a1}	139.16±3.46^{b1}	108.12±5.94^{c1}	83.51±6.15^{d1}	64.38±5.73^{e1}
GST activity in *E. eugeniae*					
Control	152.70±7.21^{a1}	140.89±7.15^{ab1}	138.70±6.36^{b1}	147.35±4.33^{ab2}	148.37±6.12^{ab3}
T1	155.49±8.21^{a1}	151.27±4.17^{ab1}	146.67±4.55^{ab1}	141.26±6.01^{b12}	140.39±4.60^{b3}
T2	143.48±4.82^{ab1}	149.00±5.48^{a1}	141.46±6.76^{ab1}	133.42±4.90^{cb1}	125.87±4.28^{c2}
T3	151.50±2.89^{a1}	188.89±5.45^{b2}	181.56±5.77^{c2}	134.81±6.88^{d1}	110.12±4.16^{e1}
GST activity in *M. posthuma*					
Control	142.62±5.36^{a1}	132.48±4.09^{b1}	129.26±4.10^{b1}	130.43±4.59^{b2}	132.60±5.54^{b2}
T1	141.97±4.56^{a1}	147.72±4.71^{a2}	139.12±5.53^{ab2}	138.00±3.80^{ab3}	129.36±7.17^{b2}
T2	148.49±3.58^{a1}	153.77±2.72^{b2}	147.29±1.24^{c3}	139.28±1.49^{d3}	132.34±0.92^{e2}
T3	143.44±5.53^{a1}	189.53±3.33^{b4}	127.49±3.81^{c1}	109.44±3.09^{d1}	91.29±7.58^{e1}

T1 (0.3mg/kg); T2 (0.6mg/kg); T3 (1.0mg/kg); Values are Mean ± S.E of triplicates; Values with at least one same numeric superscript in column do not differ significantly ($p > 0.05$) with reference to treatments. Values with at least one same alphabetic superscript in row do not differ significantly ($p > 0.05$) with reference to days.

Conclusions

It is concluded that imidacloprid is toxic to the earthworms, *Eudrilus eugeniae* and *Metaphire posthuma*, results in adverse effects on earthworm physiology. The effect of imidacloprid was found to be species specific, dose and duration dependent. Earthworm *Metaphire posthuma* was observed to be more susceptible as compared to *Eudrilus eugeniae* although the results followed similar trends.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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