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Enzymes of Earthworm as Indicators of Pesticide Pollution in Soil

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Abstract

The importance of the earthworms in the agricultural practices is well known. The increasing applications of pesticides and chemicals in the agricultural farms have adversely influenced the flora and fauna of the soil. Earthworms which immensely contribute in increasing the quality and fertility of agricultural soil are reported be worst hit organisms under such conditions. Recent reports have indicated growing interests among researchers to explore biochemical and molecular markers as indicators of accumulation of pollutants in the soil in general and pesticides in particular. The varying levels of several biomolecules in different parts of the earthworm have been reported which are indicative of sensitivity of the organisms to different xenobiotics. However, the existing information lacks the literature displaying stock of information regarding the impact of pesticides on the levels of some key enzymes regulating many crucial functions in the earthworm at one place. Keeping in view this issue, it was envisaged to bring out a mini review which illustrates updated information available on the impact of pesticides on the activities of certain key enzymes reported to be responsible for catalysing metabolic pathways concerning the neurotransmission system, energy metabolism, oxidative stress and amino acids metabolism in different body parts of the earthworms, a prospective bioindicators of pesticides contamination in the soil.

Keywords

Earthworms, Pesticides, Biomarkers, Enzymes, Oxidative Stress, Neurotransmission System

1. Introduction

It is of utmost importance to understand as how to achieve sustainable agriculture by knowing the impact of various contaminants in the soil as well as of different agricul-

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tural practices on soil ecosystems as such [1]. Now-a-days, the chemical compounds such as pesticides and fertilizers are frequently used and its excess use leads to soil, surface and ground water pollution that affect target organisms along with non-target organisms like earthworms. Earthworms are frequently available in a broad range of soil and may deposit 60% - 80% of the total soil biomass [2] [3]. They are the indispensable species in the terrestrial ecosystem which significantly influence the various processes like soil formation, organic matter breakdown, decomposition activity and nutrient cycling mineralization [4] [5].

The frequent applications of pesticides have been found to exert adverse effects on the soil development and its functioning in an ecosystem [5]. Earthworms act as bioindicator species for the ecotoxicological analysis of pesticide soil pollution [6] [7] [8]. According to a report, pesticides have detrimental effects on earthworm at various levels of organisation which involves change in the behaviour, defile metabolism and enzymatic functioning, enhance mortality, diminish fertility, hamper growth and reproduction [9]. In past and even recently, various reviews have been published on the toxic effects of pesticides on the earthworms [10] [11].

A biomarker has been defined as a biochemical, cellular, physiological or behavioural alterations that can be evaluated through a portion of tissue or fluid samples or whole individual, for the assessment of exposure and/or impact from one or more toxicants [12]. The biochemical biomarkers are being utilized these days for the analysis of pollutant toxicity, metabolization and detoxification in earthworm [13] [14] which later can be used for the detection and assessment of contaminant that influence the environmental modifications [15].

Biomarkers render evidence for the exposure or effect of pollutants on the fauna of soil which can be utilized for the assessment of pollution in environmental monitoring. Selection of biomarker is done on the basis of various criteria whether it is sensitive, acts in a dose-time dependent manner to the toxicant [16], its biochemical memory and whether its erratic response due to natural variation is known that includes temperature, sex, age, weight [17].

Earthworms play crucial role in soil ecosystem as it helps in maintaining soil structure and fertility [4]. They influence the organic matter dynamics, structure and microbial community [18] [19] [20] and hence are referred as Ecosystem engineers [21]. It has also been explained as how they enhance the soil porosity by modifying soil organic matter chemically as well as physically by mixing leaf litter with soil resulting in formation and stabilization of soil aggregates [22] [23]. Keeping in view the lack of information on the effect of xenobiotics on different biochemical and molecular indices of the earthworm, it has been endeavoured in the this mini review to present an updated information on the impact of pesticides and other xenobiotics on the enzymes from earthworms which could be of great use to those involved in soil remediation and sustainable agriculture.

2. Enzymes as Biomarkers in Earthworm

The utility of biomarkers in earthworm is gradually relevant for the assessment of impact of pesticide in soil organism. Various classes of enzymes are used as biomarkers due to their crucial role in the neurocholinergic transmission and in cell homeostasis preventing toxic action of chemicals [24] [25] [26]. Dimethoate, an organophosphate pesticide, has been reported to exert toxic effects on the profiles of protein and the cellular enzyme system as well as the testicular histomorphology of *Eisenia kinneari* [27] [28] [29].

2.1. Acetylcholinesterase in Earthworms

Acetylcholinesterase (AChE) is considered as the main cholinesterase in earthworms [30] [31]. In few earthworm species, its activity is reported and biochemically characterised [32]. It is a significant enzyme that play crucial role in the transmission mechanism of nervous system. The neurotransmission takes place at cholinergic synapses by rapid hydrolysis of acetylcholine (neurotransmitter) to choline and acetate [33]. Organophosphorus and carbamate pesticides mainly inhibit AChE. Pesticide particularly, organophosphorus inhibit the acitivity of enzyme by covalently phosphorylating the serine residue within the active site group.

It has been reported that the concentration of AChE activity was highest in the preclitellar part of earthworm and has important role in the functioning of dorsal brain present near prostomium [30] [34]. A time-dependent inhibition of AChE is found in *Eisenia fetida*, when exposed to two organophosphates, chlorpyriphos and azodrin, in the standardized paper contact test [35] [36]. The exposure of *Eisenia fetida* to carbamate pesticide, methiocarb, has been demonstrated to cause strong inhibition of AChE activity [37] [38].

2.2. Gamma Amino Butyric Acid (GABA) Amino Tranferase

The GABA is a universal inhibitory neuromuscular transmitter in most of the invertebrates [39]. The site of action of this neurotransmitter is at both the neuromuscular junctions and within the ganglia. It is known that the major target of cypermethrin, class II-pyrethroids, is GABA that regulates the chloride channels [40]. According to a report, the open state of voltage gated chloride channels is suppressed and GABA dependent uptake of chloride ions is inhibited by cypermethrin [41]. This pesticide leads to generate neurotoxicity by modulating the levels of GABA. The results of a recent study have shown that this pesticide results into reduction in the GABA level [42], which indicates that GABA may act as a sensitive biomarker to cypermethrin exposure in earthworms. The effect of cypermerthrin in brain of earthworm has been shown to be mediated via inhibition of activity of AChE involved in cholinergic neurotransmission system.

2.3. Antioxidant Enzymes

The exposure to pesticides induces oxidative stress through generation of reactive oxygen species (ROS) [43]. The production of ROS leading to oxidative stress has gained importance and has generated great interest in the field of ecotoxicology [44]. Oxidative stress may be defined as the imbalance between the oxidative as well as the antioxidative indices in the living systems. Interaction of these ROS with the essential macromolecules such as DNA, protein and lipids may cause disturbance in the physiological processes [45]. Toxicity induced by pesticides involves lipid peroxidation [46]. The treatment of earthworms with the pesticides and heavy metals leads to the production of ROS (H_2O_2 O_2^- and OH^- radicals) [47]. The cells are known to defend themselves from the oxidative damage using antioxidative enzymes and glutathione as they act as scavengers of ROS. The major enzymatic antioxidants in response to oxidative stress are: SOD, as catalase, glutathione peroxidase and glutathione reductase. In order to prevent the oxidative damage, SOD metabolizes the superoxide anion (O_2^-) into molecular oxygen and H_2O_2, which is then deactivated by catalase. In cellular protection, glutathione reductase plays key role by reducing glutathione in the oxidised form (GSSG) to GSH (reduced and active form) [48].

2.4. Glutathione-S-Transferase (GST)

Glutathione-S-Transferase (GST), a cytosolic enzyme, plays a crucial role in the detoxification and biotransformation of a number of electrophilic compounds by consumption of glutathione. The exposure of pesticides may lead to the alterations in the enzymatic activities that reflects the metabolic disturbances and cell damage in the specific tissues [31] [49]. Increased level of GST may result into better protection against toxic effects of pesticides and hence can be used as biomarker for monitoring pollution [50]. GSTs neutralise a wide range of pesticides and endogenous metabolic by-products through enzymatic *f* glutathione conjugation, glutathione-dependent peroxidase activity or isomerisation reactions [51]. The reports from different workers have shown the sensitivity of earthworm GST to the heavy metals and the pesticide exposure [52] [53] [54] [55] [56]. Earthworm, *Lumbricus rubellus*, possess a range of GSTs related to those from other taxa like nematodes and humans, with the evidence of tissue specific isoforms, activity, location, the ability to detoxify products of cellular toxicity and potential response to pollution [57].

2.5. Gut Enzymes of Earthworm

The existing information indicate that the microflora dwelling in the gut of the earthworm and the enzymes present there in such as cellulase, amylase, endoglucanase, pectinase, acid phosphatase, alkaline phosphatase and nitrate reductase have the ability to degrade the complex organic molecules (cellulose, pectin, etc.) in soil to their relatively simpler forms. Exposure of worms to deltamethrin causes reduction in cellulase activity, while exposures to lindane increased its activity. These results indicate the harmful effect of deltamethrin and the inducing effects of lindane on the biochemical metabolism of earthworms [58]. The acute exposure with these two pesticides, however, has been found to be lethal to the earthworms [58]. The presence of high activity of cellulase in the posterior region of the gut of the earthworms helps in digestion of complex plant carbohydrates and generation of energy [59]. Assays of the enzymes amylase, cellulase, invertase and pectinase from the gut of *E. eugeniae* revealed that their production was reduced in the fipronil mixed with soil as compared to that of control [60]. The carboxylesterases (CbEs) are serine hydrolases and they are involved in the detoxification of complex organic materials in soil. The CbE is secreted by earthworm in their gut lumen. In *Lumbricus terrestris*, chlorpyrifos (OP) has been shown to significantly inhibit the CbE activity [61].

3. Enzymes of Carbohydrate Metabolism in Earthworms

3.1. Lactate Dehydrogenase (LDH)

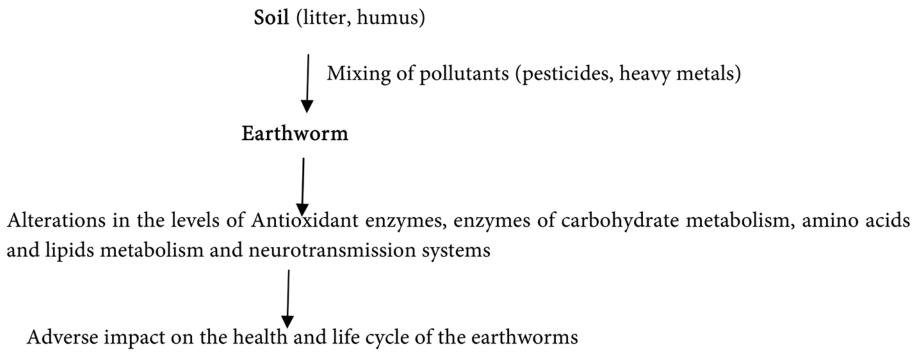
The lactate dehydrogenase (LDH) is a key glycolytic enzyme that is found in almost all the tissues of the earthworms [62] [63]. This enzyme has been used as an indicator of exposure to stress [64]. To assess toxicity of any xenobiotics including the pesticides and the heavy metals as well as the diagnosis of cell, tissue and organ damage, LDH has been widely exploited in the vertebrates. However, in the invertebrate toxicity tests, the potential of this enzyme as indicator is rarely explored [65] [66] [67].

3.2. Malate Dehydrogenase (MDH)

Malate is formed when cytosolic NADH is oxidised during reduction of oxaloacetate by the action of cytosolic MDH. Then, malate enters mitochondria by a carrier and is oxidised into oxaloacetate by the action of mitochondrial MDH. Malate is an important intermediate of TCA cycle. MDH catalyzes the interconversion of oxaloacetate to malate for energy requirements. In the present study, elevated levels of malate are observed in earthworms exposed to cypermethrin, in comparison to control earthworms. It may be due to the high energy requirement of earthworms under stress conditions caused by cypermethrin exposure [42].

4. Enzymes of Amino Acid Metabolism in Earthworms

Exposure of deltamethrin in *Metaphire posthuma* has been shown to affect the glucose and nitrogen metabolism pathway by inhibition of chief enzymes involved in the pathway: glutamine synthatase, glutamic acid decarboxylase, Acyl CoA synthase [42].



Scheme 1. A flow chart showing effect of soil pollutants on biochemical indices of the earthworms.

Table 1. Enzymes of earthworms as biomarkers of pesticide toxicity.

Species of earthworm	Pesticide	Biomarker	Effect	References
Aporrectodea caliginosa	Chlorpyrifos and azinophos-methyl	ChE	Inhibition of cholinesterase (ChE)	[14]
Eisenia andrei	Carbaryl	ChE	Inhibition of ChE observed even at lowest dose	[68]
Aporrectodea caliginosa	Chlorpyrifos and Diazinon	ChE	In juvenile, ChE activity was inhibited when exposed to 12 (75% inhibition) and 60 (90%) mg/kg of Diazinon. ChE depression of 35% and 70% was observed w. r. t. control when exposed to 4 and 28 mg/kg, respectively	[69]
Eisenia fetida	Monocrotophos	AChE	AChE inhibition was observed Dose-dependent. AChE activity co-related with morphological damage	[70]
Eisenia andrei	Carbaryl	AChE	Dose-dependent AChE activity was observed	[71]
Drawida willsi	Butachlor, Malathion and Carbofuran	AChE	When exposed to butachlor, no variation of AChE activity; on malathion exposure, maximum AChE inhibition (41% and 46%) after 9d and after 12d (54% and 62.9%) of carbofuran exposure	[72]
Pheretima peguana	Chlorpyrifos	AChE	AChE acitivity is significantly inhibited (>60%) at two concentrations of pesticide	[73]
E. fetida andrei	Benzo (a) pyrene	AChE	Increase in AChE acitivity	[74]
Eisenia fetida	Carbofuran	TChE	Protein content and total cholinesterase activity increased in low level and vice-versa	[75]
Eisenia fetida	Atrazine and chlorotoluron	SOD	Chlorotoluron is less toxic to atrazine. Synergistic effect observed when combined.SOD activity is enhanced.	[76]
Eisenia fetida	Carbofuran	SOD	When pesticide concentration is increased, SOD is reduced and vice-versa	[75]
Eisenia fetida	Chlorpyrifos and fenvalerate	SOD, Cellulase and CAT	Inhibition of SOD and cellulase whereas CAT activity increased first and then decreased.	[77]
Eisenia fetida	Fomesafen	-	Oxidative stress and peroxidation not found even at low dose	[78]
Eisenia fetida	Imidacloprid, acetamiprid, nitenpyram, clothianidin and thiacloprid Carbaryl	Cellulase	Inhibition of cellulose activity; damage epidermal and midgut cells	[79]
Eisenia fetida		Catalase LP and LPI Total GSH % GSSG	Depression of enzyme GST activity was observed	[68]
E. fetida andrei	Benzo (a) pyrene	Catalase GST LP and LPI Total GSH % GSSG	Increase in lipid peroxidation and enzyme activities. ROS involved inpesticide exposure	[74]
Lumbricus rubellus	Pyrene	Catalase	When exposed to 160 and 640 mg/kg of pyrene, catalase activity was lowered.	[80]
Eisenia kinneari	Dimethoate	-	Profound changes in testis was found due to disturbance in cellular acitivty	[81] [82]

The list of different enzymes responding to the pesticide stress in the earthworms have been summarised in the Table 1. A flow chart showing effect of soil pollutants on varying biochemical indices of the earthworms is displayed in Scheme 1.

5. Conclusion

There is growing interest on the studies concerning to monitoring or evaluation of the impact of pesticides and heavy metals on the activities of some key enzymes from different tissues in the earthworms to exploit them as potential indicators of xenobiotics contamination in the soil. The information available regarding the impact of pesticides on different species of earthworm indicated that the pesticide induced alterations in the functions of some key enzymes regulating the neurotransmission system, energy metabolism, oxidative system and amino acid metabolism of the worm. It was observed that these enzymes could serve as potential indicators of pesticide toxicity. However, still lot more is required to be done to find out more sensitive biomarkers from the earthworms to be used as specific indicator of the soil pollutants. The information may help farmers as well as the policy makers to formulate and manage better farming practices avoiding excess soil contamination with pesticides as it may significantly reduce the earthworm population in soil.

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