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Potential impact of glyphosate on morpho-behavioural changes, antioxidant system and lipid peroxidation products in the epigeic earthworm, *Eudrilus eugeniae*

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Abstract

The plants and animals in the soil ecosystem are negatively impacted by the regular use of chemical pesticides in agricultural areas. The usage of glyphosate-based herbicides (GBHs) and the discharge of glyphosate residues into the soil environment have raised serious concerns over time. This intentional release of pesticides into the soil environment highlights how important it is to keep an eye on the herbicides' ecotoxicological risk assessment and minimize any potential negative consequences. The current study examines the harmful effects of the herbicide glyphosate on the earthworm *Eudrilus eugeniae* from this perspective. In the epigeic earthworm, *E. eugeniae*, the non-enzymatic antioxidant glutathione (GSH) and the hepatic antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), and glutathione peroxidase (GPx) were selected as biomarkers. The results demonstrated that the sublethal exposure to glyphosate had a significant effect on these biomarkers' activity. Lipid peroxidation (LPO) products, such as conjugate diene and malondialdehyde, were also altered by exposure. Through its interactions with antioxidant enzymes, the current study further demonstrated that glyphosate is a chemical with oxidative potential.

Keywords: antioxidants, biomarkers, glyphosate, lipid peroxidation, oxidative stress, earthworm

1. Introduction

The use of chemical fertilisers to protect crops from diseases, pests, and weeds has increased. Up to 80–90% of applied pesticides damage non-target

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vegetation and wind up as pesticide residues in the environment, posing a major threat to the agricultural ecosystem. Applying chemical fertilisers is still thought to be the most reliable and acceptable way to protect plant crops against pests, weeds, etc (Ozkara et al. 2016). Nevertheless, only 1% of these fertilisers that is applied actually get to the intended target organism; the remaining portion ends up in the soil, water, and air, eventually finding its way into our food chain and harming non-target species like humans, animals, and plants (Lozowicka et al. 2016).

In this era of weed management, no other herbicide has influenced the industry and been more prominent than glyphosate (Duke and Powles, 2008). Its historical impact and subsequent genetically modified crops have dramatically changed the scenario of modern farming (Duke and Powles, 2009). In terms of structure, it is a phosphonomethyl derivative of the 1950-discovered amino acid glycine. Since its introduction in 1974, glyphosate has gained a prominent place in the pesticide industry. Glyphosate became the herbicide of choice for many agricultural producers due to its rapid translocation, potent mode of action, and incapacity of plants to detoxify it (Shaner, 2006). The 5-endopyruvyl-shikimate-3-phosphate synthase (EPSPS) enzyme, which breaks down the shikimate pathway, is hypothesized to be inhibited by glyphosate. This has an impact on the synthesis of three essential aromatic amino acids: tryptophan, tyrosine, and phenylamine (Matozzo et al. 2020). According to Salisbury and Ross (1994), all of this results in a disruption of protein production and growth, which ultimately leads to cellular dysfunction and death.

In addition to being used as a weed control agent, its use as a desiccant on a variety of food products has led to its residue being discovered in food products and in humans and animals, exposing them to the herbicide (Bohn et al. 2013). Although glyphosate-based herbicides have been demonstrated to be efficient in eliminating weeds from both agricultural and non-agricultural soils, glyphosate residues in the soil system have grown to be a significant problem over time (NASS, 2013). The intentional release of pesticides into the soil environment demonstrates how important it is to monitor the ecotoxicological risk assessment of the herbicides and minimise any potential negative impacts. One important and reliable tactic was the use of soil creatures, including earthworms.

An essential invertebrate found in soil that comes into direct contact with soil particles is the earthworm. Leachates from soil may penetrate the skin and digestive system, leading to toxicological consequences (Drake and Horn, 2007). Due to their significant ecological role, elevated biomass, and susceptibility to environmental contamination, earthworms have been identified as sentinel species for soil observation and evaluation of ecotoxicological hazards associated with pollutants in terrestrial environments (Landrum et al. 2006; Reinecke and Reinecke, 2007).

One technique frequently used in chemical toxicity environmental risk monitoring is biomarker assessment (McCarthy and Shugart, 1990). According to Lynn and Kathryn (2001), biomarkers provide information about the negative effects of contaminants and act as early warning signs of imminent environmental harm.

The most studied earthworm biomarkers in ecotoxicological assessment are gut enzymes, which are responsible for the digestion of food particles (Wang et al. 2015). A valuable biomarker of pollution monitoring in soil environments is glutathione S-transferase (GST), which aids in the detoxification and neutralization of pollutants, as well as changes in the activities of enzymatic antioxidants (CAT, GPx, SOD) and non-enzymatic antioxidant (GSH) defense system, which shield animal cells from oxidative damage (Pastore et al. 2003; Ribera et al. 2001; Wiegand et al. 2007). Few studies have examined biomarker responses in herbicide assessment, whereas the majority of earthworm biomarker research have been primarily focused on metal pollution assessment (Sanchez-Hernandez, 2006). Investigating the oxidative stress reactions of epigeic earthworms, *E. eugeniae*, to various sub-lethal dosages of glyphosate was deemed valuable from this angle.

2. Materials and Methods

2.1. Test chemical

The chemical used for testing was glyphosate. Probit analysis was used to determine the chemical's 96-hour LC_{50} value, based on acute toxicity studies and Organization for Economic Co-operation and Development (OECD, test number: 207) guidelines. Based on this, sub-lethal doses of glyphosate such as 1600µl/kg, 1700µl/kg, and 1800µl/kg were selected for exposure.

2.2. Experimental design

Earthworms, *E. eugeniae,* used in the experiment were procured from Integrated Farming Systems Research Station (IFSRS), Nedumcaud, Karamana, Thiruvananthapuram, Kerala. Together with the mother culture and moist soil, they were carefully transported to the lab. The earthworms were acclimated in the rearing tank (95 x 55 x 75 cm3) with a 10 cm layer of clean cow dung and moist soil (1:1) for 15 days prior to trial. A thin layer of damp, dry grass and leaves offered shade and moisture. Throughout the study period, the containers' moisture was kept between 60-70% by sprinkling them with enough water. The containers were kept in a dark, humid room at 27^0C with gunny bags over them to keep moisture from escaping.

The appropriate quantity of herbicide was dissolved in 1000µl of acetone and combined with 10g of soil for each test concentration. The acetone was allowed to evaporate in the sand for at least an hour. One kilogram of the pre-moistened soil mixture was placed on a tray. The 1 kilogram of pre-moistened soil was combined with the soil and herbicide. Four groups of twenty earthworms, each weighing between 200 to 800 mg, were placed in different trays. Similar preparations were made for Group I (control), but solely with soil mixture and water. For three, six, and nine days, earthworms in groups II, III, and IV were subjected to sub-lethal concentrations of glyphosate at 1600µl/kg, 1700µl/kg, and 1800µl/kg. Aluminium foil was used to cover the trays, and holes were made in the foil to allow for airflow. Sampling was done after the respective period of exposure.

To achieve a stable and optimal physiological state, the earthworm was starved for 24 hours before being sacrificed. In the appropriate buffers, 100mg of muscle was homogenized, and the resulting homogenates were centrifuged at the appropriate rpm (Eppendorf, Germany). Following centrifugation, the supernatant was gathered and stored for biochemical tests in a deep freezer (ROTEK) at -20^{0}C.

2.3. Protein estimation

The Bradford (1976) technique was followed to quantify the protein level for each enzyme using the same tissue extract.

2.4. Assay of antioxidant enzymes

2.4.1. Superoxide Dismutase (EC.1.15.1.1)

The Kakkar et al., 1984 procedure was used to test superoxide dismutase activity. Sodium pyrophosphate buffer (0.052M, pH 8.3), phenazine methosulphate (186µM), nitroblue tetrazolium, nicotinamide adenine dinucleotide (NADH) (780µM), and enzyme extract make up the reaction mixture. The reaction was started with NADH and ended with 1ml of glacial acetic acid after 90 seconds of incubation at 30^{0}C. The assay mixture was forcefully shaken and stirred with 4ml of n-butanol before being allowed to stand for 10 min. After that, it was centrifuged for 10 min at 2500rpm, and the butanol layer was carefully pipetted out. Using UV–visible spectrophotometer (Perkin Elmer), the colour intensity of the chromogen in butanol was evaluated at 560nm in contrast to a blank solution of butanol. One unit of enzyme activity was defined as the quantity of SOD required to reduce the optical density at 560 nm of chromogen production by 50% in 1 minute under test conditions. IU/mg protein was used to express the particular activity.

Catalase (EC.1.11.1.6)

According to Maehly and Chance (1954), catalase was measured. Following the decline in absorbance at 230 nm, the activity was measured spectrophotometrically. 3ml of phosphate buffer (0.01M, pH 7), 0.2ml of 2 mM hydrogen peroxide (H_2O_2), and 1ml of somewhat diluted extract generated by homogenizing the tissue in 10mM buffer comprised the assay combination. A system that did not have the extract was taken as blank. The precise activity was stated in terms of mg protein/minute/n moles of H_2O_2 decomposition.

2.4.2. Glutathione peroxidase (EC.1.11.1.9)

According to Lawrence and Burk (1976), GPx activity was estimated. The test mix consisted of 2ml of 0.01M phosphate buffer (pH 7.4), 300 µl of ethylenediaminetetraacetic acid (EDTA), 300µl of 1mM sodium azide (NaN3), 100µl of 0.1M NADPH, 100µl of 1mM reduced GSH, and 200µl of tissue extract. The reagent without tissue extract was called Blank. The absorbance change was measured at 340nm every 15 seconds for one minute. Enzyme activity was expressed as IU/mg protein.

2.4.3. Glutathione-S-Transferase (EC.2.5.1.18)

According to Habig et al. (1974), GST activity was assessed. 1ml of phosphate buffer (0.5M, pH 6.5), 100µl of 1-chloro-2,4-dinitrobenzene (CDNB) (30mM), 1ml of reduced GSH (30mM), and 100µl of tissue extract were included in the combination. The increase in optical density was observed for two minutes at 340nm in comparison to the blank. The activity was expressed as n moles of CDNB–GSH conjugate formed/minute/mg protein for tissue samples.

2.4.4. Glutathione content

Benke and Cheevar's (1974) method of measuring glutathione content was used. 2ml of 0.3M phosphate buffer (pH 7.4), 5µl of 5,5′-dithio-bis-2-nitrobenzoic acid (0.04%), and 200µl of tissue extract made up the assay mixture. A system was deemed blank if there was no extract. A change in absorbance at 412nm was observed within 10 minutes. The particular activity that was expressed was nmol/100g of tissue.

2.5. Estimation *of lipid peroxidation products*

2.5.1. Malondialdehyde content

According to Niehaus and Samuelsson (1968), the malondialdehyde concentration was determined. After adding 1ml of tissue homogenate to 2ml of TBA– TCA–HCl (thiobarbituric acid, trichloroacetic acid, and hydrochloric acid) reagent, the mixture was heated in a boiling water bath for 15 min. After centrifuging it for 10 min at 1000rpm, the optical density at 535nm was determined. The blank was a buffer made of TBA, TCA, and HCl. The tissue activity was reported in µmoles/g.

2.5.2. Conjugate diene content

According to Recknagel and Ghoshal (1966), conjugate diene (CD) was calculated. 1ml of tissue homogenate and 5ml of chloroform/methanol (2:1) made up the reaction cocktail, which was centrifuged for ten minutes at 1000rpm. After that, 3ml of the bottom layer was carefully pipetted out and dried in a water bath at 45°C. After adding 1.5ml of cyclohexane, the absorbance was measured at 233nm. The enzyme's activity was measured in µmoles/g of tissue.

2.6. Statistics

The data was assessed using analysis of variance. Duncan's analysis was used to test the mean differences. The significance level was $P<0.05$. The SPSS 20.0 software for Windows was used to perform all of the statistical analyses.

3. Results

3.1. Morpho-Behavioural observations

The behavioural study of *E. eugeniae,* exposed to glyphosate is demonstrated in Figure. 1. A varying degree of behavioural changes were observed in glyphosate-treated earthworms. Decreased burrowing activity and a tendency to coil were observed during experimentation on *E. eugeniae*. The intensity of behavioural changes in earthworms increased for glyphosate with respect to its increasing concentration. Throughout the experiment, no behavioral alterations were noticed in the control group of earthworms.

Figure 1: Behavioural alterations in the whole body of earthworm, *E. eugeniae,* exposed to different sub-lethal doses of glyphosate. (A) Control (B) Coiling (C) Decreased burrowing activity.

The morphological study of *E. eugeniae* exposed to glyphosate is demonstrated in Figure. 2. Morphological changes in earthworms treated with glyphosate were observed during experimentation. As their concentration increased, treated earthworms showed a range of morphological changes, including color change, body fragmentation, clitellar swelling, mucus release, tissue inflammation, haemorrhage, and body swelling. The control group of earthworms did not exhibit any morphological changes.

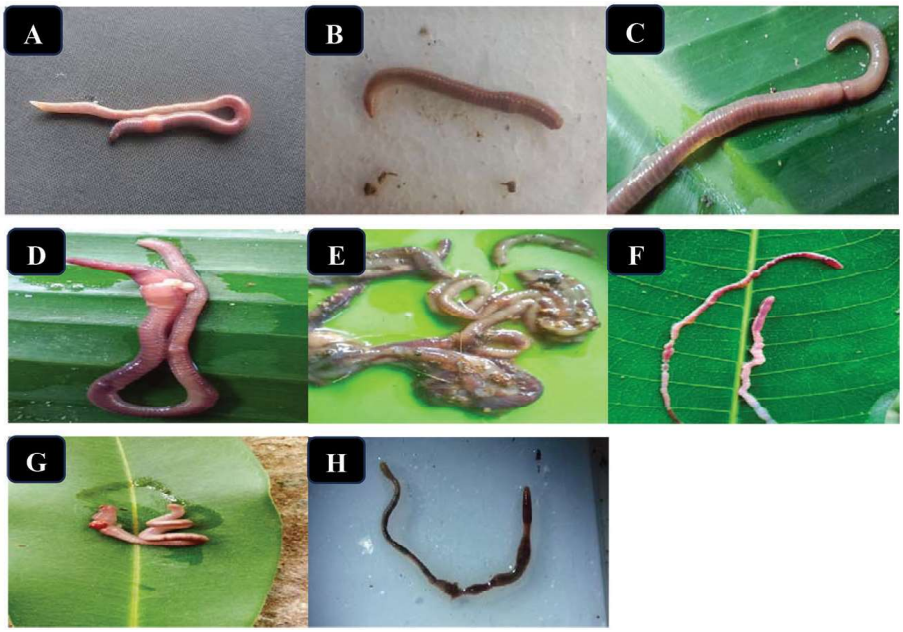

Figure 2: Morphological changes in the whole body of earthworm *E. eugeniae* exposed to different sub-lethal doses of glyphosate. (A) Control (B) Color change (C) Body fragmentation (D) Clitellar swelling (E) Mucus release (F) Tissue inflammation (G) Haemorage (H) Body swelling.

3.2. Effects of Glyphosate on the activity of antioxidants

In the current investigation, SOD activity was determined in the whole body of *E. eugeniae*, and the results are shown in Figure. 3(A). The activity of SOD increased significantly in earthworms exposed to 1600, 1700 and 1800µl/kg for 6 and 9 days. After 3 days of exposure, there is no discernible change from control in any of the sub-lethal dosages.

Catalase activity in the whole body of *E. eugeniae* exposed to glyphosate (1600, 1700 and 1800µl/kg) for 3, 6, and 9 days is shown in Figure. 3(B). Catalase activity has decreased significantly after 3 days of exposure to1600µl/kg of glyphosate. On the other hand, after 6 and 9 days of exposure to 1600, 1700, and 1800µl/kg glyphosate, a notable elevation was noted.

GPx activity significantly decreased after 3, 6, and 9 days of exposure to all selected sub-lethal doses of glyphosate (1600, 1700, and 1800µl/kg) in comparison to the control, according to a one-way ANOVA (Figure 3(C). There was a significant increase after 3 days of exposure to 1600µl/kg when compared to the control. Similarly, there is no discernible difference in 1800µl/kg after 6 days of exposure as opposed to 9 days.

The activity of GST measured with CDNB as substrate decreased significantly in the whole body of *E. eugeniae* exposed to glyphosate for 3, 6 and 9 days (Figure. 3(D). Nevertheless, no significant change was observed in 9 days of exposure when compared to 6 days of 1800µl/kg of glyphosate.

There was a notable decline in the concentration of GSH (Figure. 3(E) after 3 days of exposure to both 1600µl/kg and 1700µl/kg doses of glyphosate when contrast to the control. There was a significant increase in 6 and 9 days of exposure to 1600µl/kg and 1700µl/kg when opposed to 3 days. However, compared to the control, there was a discernible increase in glyphosate exposure of 1800µl/kg on all days.

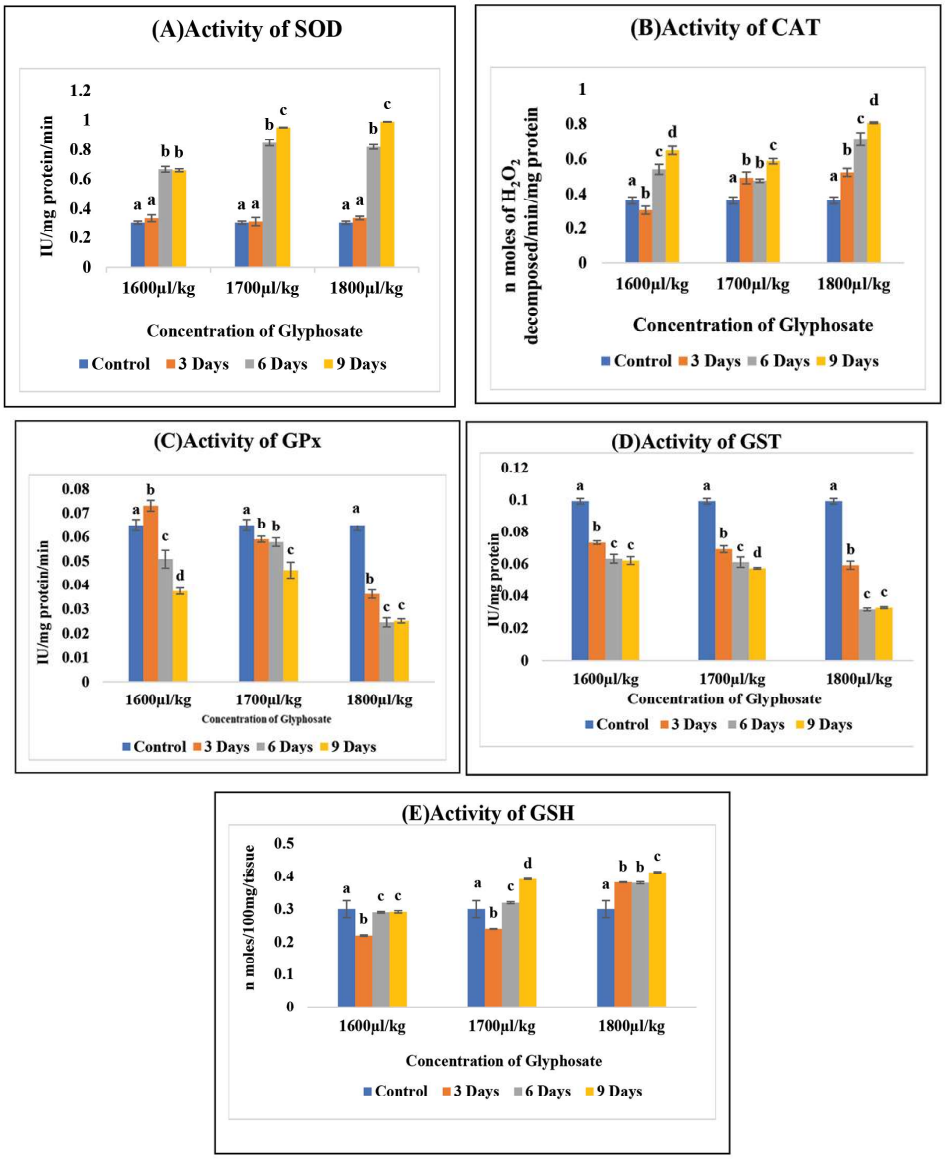

Figure 3: The effect of sublethal concentrations of Glyphosate (1600, 1700, and 1800µl/kg) on the activity of (A)SOD, (B)CAT, (C)GPx, (D)GST and (E)GSH in the whole body of *Eudrilus eugeniae* with respect to control. Each bar is mean ± SEM of six earthworm. Means with different superscript letters (a, b, c, d) for each parameter are significantly different at $p < 0.05$. SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; GST: Glutathione S Transferace; GSH: Glutathione content.

3.3. Effect of Glyphosate on the levels of lipid peroxidation products

The current investigation showed that glyphosate increased MDA and CD levels, which in turn improved lipid peroxidation. After nine days of glyphosate exposure, earthworms' hepatic concentration of MDA, a measure of the degree of lipid peroxidation, dramatically increased after six and nine days of exposure at 1700µl/kg and 1800µl/kg, respectively (Figure. 4(A). The MDA concentration remained relatively unchanged after 3 days of exposure to all sub-lethal glyphosate doses.

Conjugated diene content increased significantly in 1700µl/kg and 1800µl/kg glyphosate after 3, 6 and 9 days of exposure (Figure. 4(B). However, the CD concentration remained unchanged after 3 days of exposure to 1600µl/kg when compared to the control.

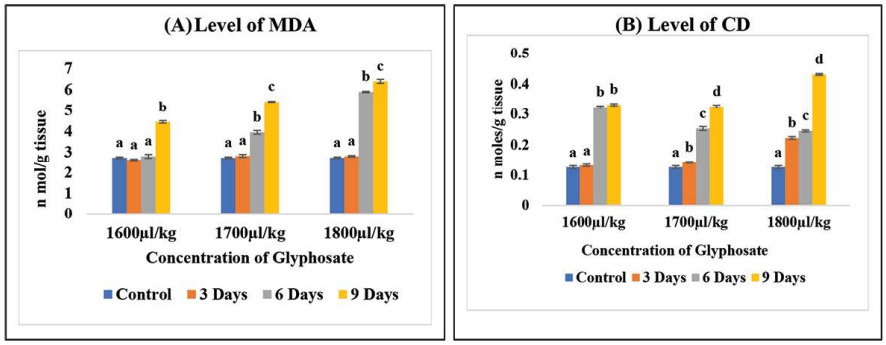

Figure 4: The effect of sublethal concentrations of Glyphosate (1600, 1700, and 1800µl/kg) on the level of MDA and CD in the whole body of *Eudrilus eugeniae* with respect to control. Each bar is mean ± SEM of six earthworm. Means with different superscript letters (a, b, c, d) for each parameter are significantly different at P<0.05. MDA: Malondialdehyde, CD: Conjugated diene.

4. Discussion

In research on environmental pollution, the evaluation of organismal biomarkers is a trustworthy method. It now accounts for a significant fraction of global environmental monitoring (Salvio et al. 2016). The active oxygen toxicology concept suggests that living organisms may adapt to changes in their environment. An imbalance between pro-oxidant and antioxidant defence mechanisms results in pesticide-induced oxidative stress, the last stage in a multi-step process (Banerjee et al. 2001).

The current study found that glyphosate induced oxidative stress in *E. eugeniae* earthworms. It was clear that exposure to glyphosate greatly raised the activities of SOD, CAT, and GSH whereas the activities of GPx and GST sharply declined.

Superoxide dismutase (SOD) is an inducible enzyme and its activity rises as an organism's need for protection against oxidative stress increases

(Achuba, 2018). When exposed to environmental contaminants or pollution-derived effluents, organisms typically show elevated levels of both oxidative and antioxidant enzymes (Kweki et al., 2018). In this study, sublethal glyphosate exposure led to a marked increase in SOD activity compared to the control group. SOD catalytically removes superoxide radicals—key contributors to oxygen toxicity—thereby shielding tissues from oxidative damage (Kadar et al., 2006). By converting superoxide into hydrogen peroxide, SOD serves as the first defensive barrier against reactive oxygen species, after which enzymes such as CAT and GPx further break down the resulting hydrogen peroxide (Kadar et al., 2006). Similar trends were observed in *Eisenia foetida* earthworms exposed to the pesticides chlorpyrifos and fenvalerate (Wang et al., 2012).

Catalase (CAT) is found in the peroxisomes, mitochondria, and cytosol, where it helps eliminate hydrogen peroxide using various co-substrates, making it a key enzyme in mitigating oxidative stress (Hegedus et al., 2001). Its activity is widely regarded as a sensitive marker of oxidative damage (Sanchez et al., 2005). In this study, CAT activity rose sharply at all sublethal glyphosate concentrations. Earlier work by Di Giulio et al. (1989) also demonstrated that CAT can be upregulated in response to oxidative stress. Likewise, Uner et al. (2001) observed elevated CAT levels in the earthworm *E. eugeniae* following malathion exposure, and studies on *D. willsi* and *L. mauritii* exposed to urea and phosphogypsum reported similar increases (Suryasikha et al., 2017). These findings align well with the present results. The heightened CAT activity suggests that the earthworms were undergoing oxidative stress, likely due to elevated H_2O_2 levels. This indicates that the elevated CAT activities following exposure were most likely a defense mechanism against oxidative damage and a metabolic adaptation to the ongoing pesticide exposure.

The present study shows that GPx activity in the whole body of *E. eugeniae* declined significantly. Glutathione peroxidase, a selenium-dependent enzyme, detoxifies organic reactive oxygen species and hydrogen peroxide (Orbea et al., 2000; Ansaldo et al., 2000). As one of the most effective defences against lipid peroxidation, GPx reduces both metabolically generated H_2O_2 and lipid hydroperoxides (Winston & Di Giulio, 1991). The reduced GPx activity observed here is consistent with the findings of Xu et al. (2013), who reported lowered GPx activity in *E. foetida* following exposure to perfluorooctane sulfonate. Similar decreases have been documented in *E. eugeniae* and *P. excavatus* after exposure to metals and pesticides (Neuhauser et al., 1995). The decline in GPx activity in the current study may be linked to elevated SOD and CAT activity, as previously suggested by Xu et al. (2013). It has been proposed that GPx primarily handles detoxification of H_2O_2 at lower concentrations, whereas CAT becomes the dominant pathway once GPx reaches substrate saturation.

Earthworms exposed to glyphosate exhibited a marked reduction in GST activity. GST's primary role is to catalyse conjugation reactions between the tripeptide GSH and various external electrophilic compounds. Because of its ability to detoxify organic lipid peroxides, GST and its isoforms have received considerable research attention in recent years (Yang et al., 2001). Studies examining xenobiotic toxicity in mammals have also identified GST as a major enzymatic target (Booth et al., 1961; Johnson, 1966). Differences in GST responses to various pesticides may stem from the nucleophilic attack involved in the enzyme-mediated conjugation of GSH (Jakoby & Habig, 1980).

A wide range of chemicals—including xenobiotics and products of oxidative stress—can serve as substrates for GST (Tjalkens et al., 1998). In this study, glyphosate exposure led to a significant reduction in GST activity in the whole body of the earthworms. Prolonged exposure to insecticides may decrease GST activity due to the depletion of reduced glutathione (Bradbury & Standan, 1959; Plummer et al., 1981). According to Krea et al. (2007), such reductions may also result from the buildup of metabolites from the first or second phase of detoxification, which can inhibit overall GST activity. These explanations are consistent with the present findings, where the test chemical clearly impacted GST activity.

The tripeptide GSH neutralises free radicals directly by donating a hydrogen atom, a property that underlies its antioxidant function. Its synthesis is tightly regulated both inside and outside cells. Kelly et al. (1998) emphasized the central role of glutathione in defending against reactive metabolites and oxidative injury. Because GSH serves as the co-substrate for GPx during the reduction of H_2O_2, its levels often shift in parallel with GPx activity (Sies, 1986). In this study, a slight increase in GSH concentration was observed after 9 days of glyphosate exposure compared to the control. Elevated GSH levels are recognized as one of the protective strategies earthworms use during pollutant exposure.

A variety of abiotic stressors can damage cellular and molecular structures either directly or indirectly through the generation of reactive oxygen species (ROS). Oxidation of membrane lipids leads to the formation of MDA, a compound commonly used as an indicator of oxidative stress levels (Guo et al., 2004). Because of this, measuring lipid peroxidation is one of the most widely used approaches for demonstrating the role of free-radical reactions in toxicological processes (Livingstone, 2001).

Two factors could account for the lack of changes in MDA content in *E. eugeniae* across all treatments in this investigation following three days of exposure. The elevated antioxidant enzymes may have temporarily compensated for the data, or oxidative stress may have been too low

to cause any discernible reaction at the sub-lethal concentrations in our investigation. But when earthworms were exposed to high levels of stress, their MDA content significantly increased in 6 and 9 days when compared to the controls. The oxidation of molecular oxygen and the generation of superoxide radicals may have occurred as a result of *E. eugeniae* being subjected to oxidative stress. H_2O_2, which starts the peroxidation of unsaturated fatty acids in the membrane and produces MDA, may also be responsible for this reaction. Research has demonstrated that the presence of MDA-induced lipid peroxidation will signal distinct stressors in aquatic and terrestrial species (Guo et al. 2004).

From the present study, it was observed that glyphosate enhanced lipid peroxidation by elevating MDA and CD levels. One of the most common arguments in favour of the role of free-radical reactions in toxicology is the identification and measurement of lipid peroxidation (Livingstone, 2001).

Grey mullet, *Mugil cephalus*, exposed to Chennai's substantially contaminated Ennore Estuary showed a considerable increase in conjugated diene, a lipid oxidation marker (Padmini et al. 2008). Xenobiotic-induced free radical peroxidation raised MDA and CD in urine and tissue samples, according to several studies conducted on experimental animals, including rats (de Zwart et al. 1997). Most of the contaminants including pulp and paper effluent (Oakes et al. 2004; Oakes and Van Der Kraak, 2003), agricultural runoff, cadmium, copper and hydrocarbons (Livingstone et al. 1993), increased LPO in different fish species. All these findings, along with the present study give an outlook on utilizing MDA and CD levels as well-suited indicators of exposure to glyphosate.

It is clear from the current study that the earthworms exposed to glyphosate suffered from oxidative stress. The study's findings suggested that there is a disruption in the delicate balance of antioxidant enzymes, which could result in variations in the redox status.

5. Conclusion

The usage of glyphosate has been rising since its launch. Herbicides containing glyphosate are used to eradicate unwanted vegetation and weeds, but because they expose non-target species to the chemical, they also have a negative impact on the ecosystem. The main factors affecting human health are the presence, duration, and exposure to herbicides containing glyphosate.

This study has shown that, in addition to the morpho-behaviour changes, glyphosate has changed the activity of enzymes linked to oxidative stress and LPO. The changes could be used to track the condition of the glyphosate-contaminated soil environment. The earthworm underwent oxidative stress

following glyphosate exposure, as evidenced by the markedly increased activities of SOD, CAT, GSH, MDA, and CD and the decreased activities of GPx and GST. Based on the results of the study, it can be said that glyphosate exposure negatively impacted earthworms' ability to function normally by interfering with their antioxidant defense systems, which led to an increase in lipid peroxidation.

Since the information may greatly lower the earthworm population in the soil, it may assist farmers and policymakers in developing and implementing better farming practices that avoid excessive pesticide contamination of the soil. It is also clear that levels of antioxidant enzyme activity can be used to study the mechanisms of environmental toxicity in soil organisms by using them as molecular biomarkers of oxidative stress.

Our research may contribute to the understanding of ecological risk monitoring and markers of soil glyphosate-based herbicide contamination. Future research must also measure the glyphosate metabolites in the soil and track alterations in the earthworm gut microbiome's activity.

Abbreviations

CAT, Catalase; CD, Conjugated Diene; CDNB, 1-chloro-2,4-dinitrobenzene; EDTA, Ethylenediaminetetraacetic acid; EPSPS, 5-endopyruvyl-shikimate-3-phosphate synthase enzyme; GPx, Glutathione peroxidase, GSH, Glutathione; GST, Glutathione S Transferase; H_2O_2, Hydrogen peroxide; IFSRS, Integrated Farming Systems Research Station; MDA, Malondialdehyde; NADH, Nicotinamide adenine dinucleotide; NaN_3, Sodium azide; OECD, Economic Co-operation and Development; ROS, Reactive Oxygen species; SOD, Super oxide dismutase; SPSS, Statistical package of social sciences; TBA–TCA–HCl, Thiobarbituric acid-trichloroacetic acid-hydrochloric acid.

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Author contribution

The experiment was conceptualised and designed by Aruna Devi C. The experiment was designed and carried out by Lekshmi Priya R. The final manuscript was written, reviewed, and approved by all authors.

Conflict of interest

The authors declare no competing interests.

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