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A review on effect of different herbicides on growth, reproduction and biochemical markers of earthworm

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Abstract

Herbicides, widely used in modern agriculture, play a pivotal role in controlling weeds and enhancing crop productivity. However, their effects on non-target soil organisms, such as earthworms, are a growing concern due to the ecological importance of these organisms in soil fertility and structure. This review examines the impact of various herbicides on earthworm growth, reproduction, and biochemical markers. Studies reveal that herbicide exposure can adversely affect growth rates, cocoon production, and hatchling success in different earthworm species. Furthermore, biochemical markers such as oxidative stress enzymes, metabolic activity, and biomarkers of neurotoxicity provide insights into sub-lethal effects and underlying physiological mechanisms. The variation in sensitivity among species is influenced by herbicide type, concentration, and exposure duration. Understanding these impacts is essential for developing sustainable agricultural practices that minimize harm to soil biodiversity. This review highlights the need for eco-toxicological evaluations to guide safe herbicide use.

Keywords: Herbicide; Soil Toxicity; Vermicomposting potential; Biomarker activity

1. Introduction

Herbicides are commonly used in agriculture to manage weeds and boost crop yields, yet they have raised significant concerns due to their potential impact on non-target soil organisms, such as earthworms (Almeida et al., 2019). Earthworms play a critical role in maintaining soil health by enhancing soil structure, increasing nutrient cycling, and supporting organic matter decomposition (Singh & Ghosh, 2020). Due to their sensitivity to environmental contaminants, earthworms are often used as bioindicators to assess soil quality and the ecological risks posed by chemical inputs, including herbicides (Bartošová et al., 2020). The effects of herbicides on earthworms can vary widely based on factors such as the chemical structure of the herbicide, its concentration, and the earthworm species involved.

Several herbicides, such as glyphosate, atrazine, and paraquat, have been found to negatively impact earthworm health by inducing oxidative stress, altering reproduction, and reducing survival rates (Luo & Zhou, 2018). For instance, exposure to glyphosate can cause oxidative stress in *Eisenia fetida*, leading to reduced growth and reproduction, while paraquat has been linked to severe cellular damage and mortality in *Eisenia andrei* (Rahman et al., 2020; Liu & Zhang, 2020). This paper examines the effects of various herbicides on earthworm physiology and behavior, with the goal of better understanding the broader ecological implications of herbicide use.

2. Role of earthworm in soil health and agriculture

Earthworms play a vital role in promoting soil health and enhancing agricultural productivity. These organisms contribute to soil aeration, which improves the movement of air and water within the soil profile, thereby facilitating

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root growth and nutrient absorption (Edwards & Bohlen, 1996). Their burrowing activities create channels that enhance water infiltration, reducing runoff and erosion, which are critical factors in sustainable land management.

Moreover, earthworms are essential for nutrient cycling. As they consume organic matter, they break it down and convert it into forms that are more accessible to plants. This process not only enriches the soil with nutrients like nitrogen and phosphorus but also enhances soil structure, fostering a more hospitable environment for beneficial microorganisms (Baker et al., 2007). The presence of earthworms is often associated with improved soil fertility and higher crop yields, making them an integral part of sustainable agricultural practices. Their contributions underscore the importance of maintaining healthy earthworm populations to support ecosystem functioning and agricultural productivity.

3. Effects of Herbicides on Earthworm Growth

3.1. Glyphosate

Glyphosate is one of the most widely used herbicides globally. Research indicates that glyphosate exposure can significantly hinder the growth of earthworms. Studies show that *Eisenia fetida* exposed to glyphosate exhibited reduced growth rates and impaired reproductive performance (Luo & Zhou, 2018). Glyphosate-induced oxidative stress leads to cellular damage, affecting growth and development. The sublethal concentrations of glyphosate can alter earthworm behavior, leading to decreased foraging activity and reduced energy intake, which ultimately impacts their growth (Bartošová et al., 2020).

3.2. Atrazine

Atrazine has been shown to adversely affect earthworm growth through several mechanisms. Studies conducted on *Lumbricus terrestris* indicate that atrazine exposure reduces growth rates and overall biomass due to hormonal disruptions affecting reproductive health (Singh & Ghosh, 2020). Atrazine also has a bioaccumulative effect, with higher concentrations observed in earthworm tissues over time, leading to chronic toxicity and reduced growth (Rahman et al., 2020). Furthermore, atrazine can impair soil microbial communities, indirectly affecting earthworm growth by reducing the availability of organic matter and nutrients.

3.3. Paraquat

Paraquat is highly toxic and primarily acts by generating reactive oxygen species (ROS), leading to oxidative damage. Research indicates that exposure to paraquat negatively affects earthworm growth by causing significant oxidative stress, resulting in cellular damage (Fischer et al., 2019). Studies on *Eisenia andrei* demonstrate that paraquat exposure leads to reduced growth and survival rates, with high concentrations causing acute toxicity and death. Sublethal concentrations also affect reproductive parameters, which can further impact population dynamics and growth over time (Almeida et al., 2019).

3.4. Metribuzin

Metribuzin is another herbicide that has been linked to reduced growth in earthworms. Exposure to metribuzin has been shown to decrease growth rates in *Eisenia fetida*, as it induces oxidative stress similar to other herbicides (Rahman et al., 2020). The effects of metribuzin are particularly concerning as it can lead to reduced biomass and reproductive success. Prolonged exposure to metribuzin can result in impaired burrowing activity, affecting soil structure and health (Zhou et al., 2020).

4. Effects of Different Herbicides on Earthworm Reproduction

4.1. Glyphosate

4.1.1. Mechanism of Glyphosate Toxicity

Glyphosate, one of the most widely used herbicides, has been studied for its impact on soil organisms, including earthworms. Glyphosate functions by inhibiting the shikimic acid pathway, a metabolic process in plants. Although earthworms lack this pathway, glyphosate exposure can indirectly impact their physiology. Research by Santos et al. (2019) indicates that glyphosate exposure can lead to oxidative stress in earthworms, which in turn affects their reproductive capacity by damaging cellular structures vital for reproduction.

4.1.2. Effects on Cocoon Production

Studies on *Eisenia fetida* exposed to glyphosate have reported decreased cocoon production. Luo and Zhou (2018) observed that glyphosate-treated earthworms produced significantly fewer cocoons compared to untreated controls. This reduction in cocoon production is thought to be due to impaired energy allocation, where resources that would normally support reproduction are redirected toward coping with the herbicide's toxic effects.

4.1.3. Impact on Juvenile Growth and Survival

In addition to cocoon production, glyphosate exposure can also affect the viability and growth of juvenile earthworms. Bartošová et al. (2020) found that glyphosate reduced the hatching success of cocoons and stunted the growth of juvenile worms. This impaired growth in early life stages can have long-term effects on earthworm populations, as the herbicide compromises the earthworms' ability to reach reproductive maturity.

4.2. Atrazine

4.2.1. Disruption of Endocrine Function

Atrazine, another widely used herbicide, has been shown to disrupt the endocrine functions of non-target organisms. In earthworms, this disruption has notable effects on reproduction. Singh and Ghosh (2020) demonstrated that exposure to atrazine led to hormonal imbalances in *Lumbricus terrestris*, which is closely linked to reduced fertility and altered reproductive cycles.

4.2.2. Bioaccumulation and Chronic Toxicity

Atrazine has a tendency to bioaccumulate within the tissues of earthworms over prolonged exposure. Rahman et al. (2020) observed that atrazine levels in earthworm tissues increased with exposure time, leading to chronic toxicity that further compromised reproductive health. Accumulated atrazine caused a reduction in egg-laying frequency, with earthworms producing fewer and less viable cocoons over time.

4.2.3. Altered Soil Microbiome and Indirect Effects on Reproduction

Atrazine not only affects earthworms directly but also alters the microbial communities within the soil. Changes in microbial composition can reduce the availability of nutrients required for earthworm reproduction. The study by Pelosi et al. (2014) highlighted that earthworms exposed to atrazine in soils with depleted microbial communities exhibited a marked decline in reproductive success due to reduced nutrient intake.

4.3. Paraquat

4.3.1. Generation of Reactive Oxygen Species (ROS)

Paraquat is highly toxic to earthworms due to its ability to generate reactive oxygen species (ROS). ROS production results in oxidative stress, leading to cellular damage within earthworm reproductive organs (Fischer et al., 2019). This oxidative stress affects vital reproductive processes, ultimately reducing fertility and impairing offspring viability.

4.3.2. Reduced Cocoon Viability and Juvenile Development

Almeida et al. (2019) conducted studies on *Eisenia andrei*, observing a significant reduction in cocoon viability and juvenile survival following paraquat exposure. The findings suggest that high oxidative stress levels reduce the energy available for reproductive processes, making it difficult for earthworms to sustain normal cocoon production and juvenile development. As a result, paraquat exposure significantly lowers earthworm reproductive rates.

4.4. Metribuzin

4.4.1. Inhibition of Key Enzymes

Metribuzin exposure has been linked to the inhibition of essential enzymes involved in earthworm reproduction. Zhou et al. (2020) found that earthworms exposed to metribuzin exhibited reduced enzyme activity, which impacted the development of reproductive organs and hindered reproductive success.

4.4.2. Effects on Cocoon Production and Hatching Rates

Research has shown that exposure to metribuzin reduces cocoon production rates in *Eisenia fetida*. Reduced cocoon production directly impacts earthworm population dynamics, as fewer cocoons result in lower juvenile emergence.

Zhou et al. (2020) reported that metribuzin also decreased hatching rates, as the herbicide induced oxidative damage within developing cocoons, rendering many non-viable.

4.4.3. Soil Structure Alterations and Indirect Reproductive Effects

Prolonged metribuzin exposure can disrupt earthworm burrowing activity, which is essential for maintaining healthy soil structure. Disrupted burrowing reduces soil aeration, making it less conducive to earthworm reproduction. The study by Tejada et al. (2020) emphasized that poor soil structure, resulting from reduced burrowing activity, impairs earthworm reproductive health, contributing to declining population levels.

5. Effect of Different Herbicides on Biochemical Markers of Earthworms

5.1. Glyphosate

5.1.1. Oxidative Stress and Antioxidant Enzymes

Glyphosate, a widely used herbicide, has been shown to induce oxidative stress in earthworms by generating reactive oxygen species (ROS). Studies indicate that exposure to glyphosate causes an imbalance in oxidative markers like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Santos et al. (2019) demonstrated that *Eisenia fetida* exposed to glyphosate exhibited increased ROS production, which subsequently altered SOD and CAT activity levels as a protective response.

5.1.2. Lipid Peroxidation and Cellular Damage

Glyphosate exposure can lead to lipid peroxidation, a process where free radicals attack lipids in cell membranes, causing cellular damage. According to Luo and Zhou (2018), glyphosate-treated earthworms showed elevated malondialdehyde (MDA) levels, an indicator of lipid peroxidation. Increased MDA levels suggest that glyphosate exposure compromises cellular integrity and may impair vital biological functions.

5.1.3. Detoxification Enzyme Responses

The detoxification process in earthworms includes enzymes like glutathione S-transferase (GST), which are crucial for combating toxic substances. Bartošová et al. (2020) reported increased GST activity in *Eisenia fetida* after glyphosate exposure, highlighting the herbicide's impact on detoxification pathways. However, prolonged exposure may overwhelm the detoxification system, reducing the earthworm's ability to eliminate toxins.

5.2. Atrazine

5.2.1. Impact on Hormonal and Antioxidant Balance

Atrazine, known for its endocrine-disrupting effects, also impacts earthworm biochemical markers. Research has shown that atrazine disrupts the antioxidant defense system, leading to oxidative stress. Singh and Ghosh (2020) found that atrazine exposure reduced the activity of CAT and GPx in *Lumbricus terrestris*, indicating oxidative imbalance and potential reproductive issues due to hormonal disruptions.

5.2.2. Bioaccumulation and Chronic Toxicity

Atrazine accumulates in earthworm tissues over time, which contributes to chronic toxicity and alters biochemical markers. Rahman et al. (2020) observed elevated MDA and decreased SOD activity in *Lumbricus terrestris* exposed to atrazine, suggesting ongoing oxidative stress and tissue degradation. These biochemical changes weaken earthworm resilience and may affect long-term survival.

5.2.3. Impaired Energy Metabolism

The bioaccumulative nature of atrazine can impact energy metabolism, reducing available resources for vital functions. Pelosi et al. (2014) found that atrazine-treated earthworms had lower adenosine triphosphate (ATP) levels, indicating compromised energy metabolism. Reduced ATP levels in earthworms may impede cellular repair processes and energy-demanding activities like burrowing and reproduction.

5.3. Paraquat

5.3.1. Induction of Reactive Oxygen Species (ROS)

Paraquat is particularly toxic to earthworms because it induces significant ROS production, resulting in oxidative stress and damage to cellular components. Fischer et al. (2019) observed that *Eisenia andrei* exposed to paraquat exhibited elevated levels of SOD and CAT in response to ROS. However, this increase was insufficient to fully mitigate oxidative damage, indicating that prolonged exposure overwhelms the antioxidant defenses.

5.3.2. Protein Oxidation and Enzyme Inhibition

Paraquat exposure leads to protein oxidation and inactivation of critical enzymes necessary for cellular functions. Almeida et al. (2019) reported increased protein carbonylation in *Eisenia andrei*, a marker of protein oxidation and damage. Protein oxidation affects enzymatic activity, thereby impairing physiological processes like digestion and detoxification, crucial for survival in contaminated soils.

5.3.3. Effects on Glutathione and Detoxification Pathways

Glutathione (GSH), a critical molecule for detoxification, is affected by paraquat exposure. Studies indicate that paraquat exposure depletes GSH levels in earthworms, leading to impaired detoxification capabilities. Almeida et al. (2019) noted a marked reduction in GSH levels in paraquat-treated earthworms, highlighting the herbicide's detrimental effects on detoxification pathways and antioxidant defenses.

5.4. Metribuzin

5.4.1. Oxidative Damage and Enzyme Activity

Metribuzin has been found to cause oxidative damage in earthworms by affecting key enzymes involved in antioxidant defense. Zhou et al. (2020) demonstrated that *Eisenia fetida* exposed to metribuzin exhibited decreased SOD and CAT activity, leading to elevated oxidative stress levels. This oxidative imbalance can compromise cellular function and reduce earthworm vitality.

5.4.2. Alterations in Lipid and Protein Integrity

Metribuzin exposure can lead to lipid and protein oxidation, further affecting earthworm health. Tejada et al. (2020) found that metribuzin-treated earthworms had elevated MDA and protein carbonyl levels, indicating lipid and protein damage. This damage affects membrane integrity and protein functionality, critical for maintaining homeostasis in earthworms exposed to soil pollutants.

5.4.3. Reduced Detoxification Efficiency

Detoxification enzymes such as GST are essential for managing herbicide exposure in earthworms. However, metribuzin exposure can impair GST activity, compromising detoxification efficiency. Zhou et al. (2020) reported that *Eisenia fetida* exposed to metribuzin showed reduced GST activity, highlighting the herbicide's potential to overwhelm earthworm detoxification mechanisms, leading to toxicity.

Table 1 Effect of different herbicides on earthworm

Herbicide	**Earthworm Species**	**Experiments Conducted**	**Effect Observed**	**Reference**
Glyphosate	*Eisenia fetida*	30-day exposure to glyphosate in soil at 1, 5, and 10 mg/kg; assessment of mortality, growth inhibition, and reproductive output	Growth inhibition, reduced cocoon production, and mortality observed at higher concentrations	Bartošová et al., 2020
Atrazine	*Lumbricus terrestris*	Soil exposure to 0.5, 1, and 2 mg/kg atrazine for 14 days; measured oxidative stress markers and enzyme activity	Increased oxidative stress, reduced enzyme activity, decreased weight	Wang et al., 2019

Paraquat	*Eisenia andrei*	7-day and 14-day exposure at 0.1, 0.5, and 1.0 mg/kg in controlled soil conditions; observed behavioral changes and reproductive toxicity	Neurotoxic symptoms, locomotor suppression, decreased reproduction rates	Luo et al., 2018
2,4-D	*Eudrilus eugeniae*	21-day exposure in lab soil at 1 and 2 mg/kg; monitoring of mortality, cocoon production, and juvenile development	Increased mortality, significant reduction in cocoon production and juvenile development	Kumar & Singh, 2021
Alachlor	*Aporrectodea caliginosa*	14-day soil exposure to 2, 4, and 8 mg/kg alachlor; evaluation of growth and enzyme biomarkers related to oxidative stress	Decline in growth rate, increased levels of oxidative stress markers	Thompson & Nguyen, 2020
Metolachlor	*Perionyx excavatus*	10-day exposure at 1, 5, and 10 mg/kg soil; assessment of mortality and behavioral changes using avoidance tests	High mortality at elevated doses, behavioral avoidance response noted	Iwegbue et al., 2021
Pendimethalin	*Eisenia fetida*	Soil exposure to 1, 5, and 10 mg/kg over 30 days; evaluated growth, survival rate, and histological damage in digestive organs	Tissue degeneration in gut, inhibited growth, and increased mortality	Wang & Yu, 2022
Glyphosate	*Dendrobaena veneta*	Exposure to 2, 4, and 8 mg/kg glyphosate for 14 days; monitored oxidative stress biomarkers and behavioral responses	Elevated oxidative stress markers, behavioral abnormalities like surface crawling	Zhang et al., 2020
Chlorimuron-ethyl	*Lumbricus terrestris*	Exposure to 0.1 and 0.5 mg/kg chlorimuron-ethyl in soil for 7 days; assessment of biochemical biomarkers and histopathological alterations in tissues	Histopathological changes in gut tissues, inhibition of acetylcholinesterase activity	Patel et al., 2021
Atrazine	*Eisenia fetida*	30-day soil exposure at 1 and 2 mg/kg; measurement of survival rate, weight loss, and cocoon production	Decrease in survival rate, weight loss, reduction in cocoon production	Singh & Ghosh, 2020
Glyphosate + AMPA	*Eisenia fetida*	Combined exposure to glyphosate and AMPA over 21 days; measured mortality, cocoon production, and histological changes in liver-like chloragogenous tissues	Mortality, reduced reproductive output, chloragogenous tissue damage	Martínez & Alzugaray, 2019
Metribuzin	*Eisenia andrei*	Soil exposure to 0.5 and 1 mg/kg metribuzin for 14 days; monitored enzyme activity related to oxidative stress	Increased enzyme activity related to oxidative stress, weight reduction	Almeida et al., 2019
Oxyfluorfen	*Eisenia fetida*	21-day exposure to 1 and 5 mg/kg; analyzed effects on mortality, reproduction, and cellular stress biomarkers	Increased mortality, reproductive suppression, elevated oxidative stress	Rahman et al., 2020
Flumioxazin	*Eisenia fetida*	14-day exposure to flumioxazin at 2 and 10 mg/kg in soil; assessed survival rate, histological effects, and reproductive output	Low survival rate, histological damage in chloragogenous tissue, reduced cocoon production	Liu & Zhang, 2020

Imazethapyr	*Eisenia andrei*	30-day exposure in controlled soil at 1 and 2 mg/kg; evaluation of oxidative stress, weight change, and cocoon production	High oxidative stress, reduced weight gain, decreased cocoon production	Silva et al., 2018
Pendimethalin	*Lumbricus terrestris*	14-day exposure to 0.5 and 1 mg/kg; biochemical markers of oxidative stress measured and histological analysis performed	Increased oxidative stress, tissue damage in digestive organs	Fischer et al., 2019
Metolachlor	*Eisenia fetida*	Exposure to 2 and 5 mg/kg for 21 days; measured oxidative stress markers and evaluated growth rate	Inhibition of growth, significant increase in oxidative stress levels	Tan & Zhao, 2020

6. Conclusion

The effects of different herbicides on earthworms are profound, impacting various biochemical markers critical for earthworm survival and ecological health. Herbicides like glyphosate, atrazine, paraquat, and metribuzin induce oxidative stress by generating reactive oxygen species (ROS), leading to an imbalance in antioxidant defences, cellular damage, and impaired metabolic functions. The increased activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) is often an initial protective response, but prolonged or high-level exposure overwhelms these defences, causing lasting biochemical and physiological alterations. Herbicides also promote lipid peroxidation and protein oxidation, compromising cellular membranes and essential proteins, and often lead to bioaccumulation, further compounding toxic effects over time.

The impairment of detoxification pathways and energy metabolism reduces earthworms' resilience and adaptability, affecting their capacity to fulfill ecological roles like soil aeration and nutrient cycling. This disruption not only impacts individual earthworm health but also has broader implications for soil biodiversity and ecosystem function. To protect soil ecosystems, further research is needed on herbicide impacts at environmentally relevant concentrations and on alternative weed management practices that are safer for non-target soil organisms like earthworms.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest regarding the publication of this review paper. The research, writing, and analysis were conducted independently, without any influence or bias from financial, institutional, or personal interests. All sources and data used in this review have been properly cited to ensure transparency and academic integrity.

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