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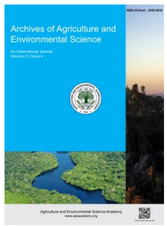

REVIEW ARTICLE

A review on the impact of commonly used pesticides on the biology of earthworms

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ABSTRACT

Earthworms are considered important bio-indicators of chemical contamination in the soil ecosystem. Being an important biotic factor of soil ecosystem, earthworms play a vital role in the functioning of soil ecosystems and maintenance of soil fertility. The present review encompasses the diverse effects of chemical contaminants like pesticides on earthworm biology, considering both direct toxicity and indirect impacts on ecosystem functions. Through a comprehensive review of existing literature, we assess the varying impacts of different classes of pesticides on earthworms. Several studies included in this review shed light on how pesticide exposure affects earthworm behaviour, reproduction, regenerative capacity, histology, gut microbial diversity, and nutrient transition, among other adverse effects, which consequently affect the soil ecosystem dynamics. Furthermore, we discuss the implications of these findings for agricultural practices, soil health, and biodiversity conservation. This study discusses the impact of pesticides on different facets of earthworm biology and emphasizes the necessity of sustainable pest management strategies to maintain the productivity and adaptability of ecosystems by enhancing our understanding of the complex interactions that occur between soil organisms, like earthworms, and foreign chemicals, or xenobiotics, like pesticides.

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INTRODUCTION

Earthworms are distributed worldwide, and their population is considered to make up approximately 8% of the total biomass of the soil organisms (Sinha *et al*., 2013). It is a tube-shaped, long, cylindrical, segmented soil-dwelling creature belonging to the class Oligochaeta of Phylum Annelida. These soil organisms play various significant roles in maintenance and development of the nutritional value and quality of soil by turning organic waste and biodegradable material into nutrient-rich worm manure (Bardiya & Dixit, 2024). The cast produced by earthworms have higher organic content than that of the surrounding soil in which earthworms remain present (Bhadauria & Saxena, 2010). Being a vital biotic component of the soil ecosystem, earthworms significantly contribute to enhancing soil fertility and maintaining the soil ecosystem dynamics by increasing nutrient availability through their potential role in organic matter mineralization and decomposition (Aina, 1984; Edwards & Bohlen, 1996; Abdul & Bouché, 1997; Brown *et al*., 2003). Therefore, any factor that can adversely affect the growth, survival, biological parameters, or ecological functioning of earthworms might have a detrimental impact on soil quality and soil ecosystem dynamics. The rapid increase in human population has a mandatory demand for a rapid and corresponding increase in crop production, which has resulted in the irrational use of different chemical agents like pesticides for crop protection and better crop yield. With the onset of Green revolution in 1960s the use of synthetic chemical agents like pesticides has increased rapidly across the globe including India (Alavanja, 2009). The total amount of pesticides used worldwide is approximately 3.39 million tonnes, whereas in India it is approximately 61,702 tonnes (Reddy & Mathur, 2024). In addition, Reddy & Mathur (2024) noted that while pesticide use in India has increased by about 46% overall, there has been a notable spike in use in a few states, including Jharkhand (an increase of 833%), Chhattisgarh (247%), Andhra Pradesh (265%), and Maharashtra (253%). However, these chemical agents can also have adverse impact on beneficial and non-target soil organisms, most notably earthworms (Gowri & Thangaraj, 2020). The earthworms are considered significant bio-indicators of contamination of soil by undesirable agents, including pesticides (Edwards & Thompson, 1973; Greig-Smith *et al*., 1992). There are a number of pesticides that are extensively used in agriculture, such as imidacloprid, benomyl, and metribuzin among many others which are reported to have a harmful or adverse impact on earthworms (Astaykina *et al*., 2022).

Pesticides are categorized into four primary groups based on their chemical composition: Carbamates, Pyrethrin and Pyrethroids, Organochlorines, and Organophosphorus (Buchel, 1983). Pesticides reduce biodiversity even at concentrations that don't go above the Maximum Residue Levels (MRLs) (Hole *et al*., 2005). In addition to their role in improving crop yield, pesticides can adversely affect the environment in several ways. According to Reinecke & Reinecke (2007), the usage of organophosphate insecticides has caused significant changes in earthworm population densities in South Africa. Another study from Mahmood *et al*. (2016) has reported that excessive pesticide use may result in the eradication of biodiversity. The target animal (or organism) and chemical structure (organic, inorganic, synthetic, and biological compounds) are used to categorize pesticides (Amenyogbe *et al*., 2021). Implementing appropriate corrective actions contributes to fewer cases of pesticide poisoning and other health problems associated with pesticide use (Rani *et al*., 2021). Persistent pesticides degrade slowly and can linger in the environment for extended periods and the soil conditions can also impact pesticide transport (Anjum *et al*., 2017). Previous studies have found that class I and II pesticides (WHO, 2005), extremely to moderately hazardous and banned in other countries, are still available in India (Bond *et al*., 2009). Many studies have been carried out in the last few decades on how different pesticides affect different aspects of earthworm biology, and only a small number of these pesticides are no longer in use. The employment of novel chemical combinations as pesticides, however, is possible everywhere in the world, especially in India. Understanding the effects of commonly used pesticides on earthworm biology and ecology is crucial, especially considering the significant roles that earthworms play in soil ecology and the widespread use of pesticides. It is quite evident that a wide range of pesticides are employed in agricultural fields, and the majority of these pesticides have not been tested for their effects on earthworm biology or ecology. As the survival of earthworms and its contributions in soil ecosystem have many facets therefore it is necessary to understand the impact of any pesticide in all such aspects which can affect the survival and ecological role of earthworms. Wang *et al*. (2012) have studied almost 45 different types of pesticide compositions on a single earthworm species, *Eisenia fetida*, and it can be viewed in correlation with different types of earthworm species like epigeic, anecic, and endogeic, as well as a significant biodiversity of different earthworm species. Additional research in this area is necessary to better understand earthworm-pesticide interactions because of the wide range of earthworm species, the various pesticide compositions in use, and the need to understand how each pesticide affects various aspects of earthworm biology. Therefore, a comprehensive understanding of the impact of chemical agents on soil ecosystem dynamics and soil ecology is essential. The present review encompasses the scientific reports and evaluation of the impact of some commonly used pesticides on some selected parameters of biology and ecological role of earthworms and might open the ways of new or further research in this field so that the use of any kind of chemical agent to increase crop yields will be made in a sustainable way to protect the soil faunal diversity and ecology, especially the earthworms.

METHODOLOGY

The primary objective of the present study is to evaluate the inferences drawn from existing scientific research works in the field of pesticide-earthworm interactions and to assess the gaps in understanding the impact of varieties of pesticides on diverse facets of earthworm biology, from molecular to ecological levels. We started our work by collecting the data on pesticide use from official sources like Food and Agriculture Organization Statistics (FAOSTAT) and a few Indian government publications. Later on, we went through the results of previous research works on pesticide-earthworm interactions through a survey of available and current scientific literature. We then categorized the major facets of earthworm biology that have been worked out to evaluate the impact of selected pesticides and then designed our work to focus on the selected aspects of earthworm biology, such as growth and development, reproduction, regeneration, oxidative stress, gut microbial diversity, and histological impacts, for evaluation of the impact of different pesticides. From the available scientific works, it was quite evident that during the past few decades till date, a number of chemical combinations have been utilized as pesticides for crop protection; on the contrary, there are different ecological groups and varieties of different species of earthworms. Neither each of the pesticides is being assessed for its impact on all the significant aspects of earthworm biology, nor are all the different varieties of earthworm species being evaluated to see the impact of a single pesticide. Further, the impact of a single pesticide on different ecological groups of earthworms, such as epigeic, anecic, and endogeic, found in the same geographical area, is also not worked out. The lack of a comprehensive and comparative analysis of the impact of widely used pesticide chemical combinations on different facets of earthworm biology is existing, and therefore the present study has been undertaken to address the available scientific knowledge and the gaps in the field of pesticide-earthworm interactions.

Effects of some commonly used pesticides on earthworms

Pesticides have the potential to negatively affect earthworms at various organizational and biological levels. These effects may include behavioral modifications, altered growth and development, altered fertility and reproduction, increased mortality, changes in metabolic features such as enzyme function, and even changes in gene expression (Zhang *et al.*, 2013; Velki *et al.*, 2014; Velki & Ečimović, 2015; Liu *et al.*, 2017, 2018). The potential impact of pesticides on earthworms depends upon various factors such as earthworm species, type and concentration of contaminants, soil properties, etc. (Rodriguez-Campos *et al.*, 2014). The epigeic and anecic earthworm species are found in the upper soil layers near the surface, whereas the endogeic species remain present in the inner soil strata, and therefore the epigeic and anecic species are more prone to facing the adverse effects of pesticides since the pesticides are used to spray on the soil surface (Singh *et al.*, 2016). Several studies have been conducted to evaluate the impact of commonly used pesticides on a range of biological parameters of various earthworm species, including histopathological effects, enzyme activities and oxidative stress assessment, growth and reproduction (including gamete viability, cocoon production, etc.), gut microbial diversity, cast quality and production, cytotoxic activities, behavioural changes like burrowing activities, etc. (Datta *et al.*, 2016). Some of the scientific reports of commonly used pesticides and their impact on different parameters of earthworm species are listed in Table 1.

Table 1. Commonly used pesticides and their impact on different biological parameters of selected earthworm species.

S. No.	Pesticide	Earthworm species	Effect	Reference
1.	Carbaryl and Dieldrin	*Eisenia fetida*	Growth and cocoon production inhibited.	Neuhauser & Callahan (1990)
2.	Benomyl	*Eisenia fetida*	Sperm were undeveloped or had abnormal acrosome formation.	Sorour & Larink (2001)
3.	Carbaryl	*Eisenia fetida*	Cholinesterase (ChE) inhibition was observed even at low doses.	Ribera *et al.* (2001)
4.	Butachlor, Malathion and Carbofuran	*Drawida willsi*	Butachlor did not affect AChE activity. Maximum AChE inhibition occurred after 9 days of malathion treatment and 12 days of Carbofuran exposure.	Booth & O'Halloran (2001)
5.	Chlorpyriphos, Atrazine, Cyanazine	*Eisenia fetida*	Chlorpyrifos binary combinations with Atrazine and Cyanazine were more harmful than additives.	Lydy & Linck (2003)
6.	Endosulphan and Aldicarb	*Lumbricus terrestris*	Weight loss and reduced growth rate. Compared to Endosulfan, Aldicarb is more hazardous and toxic.	Mosleh *et al.* (2003)
7.	Azodrin	*Eisenia fetida*	Dose-dependent suppression of AChE activity. AChE a ctivity is associated with morphological damage.	Rao & Kavitha (2004)
8.	Butachlor, Malathion and Carbofuran	*Drawida willsi*	No variation in AChE activity when exposed to Butachlor; maximum AChE inhibition was observed (41% and 46%) after 9 days when exposed to malathion and after 12 days of Carbofuran exposure (54% and 62.9%).	Panda & Sahu (2004)
9.	Malathion	*Eisenia fetida*	Significant loss of body mass and decline in sperm viability.	Navarro & Obregon (2005)
10.	Imidacloprid	*Aporrectodea nocturna and Allolobophora icterica*	The LC50 for *Aporrectodea nocturna* and *Allolobophora icterica* was 2–4 mg kg^{-1} dry soil. Additionally, considerable weight loss was noticed.	Capowiez *et al.* (2005)
11.	Acetochlor	*Eisenia fetida*	The growth and number of juveniles within each cocoon were greatly impacted.	Xiao *et al.* (2006)
12.	Carbofuran	*Eisenia fetida*	Total Cholinesterase Activity and protein content both increased at low levels, and vice versa. When the concentration of Carbofuran rose, SOD decreased and vice versa.	Ling (2006)
13.	Imidacloprid	*Aporrectodea nocturna and Allolobophora icterica*	Both species disrupted the continuity of their burrow systems. There was a decrease in gas diffusion through the soil cores of *Aporrectodea nocturna*, while there was no change in gas diffusion in the soil cores of *Allolobophora icterica*.	Capowiez & Berard (2006)
14.	Benomyl	*Eisenia fetida*	The toxicity of Benomyl was lower in tropical than temperate artificial soils.	Rombke *et al.* (2007)
15.	Carbendazim, Dimethoate, and Glyphosate	*Eisenia fetida*	Both independently and in combination are harmful to reproduction and development.	Yasmin & D'Souza (2007)
16.	Chlorphyrifos	*Eisenia fetida*	Adverse effects on growth and development	Zhou *et al.* (2007)
16.	Lindane and deltamethrin	*Eisenia fetida*	Lindane was found to be more harmful than deltamethrin. There were significant effects on growth and cellulase activity.	Shi *et al.* (2007)
17.	Glyphosate and Chlorpyrifos	*Eisenia fetida andrei*	Glyphosate decreased cocoon production. The NRRT (Neutral red retention test) and Comet assays demonstrated changes at the subcellular level.	Casabé *et al.* (2007)
18.	Carbaryl	*Eisenia fetida andrei*	AChE activity was seen to be inhibited dose-dependently.	Gambi *et al.* (2007)
19.	Chlorpyriphos and Azinphos methyl	*Aporrectodea caliginosa*	Inhibition of ChE.	Reinecke & Reinecke (2007)
20.	Cypermethrin	*Eisenia fetida*	Notable decrease in the production of cocoons. Juveniles were more sensitive than adults.	Shi-ping *et al.* (2008)

Table 1. *Contd…..*

21.	Cypermethrin	*Eisenia fetida Andrei*	Impacts the reproductive functions and reduction in cocoon production. Juveniles more sensitive than adults.	Ahmad *et al.* (2012); Lu *et al.* (2021)
21.	Butachlor	*Eisenia fetida*	Using the chloragogen tissue's reserve energy results in less biomass and cocoons.	Gobi & Gunasekaran (2010)
22.	Imidacloprid	*Lumbricus terrestris and Aporrectodea caliginosa*	Body mass loss and increased cast formation at higher concentrations.	Dittbrenner *et al.* (2010)
23.	Chlorpyrifos, Carbofuran and Mancozeb	*Perionyx excavatus*	The decline in toxicity: Carbofuran > Chlorpyrifos > Mancozeb	De Silva *et al.* (2010)
24.	Cypermethrin, Endosulfan, Carbaryl, Chlorpyrifos, Aldicarb, Monocrotophos	*Perionyx excavatus*	Toxicity order Monocrotophos >Aldicarb > Chlorpyrifos > Carbaryl > Cypermethrin > Endosulfan	Gupta *et al.* (2010)
25.	Imidacloprid	*Pheretima posthuma*	Protein concentration increased in the clitellum, some proteins were inhibited in the head, and there was no change seen in abdomen.	Faheem & Khan (2010)
26.	R-metalaxyl and rac-metalaxyl	*Eisenia fetida*	Metalaxyl bioaccumulation was found to be enantioselective in earthworms, with preferential accumulation of the Senantiomer.	Xu *et al.* (2011)
27.	Imidacloprid	*Lumbricus terrestris and Aporrectodea caliginosa*	*Aporrectodea caliginosa* exhibits burrowing effects even at lower concentrations, but *Lumbricus terrestris* exhibits burrowing effects only at greater concentrations.	Dittbrenner *et al.* (2011)
28.	Mixture of Ni and Chlorpyriphos	*Lumbricoid*	The combination of Ni and chlorpyrifos is harmful to earthworms. Worms store Ni and chlorpyriphos in their tissues.	Lister *et al.* (2011)
29.	Chlorpyrifos and Fenvalerate	*Eisenia fetida*	Cellulase and SOD activity were inhibited, while CAT activity initially increased and later decreased.	Wang *et al.* (2012)
30.	Azinphos methyl	*Eisenia fetida andrei*	Reduced burrowing activity and inhibition of cholinesterase activity.	Jordaan *et al.* (2012)
31.	Dimethoate	*Eisenia kinneari*	A disturbance in the cellular enzyme system which led to profound changes in testes.	Leena *et al.* (2012)
32.	Fomesafen	*Eisenia fetida*	Low doses may not cause oxidative stress and peroxidation.	Zhang *et al.* (2013)
33.	Chlorpryriphos	*Eisenia fetida*	With increasing doses, the earthworm body began to melt and disintegrate, and thus hindering the development, growth, reproduction, and the digging process.	Pawar & Ahmad (2014)
34.	Dimethoate	*Eudichogaster kinneari*	Effects on neurosecretory cells of the brain.	Lakhani (2015)
35.	Imidacloprid	*Aporrectodea caliginosa, Lumbricus terrestris, Eisenia fetida*	Severe impacts on CNS also inhibited cellulose and SOD activity, and induced sperm deformity also found serious DNA damage.	Taillebois *et al.* (2018); Zhao *et al.* (2018); Wang *et al.* (2016)
36.	Chlorpyrifos, Cypermethrin	*Eudrilus eugeniae*	Variation in morpho-behavioral changes like coiling, clitellar enlargement, mucus production, bleeding, and body segmentation. Significant alterations in stress markers such as AChE, SOD, CAT, and GST.	Tiwari *et al.* (2019)
37.	Triazophos and Deltamethrin	*Eudrilus eugeniae*	Significant changes in oxidative stress indicators, including non-enzymatic (MDA, GSH) and enzymatic (SOD, CAT, GST, LDH, ACP, ALP, ALT, and AST).	Singh *et al.* (2020)
38.	Malathion	*Eudrilus eugeniae*	Effect on anatomical symptoms such as coiling, unusual swelling, mucous production, bleeding, and fragmentation was observed after 24 and 48 hours of exposure. AChE enzyme activity was inhibited.	Jeyaprakasam *et al.* (2021)
39.	Chlorpyrifos, Cypermethrin and Glyphosate	*Eudrilus eugeniae*	Significant increase in glutamic acid (~91.81%), lysine (~92.20%), asparagine (~94.20%), leucine (~90.20%), malic acid (~93.37%), turanose (~95.04%), Oleic acid (~93.47%), methionine (~92.27%), maltose (~92.36%), cholesta-3,5-diene (~86.11%), and galactose (~).	Malla *et al.* (2023)
40.	Quinalphos	*Eudrilus eugeniae*	Significant changes in stress markers. Morphological anomalies like bulging, coiling, and bleeding.	Sujeeth *et al.* (2023)

Impact of pesticides on growth, development and reproduction of earthworms

The stress brought on by the presence of a contaminant, such as a pesticide, may induce the organism to redirect its energy from growth and development to survival (Gibbs *et al*., 1996). In order to evaluate any adverse effects of pesticides on earthworms, many scientists have considered the growth, development, and survival of earthworms as a crucial metric (Yasmin & D'Souza, 2010). Several previous works have reported the adverse effect of selected pesticides on the growth, development, and reproduction of earthworms. Mosleh *et al.* (2003) assessed the impact of cypermethrin, aldicarb, chlorfluazuron, profenofos, metalaxyl, and atrazine, and Booth *et al.* (2000) investigated the effect of two organophosphate pesticides diazinon and chlorpyrifos on the earthworm *Aporrectodea caliginosa* and found a decrease in growth rate in the pesticide-treated worms. Another investigation by Mosleh *et al.* (2002, 2003) assessed the impact of endosulfan and aldicarb on the earthworm *Lumbricus terrestris* and found that the pesticides exposure has resulted into an adverse effect on the growth of the earthworms. Booth & O'Halloran (2001) and Zhou *et al.* (2007) separately concluded that the adverse effects of pesticides are more prominent in the juvenile stage in comparison to the adult stage. According to Zhou *et al.* (2006), earthworm weight was a more sensitive indicator of acetochlor and methamidophos toxicity than death. In another study, it was reported that weight loss also appears to be a valuable indicator of physiological stress, related to the degree of intoxication and time of exposure (Frampton *et al.*, 2006).

Another symptom observed in 100% of the worms treated with Parathion was coiling, which is associated with weight loss and is thought to be the result of a change in muscle function brought on by organophosphate pesticides. This may account for the worms' difficulty moving around and relative incapacity to feed themselves (Bustos-Obregón & Goicochea, 2002). Jordaan *et al.* (2012) reported that the pesticide azinphos-methyl has an adverse effect on maturation, growth, reproduction, and burrowing activity and could result in cholinesterase (ChE) inhibition in *E. andrei*. According to Yasmin & D'Souza (2007), pesticides have a dose-dependent influence on worm reproduction, which may result in longer incubation times, fewer hatchlings per cocoon on average and maximum, and more worms creating cocoons overall. Several reproductive parameters have been assessed for the impact of pesticides on earthworms, like sperm maturation and viability, cocoon and hatchling production, and viability of hatchlings (Robidoux *et al.*, 2000; De Silva *et al.*, 2010). Several previous works, like Van Gestel (1992) and Gupta & Saxena (2003), have reported that cocoon hatchability was found to be a sensitive parameter for toxicity assessment of parathion carbendazim, pentachlorophenol, and copper oxychloride, whereas cocoon production was found to be a significant parameter for the assessment of toxic impacts of dieldrin, paraquat, fentin, benomyl, carbaryl, phenmedipham, and copper oxychloride. Bustos-Obregón & Goicochea (2002) evaluated the effects of parathion exposure on *Eisenia fetida* reproductive parameters, such as genotoxicity on male germ cells and the production of sperm and cocoons. They discovered that changes in these parameters were evident in the number of worms, sperm, and cocoons that were born. Xiao *et al.* (2006) reported the number of juveniles per cocoon as a sensitive parameter for assessment of the impact of acetachlor on earthworms. Dasgupta *et al.* (2012) reported that the toxic impact of carbaryl, chlorpyrifos, and endosulfan has resulted in a decrease in cocoon and juvenile production.

Likewise, Gupta & Saxena (2003) reported that the toxic impact of carbaryl on the earthworm *Metaphire posthuma* could produce abnormalities in the sperm head, including a wavy head, in lower concentrations, whereas at higher concentrations the sperm head becomes amorphous and the nucleus is converted into granules deposited within the region of the wavy head. Espinoza-Navarro & Bustos-Obregon (2005) reported that malathion contamination resulted in modifications in DNA of sperms, altered the cell proliferation, and decreased the sperm viability in *Eisenia fetida*. Another study by Muangphra *et al.* (2016) reported that chronic exposure of chlorpyrifos to the earthworm *Pheretima penguana* resulted in a reduction in cocoon production and viability. Gowri & Thangraj (2020) have assessed the impact of pesticide Monocrotophos on earthworms *Perionyx barotensis* and *Eudrilus eugeniae* and reported that the toxic impact of pesticide had resulted in the production of defective cocoons, increased mortality in the earthworms, and abnormalities in sperm, including oligopermia, necrospermia, and asthenospermia. Additionally, the growth, development, and reproduction in earthworms are crucial for their survival and ecological contributions. These processes involve a number of interrelated parameters that need to be closely observed. Even though a lot of research has been done in this field so far, a number of different earthworm species and types still need to be examined, in contrast to a lot of various pesticide combinations. With crop yields becoming more and more dependent on agrochemicals like pesticides worldwide, there is still more study to be done in this area to better understand how the growth, development, and reproduction of the ecologically significant earthworm species interact with agrochemicals like pesticides.

Impact of pesticides on histological parameters of earthworms

Some of the commonly used pesticides and their impact on histological parameters of earthworm species are listed in Table 2. Earthworms are extremely sensitive to changes in their surrounding environment, and therefore exposure to any chemical toxicant may produce several adverse effects, which prominently include alterations in the histological features of the earthworms. Singh *et al.* (2020) studied the histopathological impact of sublethal concentrations of triazophos on earthworm *E. eugenia* and reported that the earthworms exposed to the pesticides displayed both significant morphological changes in the earthworm body and histological changes in the post-clitellar region. Nunes *et al.* (2016) reported that the exposure of Abamectin to earthworm *Eisenia Andrei* resulted in skin thinning and discolouration, body constriction, posterior segment fragmentation, etc. In another study, Oluah *et al.* (2010) observed that the earthworm species *N. mbae* subjected to the pesticide atrazine had histopathological effects such as pyknotic cells, glandular growth of the epithelial tissues, and damage to the chloragogenous layer and epithelial tissues. Furthermore, Kavitha *et al.* (2020) reported significant histopathological changes in the intestine of *L. mauritii* after application of a lower sublethal concentration of monocrotophos; prominent damages were observed on the first few days (1^{st} and 5th day), which thereafter slowly recovered (30th day). The intestinal tract in this investigation displayed cell fusion, pyknotic nuclei development, damaged villi, and loss of the thick epithelial lining on the first day of the experiment. On the fifth day, the cytoplasm developed vacuoles, which led to space formation, blood sinus congestion, and significantly damaged nuclei, along with extensive damage to the gut. By the thirty-first day, the epithelial cells had been regenerated and were more or less evenly spaced across the epithelial layer. Moreover, the detrimental effects of the herbicide butachlor on the earthworm species *Perionyx sansibaricus* and *Eisenia fetida* were investigated by Muthukaruppan *et al.* (2005) and Gobi & Gunasekaran (2010), respectively. They observed enlargement of glandular cells, visible epithelial cell damage, and cellular enlargement, along with the loss of chromatin material in the cells, demonstrating the histological as well as cytological impact of the pesticides on earthworms. Moreover, in another experiment with earthworm species *E. fetida*, the epithelium layer of the earthworm was reported to be severely damaged, resulting in fused cells and excessive villous development. There were necrotic cell ruptures, causing widespread dispersion of cell debris. The chloragogen tissue was also severely damaged with minimal reserve inclusion. It was also observed that with the increase in the dose concentration of the pesticide, the villi continued to get fused while a distinct cavitation and pyknotic nuclei were observed in the epithelial layer of the gut of the earthworm. Also, the previous studies have clearly demarcated the histological alterations and damage caused by pesticide exposure. However, further research is required to fully comprehend how histological changes are related to the type and dosage of pesticides that are commonly used, especially in agricultural fields, as new types of chemical combinations of pesticides are regularly emerging for crop protection. The histological impacts of any pesticide may be assessed in different tissue types, or the comparative histological impacts may be analyzed on a single tissue type following the exposure to different pesticide combinations. Further, the molecular mechanisms and gene expression analysis may be done to comprehend how a pesticide is triggering the molecular mechanisms leading to necrosis or cell death.

Impact of pesticides on regeneration of earthworms

Earthworms are soil-dwelling animals; hence, they are very much prone to serious damage and wounds inflicted on them, and therefore they have a great regenerative capacity, which helps in their survival. Numerous reports have demonstrated the adverse effects of pesticides on earthworms, but only a few works have been conducted to study the pernicious effects of pesticides on the regenerative capacity of earthworms. According to an experiment conducted by Kumari & Sinha (2011), different doses of malathion on all the segments at different intervals significantly (p<0.01) affected the process of regeneration in the earthworm *D. willsi*, a cropland earthworm. In another experiment, Zoran *et al.* (1986) observed the teratogenic effects of benomyl on the earthworm species *Eisenia fetida* and reported that there was a reduction in the number of regenerated segments and an increased frequency of segmental groove anomalies, followed by a variety of other abnormalities. The effects of the pesticide benomyl treatment on the number of regenerated segments and frequency of anomalies were found to be dose-dependent within a narrow concentration range (approximately 0.2–5.0 mg/litre) and at higher concentrations (e.g., 25 mg/litre); teratogenic effects were less frequent because of the delayed onset of segmental delineation until after exposure. Furthermore, Yuan *et al.* (2024) evaluated the impact of Carbamates, Metolcarb, and Fenoxycarb on *Eisenia fetida* and reported that though the precise mechanisms of harmful effects of CAR's (Carbamates) on earthworms remain unclear, especially from a regenerative standpoint, MEB (Metolcarb) and FEB (Fenoxycarb) treatments resulted in delayed posterior renewal of amputated earthworms compared to the control group, resulting in compromised morphology, dwindling segments, and increased cell apoptosis in blastemal tissues. This was mediated by rising Sox2 (sex-determining region Y-box 2) and decreasing TCTP (translationally controlled tumor protein) levels. These findings provide baseline toxicity cues for MEB and FEB exposure against earthworms, as well as mechanistic insights into regenerative toxicity during CAR exposure. Previous studies in

Table 2. Impact of some commonly used Pesticides on histological parameters of earthworms.

S. No.	Pesticide	Earthworm species	Effect	References
1.	Benomyl	*Eisenia fetida*	Hypodeveloped sperms; sperms with abnormal acrosome development.	Sorour & Larink (2001)
2.	Butachlor	*Perionyx sansibaricus*	Glandular cell enlargement at all exposed concentrations.	Gobi *et al.* (2005)
3.	Malathion	*Eisenia fetida*	Decreased spermatic viability in spermatheca, altering the cell proliferation and modifying the DNA structure of spermatogonia.	Espinoza-Navarro & Bustos-Obregón (2005)
4.	Carbofuran	*Eisenia fetida*	Protein content and total cholinesterase activity (TChE) increased in low level.	Ling (2006)
5.	Profenofos	*Eisenia fetida*	Body ruptures and bloody lesions.	Reddy & Rao (2008)
6.	Butachlor	*Eisenia fetida*	The epithelium layer of the earthworm was reported to be severely damaged, resulting in fused cells and excessive villous development.	Gobi *et al.* (2010)
7.	Atrazine	*Nsukkadrilus mbae*	Chloragogenous layer and epithelial tissue damage; prominent vacuolations and pyknotic cells.	Oluah *et al.* (2010)
8.	Triplen, Ridomil, Cyren and Mamba	*Lumbricus terrestris*	Symptoms of toxicity found were body swellings, coelomicfuid discharge, slow movements, coiling etc..	Ahmed (2013)
9.	Imidacloprid, Acetamiprid, Nitenpyram, Clothianidin and Thiacloprid	*Eisenia fetida*	Significantly inhibit fecundity and cellulase activity and also damage the epidermal and midgut cells.	Wang *et al.* (2015)
10.	Abamectin	*Eisenia andrei*	Skin thinning and discoloration, body constriction and posterior segment fragmentation.	Nunes *et al.* (2016)
10.	Monocrotophos	*Lampito mauritii*	Intestinal tract showed loss of dense epithelial lining, damaged villi, cell fusion, and formation of pyknotic nuclei.	Kavitha *et al.* (2020)
11.	Monocrotophos	*Eudrilus eugeniae* and *Perionyx barotensis*	Increase in mortality and abnormal sperm (asthenospermia, necrospermia, and oligospermia). Rupture of chloragogenous tissue, longitudinal muscle, fused and extra-villous growth and necrotic cell rupture in earthworm's body wall (epidermis, circular and longitudinal muscles) were observed.	Gowri & Thangaraj (2020)
12.	Chlorpyrifos	*Eudrilus euginae*	The gut region showed mild degeneration of intestinal epithelium, epithelial cells, and fusion of villi. Epidermal hyperplasia was also observed.	Krishnaswamy *et al.* (2021)

molecular mechanisms involved in regeneration reveal that major proteinases, like Plasmin, Plasminogen activator, and Elastases play a crucial role in spatiotemporal distribution and hydrolysis of extracellular matrix components during regeneration (Sun *et al*., 2001). The expression of Sox 2 protein and translationally controlled tumour proteins (TCTPs) rises significantly during regeneration (Subramaniam *et al*. 2017; Jing Tao *et al*. 2018). Other significantly involved proteins in the regeneration process include Wnt 3a (stem cell marker), PCNA (cell proliferation), and YAP 1 (hippo signalling), which are expressed in response to THTPs (Rajgopalan *et al*. 2022). The regeneration mechanism includes a group of intricately related mechanisms at molecular, cellular, and tissue levels, and therefore a deep understanding of how a pesticide affects the process of regeneration is very essential. But, still, not much work has been done on this aspect, and hence further studies are required to comprehend how various pesticides affect the physiological, anatomical, or molecular processes involved in earthworm regeneration.

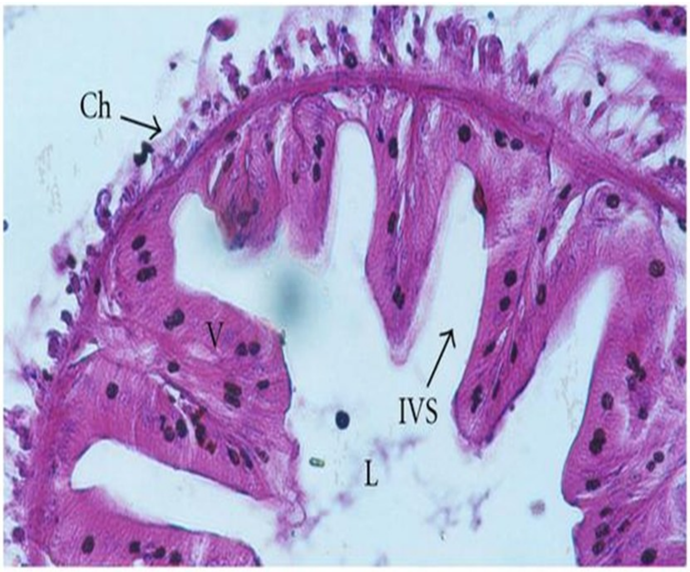

Figure 1. *Cross section of earthworm E. fetida normal intestine and chloragogen tissue at control. L: Lumen; V: Villi; Ch: chloragogen tissue; IVS: Inter villious space (Courtesy: Gobi & Gunasekaran, 2010).*

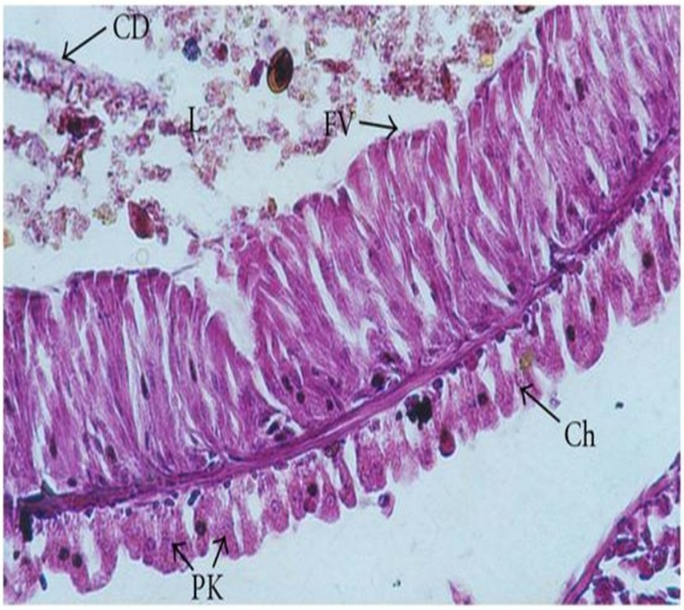

Figure 2. *Cross section of earthworm E. fetida showing intestine and chloragogen tissue at 0.2575 mg kg^{-1} concentration of herbicide. PK: pyknotic nuclei; CD: Cellular Debris; L: Lumen; FV: Fused Villi; Ch: chloragogen tissue (Courtesy: Gobi & Gunasekaran, 2010).*

Impact of pesticides on biochemical and physiological parameters of earthworms

Some of the commonly used pesticides and their impact on biochemical parameters of earthworm species are listed in Table 3. Through the course of various studies and research, a variety of remarkable observations were made regarding the impact of pesticides on different biochemical and physiological parameters of earthworms. According to Datta *et al.* (2016), earthworms constitute up to 60–80% biomass in the soil macrofauna and are prone to the non-target effects of soil pesticide contamination as it's widely distributed in agroecosystems. Almost all organophosphate pesticides are neurotoxic, and generally, upon acute exposure, acetylcholinesterase (AChE) enzyme activity is inhibited, resulting in the accumulation of acetylcholine at nerve and muscle endings, which stimulates both muscarinic and nicotinic receptors in the nerves and muscles (Thompson & Richardson, 2004). The exposure of CARs like carbofuran (Ferreira *et al.*, 2015; Kanedi, 2017a, 2017b), carbaryl (Lima *et al.*, 2015), aldicarb (Mosleh *et al.*, 2003), and methomyl (Ibtissem *et al.*, 2012) results in negative physiological responses of earthworms, which involve avoidance behaviour, decreased reproduction, weight loss, reduced survival rates, and impaired enzymatic system. According to Wang *et al.* (2015), the DNA in the cell nucleus, which was supposedly exposed to the highest concentration (1.00 mg/kg imidacloprid), was fluffy, scattered, and dusky, with the DNA migrating into the tail region as a result of extensive strand breakage, and as the concentrations decreased, the DNA migrating extended in the cell nucleus. It was also seen that the nuclear DNA migrated the most significantly from the cell nuclear space after it was exposed to the lowest concentration (0.10 mg/kg imidacloprid). In another work carried out by Qiao *et al.* (2022), it was observed that the subchronic toxicity of FLU (Flupyradifurone) resulted in an increase in the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione-S transferase (GST), while the peroxidase (POD) and acetylcholinesterase (AChE) activities decreased. In this study, it was also revealed that the reactive oxygen species (ROS) level and malondialdehyde (MDA) content in earthworms were increased by FLU, resulting in DNA damage. Based on several scientific reports, it has been established that the pesticides may produce oxidative stress, which might result in severe adverse effects like DNA damage and abnormal levels of antioxidant enzymes in the body, among others, which can adversely affect growth, survival, and other facets of earthworm biology. Therefore, more work is needed in this area to understand how different free radicals are generated as a result of exposure to different pesticides and how they damage or hamper vital molecular and physiological mechanisms within the cells.

Table 3. Impact of pesticides on biochemical parameters of earthworms.

S. No.	Pesticide	Earthworm species	Enzymes	Effect	References
1.	Azoxystrobin	*Eisenia fetida*	SOD and GST	Both the SOD and GST activities were increased.	Han *et al.* (2014)
2.	Imidacloprid	*Eisenia fetida*	SOD	SOD activity increased at lower dose but decreased at higher dose.	Zhang *et al.* (2014)
3.	Imidacloprid	*Eisenia fetida*	AChE	AChE activity was suppressed.	Wang *et al.* (2015)
4.	Carbaryl	*Eudrilus eugenie, Perionyx ceylanensis and Perionyx excavatus*	SOD, CAT, GST	SOD and CAT activity was suppressed whereas there was an increase in GST.	Jeyanthi *et al.* (2016)
5.	Carbendazim and Captan	*Eisenia fetida*	ACP	ACP activity was inhibited.	Mandal *et al.* (2017)
6.	Clothianidin	*Eisenia fetida*	GST	GST activity was decreased at higher concentration.	Liu *et al.* (2017)
7.	Glyphosate	*Dendrobaena veneta*	AChE	AChE activity was significantly increased.	Hackenberger *et al.* (2018)
8.	Fluoxastrobin	*Eisenia fetida*	SOD and CAT	SOD activity was increased, whereas CAT activity was suppressed.	Zhang *et al.* (2018)
9.	Chlorpyrifos and Cypermethrin	*Eudrilus eugeniae*	AChE	Inhibition of AChE activity was region and dose-dependent.	Tiwari *et al.* (2019)
10.	Pyraclostrobin	*Eisenia fetida*	SOD	SOD activity was increased in beginning and reduced with time.	Ma *et al.* (2019)
11.	Sulfentrazone	*Eisenia fetida*	SOD, CAT, GST	SOD was greatly suppressed at the beginning but steadily enhanced with increasing concentration. GST activity was higher in comparison to control among all the three enzymes.	Li *et al.* (2020)
12.	Triazophos and Deltamethrin	*Eudrilus eugeniae*	ACP and ALP	Both ACP and ALP activities were significantly increased.	Singh *et al.* (2020)
13.	Tebuconazole	*Eisenia fetida*	CAT	No changes were reported.	Zhang *et al.* (2020)
14.	Sulfoxafor	*Eisenia fetida*	GST	GST activity was enhanced.	Zhang *et al.* (2020)
15.	Glyphosate	*Alma millsoni, Eudrilus eugeniae and Libyodrilus violaceus*	AChE	No significant changes were observed.	Owagboriaye *et al.* (2020)
16.	Trifoxystrobin	*Eisenia fetida*	SOD	SOD activity did not vary much at low concentrations but improved considerably at higher dose.	Liu *et al.* (2020)
17.	Chlorpyrifos	*Eisenia fetida*	CAT	CAT activity was severely inhibited at higher concentrations, whereas there was no significant difference seen at low concentrations.	Zhu *et al.* (2020)
18.	Thifuzamide	*Eisenia fetida*	SOD and GST	SOD activity was inhibited at higher doses whereas GST activity was dose dependent.	Yao *et al.* (2020)
19.	Tebuconazole	*Eisenia fetida*	CAT and SOD	SOD activity considerably decreased and CAT activity showed no effect.	Zhang *et al.* (2020)
20.	Atrazine	*Eisenia fetida*	ALP	ALP activity was decreased.	Lammertyn *et al.* (2021)
21.	Trifoxystrobin	*Eisenia fetida*	CAT	CAT activity was decreased.	Wu *et al.* (2021)

Impact of pesticides on gut microbiota of earthworms

The unique features of the gut ecosystem in earthworms play an important role in improving the soil fertility parameters during the gut transition of soil. It contains an array of enzymes produced endogenously by the residing microbes, used for the digestion of organic matter (Drake & Horn, 2007). The gut microbiota of earthworms contains bacteria, fungi, protozoa, and other microbes that play an important part in nutrient metabolism, digestion, and even immune functions by contributing to organic matter breakdown, nutrient release, and maintaining gut homeostasis (Furlong *et al*., 2002). Depending on the ecological trophic group, earthworms can contribute to further movement of pollutants into the soil (Kuzyakov & Blagodatskaya, 2015). For instance, *Lumbricus terrestris*, an anecic earthworm (Bouché, 1977; Lavelle *et al*., 1989), is capable of transporting pesticides from the surface to the mineral horizons. Moreover, *L. terrestris* digestion can take up to 6 hours (Nechitaylo *et al*., 2010) due to the structural features like a longer intestinal tract; consequently, when pesticides enter this digestive tract, they can have long-term impacts on gut microorganisms. According to Astaykina *et al.* (2022), pesticide-contaminated soil greatly affects the microbial community that lives in the guts of earthworms. They found that pesticides, imidacloprid, benomyl, and metribuzin, reduced the total bacterial diversity in the earthworm's gut even at the recommended application rate. Under the administered pesticides, the structure of the gut prokaryotic community experienced alterations in the relative abundance of the phyla proteobacteria, actinobacteria, acidobacteria, planctomyces, verrucomicrobia, and cyanobacteria. Furthermore, pesticides influenced the amount of *Verminephrobacter*—the earthworms' nephridia-specific symbionts.These findings demonstrated that the earthworm's gut microbial population is extremely vulnerable to pesticide contamination in soil. Krishnaswamy *et al.* (2021) studied the impact of pesticide Chlorpyrifos (CPF) toxicity on the gut microbiome of *E. euginae* by using a long-amplicon Nanopore sequencing method. The results showed no fluctuations with Firmicutes and Bacteroidetes, which were shown to be dominant at the bacterial phylum level, while significant differences were observed at the genus level. Chang *et al.* (2021) stated that the pesticide Fomesafen not only targeted and significantly altered the gut microbial phyla but also that exposure to this bioscide lowered the earthworms' energy resources and activated the antioxidant system, both of which were strongly linked with gut microbial diversity. Similarly, three species of earthworms, namely *Alma millsoni*, *Eudrilus eugeniae*, and *Libyodrilus violaceus*, when exposed to a glyphosate-based herbicide, showed a significant shift in bacterial populations, with Proteobacteria becoming the dominant phylum; the affected bacterial genera mostly belonged to genera Pantoea, Pseudomonas, and Enterobacter (Owagboriaye *et al.*, 2021). Moreover, Kavitha *et al.* (2020) worked on the impact of monocrotophos on *Lampito mauritii*, an Indian earthworm species commonly known as Kinberg, and observed that after 30 days of exposure, microbial populations returned to their normal levels after an initial decrease in microbiota. This work revealed that only six bacterial and three fungal species survived exposure to monocrotophos out of eight bacterial and five fungal species. On the first and fifth days of the experiment, the gut showed severe pathological alterations, including vacuolization, degraded nuclei, damaged villi, and blood sinus congestion. But on the 30th day, the damages were slowly recovered due to the degradation of monocrotophos by the presence of some pesticide-degrading bacterial and fungal species and the regenerative capability of chloragogen cells in the intestine. Prior research has demonstrated that interactions between gut microbiota and the transient soil in the gut of earthworms have a significant role in improving soil fertility. Any external factor, such as pesticides, that can adversely affect the microbial environment or microbial diversity of the earthworm gut will hamper soil processing through the earthworm gut, consequently affecting the biology and ecological role of earthworms. Further, the microbial diversity of earthworm gut has been found to play a role in mitigation of pesticide contamination, and therefore more studies in this area are required for a better understanding of the interaction between gut microbiota and the chemical contaminants like pesticide exposure to the earthworms. Despite earlier studies in this area, additional research is necessary to fully understand how different pesticides can alter earthworm gut microbiota and what impact this interaction may have on the role of earthworms in soil ecosystem dynamics.

Conclusion

Even though pesticides tend to play a significant role in better crop yield, it also significantly destroys and handicaps the natural biological soil fertility creatures which include the earthworms. Studies consistently demonstrate adverse effects of pesticides in earthworms, such as reduced population, altered behavior, histopathological alterations, impaired growth, development and reproduction, altered regenerative capacities and adverse changes in gut microbiota among other marked effects. It is quite evident that there are numerous chemical combinations that are used as pesticides and on the other hand there are different ecological groups and species of earthworms. A thorough understanding of different aspects of the effects of pesticides on earthworm biology, from the molecular, cytological, histological, physiological, individual, and ecological levels, is still lacking despite a number of earlier studies. Any factor that can adversely affect the earthworm biology can have cascading effects on other soil organisms, plant communities, soil structure, and ultimately, ecosystem health. These findings underscore the need of further research in various aspects of earthworm-pesticides interactions which will prove to be significant for sustainable agricultural practices to mitigate harm to earthworm populations and maintain soil biodiversity, fertility and soil ecosystem dynamics.

DECLARATIONS

Author contribution statement

Conceptualization: A.K.; Methodology: A.K. and N.S.; Software and validation: N.S. and K.A.; Formal analysis and investigation: A.K. and N.S.; Resources: N.S. and S.F.; Data curation: N.S., K.A. and S.F.; Writing-original draft preparation: N.S., K.A. and S.F.; Writing-review and editing: A.K.; Visualization: K.A., S.F. and N.S.; Supervision: A.K.; Project administration: A.K.; Funding acquisition: A.K.; All authors have read and agreed to the published version of the manuscript.

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