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Assessment of the Effects of Chemical Fertilizers and Insecticides on Earthworm Species

Aanchal Bargoti¹ and Hridayesh Arya¹

¹Department of Zoology, N.R.E.C. College, Khurja, Bulandshahr. Affiliated to Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India

* Corresponding author. E-mail- aanchalbargoti2000@gmail.com

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ABSTRACT

Earthworms are considered important bioindicators of soil health due to their significant role in nutrient cycling, aeration, decomposition, and maintenance of soil fertility. The excessive use of chemical fertilizers and insecticides in modern agriculture has raised concerns regarding their adverse effects on soil biodiversity and ecosystem functioning. The present study was conducted to assess the effects of selected chemical fertilizers and insecticides on earthworm species under controlled experimental conditions. Earthworms were exposed to different concentrations of urea fertilizer and chlorpyrifos insecticide for a period of 30 days. Various biological parameters including survival rate, body weight, behavioral changes, burrowing activity, and mortality were observed at regular intervals. The results revealed that exposure to agrochemicals caused significant physiological and behavioral alterations in earthworms. Increased concentrations of fertilizers and insecticides resulted in reduced body weight, sluggish movement, decreased burrowing activity, and higher mortality rates compared to the control group. Chlorpyrifos exhibited greater toxicity than urea fertilizer. Histological observations also indicated tissue degeneration and damage in treated groups. The findings suggest that indiscriminate use of agrochemicals may negatively affect earthworm populations and ultimately deteriorate soil quality and ecological balance. Sustainable agricultural practices and eco-friendly alternatives are therefore recommended to minimize environmental hazards and conserve beneficial soil organisms.

Introduction

Earthworms are considered important bioindicators of soil health due to their significant role in nutrient cycling, aeration, decomposition, and maintenance of soil fertility. The excessive use of chemical fertilizers and insecticides in modern agriculture has raised concerns regarding their adverse effects on soil biodiversity and ecosystem functioning. The present study was conducted to assess the effects of selected chemical fertilizers and insecticides on earthworm species under controlled experimental conditions. Earthworms were exposed to different concentrations of urea fertilizer and chlorpyrifos insecticide for a period of 30 days. Various biological parameters including survival rate, body weight, behavioral changes, burrowing activity, and mortality were observed at regular intervals. The results revealed that exposure to agrochemicals caused significant physiological and behavioral alterations in earthworms. Increased concentrations of fertilizers and insecticides resulted in reduced body weight, sluggish movement, decreased burrowing activity, and higher mortality rates compared to the control group. Chlorpyrifos exhibited greater toxicity than urea fertilizer. Histological observations also indicated tissue degeneration and damage in treated groups. The findings suggest that indiscriminate use of agrochemicals may negatively affect earthworm populations and ultimately deteriorate soil quality and ecological balance. Sustainable agricultural practices and eco-friendly alternatives are therefore recommended to minimize environmental hazards and conserve beneficial soil organisms.

Keywords: Earthworm, Chemical Fertilizers, Insecticides, Soil Toxicity, Chlorpyrifos, Soil Health, Agrochemicals, Bioindicator

Introduction

Earthworms are among the most important soil invertebrates and are widely recognized as key indicators of soil fertility and

environmental health. They play a significant role in maintaining soil structure, nutrient cycling, organic matter decomposition, aeration, and microbial activity. Due to their ecological importance, earthworms are often referred to as “ecosystem engineers” because their burrowing and feeding activities improve soil productivity and agricultural sustainability (Lavelle & Spain, 2001). The presence of healthy earthworm populations generally reflects fertile and biologically active soil ecosystems, whereas a decline in their population indicates environmental degradation and soil pollution. Agriculture is the backbone of the economy in many developing countries, including India. To increase agricultural productivity and fulfill the food demands of a rapidly growing population, farmers increasingly rely on chemical fertilizers and insecticides. Chemical fertilizers such as urea, ammonium sulfate, superphosphate, and potassium nitrate are extensively used to enhance crop growth and yield by supplying essential nutrients like nitrogen, phosphorus, and potassium. Similarly, insecticides are commonly applied to protect crops from insect pests and improve agricultural production. Organophosphate insecticides such as chlorpyrifos, malathion, and monocrotophos are frequently used in agricultural fields because of their broad-spectrum insecticidal properties (Tomlin, 2009). Although these agrochemicals contribute significantly to crop production, their excessive and indiscriminate use has become a major environmental concern.

Soil is a complex living ecosystem inhabited by numerous microorganisms and soil fauna, including earthworms, nematodes, insects, fungi, and bacteria. The application of agrochemicals directly affects these non-target organisms and may alter soil biodiversity and ecological balance. Earthworms are particularly vulnerable to soil contaminants because they continuously ingest soil particles and organic matter while maintaining direct contact with the soil through their moist body surface. Therefore, they

readily absorb toxic substances present in contaminated soil (Edwards & Bohlen, 1996). Exposure to agrochemicals can adversely affect earthworm survival, growth, reproduction, burrowing activity, and physiological functioning.

Chemical fertilizers, when applied in excessive amounts, may alter soil pH, salinity, and nutrient composition. High concentrations of nitrogenous fertilizers can increase soil acidity and ammonia levels, thereby creating unfavorable conditions for earthworm survival. Fertilizer-induced changes in soil physicochemical properties may also reduce microbial biomass and organic matter availability, which serve as major food sources for earthworms (Suthar, 2008). Long-term use of synthetic fertilizers has been associated with reduced earthworm density and biomass in agricultural soils. Furthermore, excessive fertilizer application can lead to nutrient leaching, groundwater contamination, and ecological disturbances.

Insecticides are considered more hazardous to soil organisms because they are specifically designed to kill living organisms. Organophosphate insecticides such as chlorpyrifos act by inhibiting acetylcholinesterase activity in the nervous system, leading to accumulation of acetylcholine at nerve synapses and disruption of normal nerve impulse transmission. Earthworms exposed to insecticides often exhibit behavioral abnormalities such as coiling, sluggish movement, mucus secretion, surface crawling, and paralysis (Yasmin & D'Souza, 2010). Prolonged exposure may result in oxidative stress, tissue damage, impaired reproduction, and mortality. Studies have shown that insecticides can significantly reduce earthworm population density and negatively impact soil ecosystem functioning.

Earthworms contribute significantly to soil aeration and nutrient mixing through their burrowing activities. Their tunnels facilitate water infiltration, root penetration, and gaseous exchange in the soil. In addition, earthworms accelerate decomposition of organic matter by ingesting plant residues and converting them into nutrient-rich casts. These casts contain higher concentrations of nitrogen, phosphorus, potassium, calcium, and beneficial microorganisms compared to surrounding soil (Lavelle & Spain, 2001). Therefore, any decline in earthworm population may adversely affect soil fertility and agricultural productivity.

Modern agricultural practices often involve combined application of fertilizers and pesticides, which may produce synergistic toxic effects on soil organisms. Combined exposure to multiple agrochemicals can increase physiological stress and toxicity in earthworms compared to individual chemical exposure. Agrochemical mixtures may interfere with metabolic pathways, enzymatic activities, and antioxidant defense systems, ultimately leading to cellular damage and mortality. Earthworms exposed to contaminated soil often show reduced feeding activity and body weight due to impaired digestion and metabolic dysfunction. Several researchers have reported toxic effects of chemical fertilizers and pesticides on earthworm species. Gupta *et al.* (2007) observed that chlorpyrifos exposure significantly reduced survival and reproductive capacity in earthworms. Reinecke and Reinecke (2007) reported histological damage and oxidative stress in earthworms exposed to organophosphate insecticides. Pelosi *et al.* (2014) reviewed the ecological impacts of pesticides on earthworm diversity and concluded that pesticide exposure can severely disrupt soil biological communities. Similarly, Suthar (2008) demonstrated that high fertilizer concentrations negatively affect earthworm growth and burrowing activity. Apart from physiological toxicity, agrochemicals also influence earthworm behavior, which serves as an early indicator of environmental stress. Behavioral responses such as avoidance behavior, reduced burrowing, surface migration, and decreased feeding activity are commonly observed in contaminated soils. These behavioral changes may affect soil mixing and organic matter decomposition processes. Burrowing reduction can decrease soil porosity and water infiltration, thereby affecting plant root development and crop productivity. Earthworms are widely used in ecotoxicological studies because of their sensitivity to environmental pollutants and their ecological importance in soil ecosystems. International organizations such as the Organisation for Economic Co-operation and Development

(OECD) recommend earthworm toxicity tests for evaluating soil contamination and pesticide safety. Earthworm-based toxicity assessments provide valuable information regarding the environmental risks associated with agrochemical use.

In India, intensive agricultural practices have resulted in increased use of fertilizers and insecticides, particularly in regions dominated by commercial farming. Continuous application of agrochemicals without proper management may lead to accumulation of toxic residues in soil and negatively affect beneficial soil fauna. Despite the agricultural importance of earthworms, limited studies have been conducted regarding the combined effects of fertilizers and insecticides on earthworm health under Indian environmental conditions. Therefore, understanding the toxicological effects of agrochemicals on earthworm species is essential for sustainable soil management and environmental conservation. The present study was therefore undertaken to assess the effects of chemical fertilizers and insecticides on earthworm species under controlled experimental conditions. The study aims to evaluate changes in survival, body weight, behavior, and burrowing activity of earthworms exposed to agrochemicals. The findings of the study may help in understanding the ecological risks associated with excessive agrochemical application and promote environmentally sustainable agricultural practices.

Review of Literature

Earthworms are ecologically important soil organisms that contribute significantly to soil fertility, organic matter decomposition, nutrient recycling, and maintenance of soil structure. Due to their sensitivity toward environmental contaminants, earthworms are widely used as bioindicators in ecotoxicological studies. Several researchers have investigated the effects of chemical fertilizers and insecticides on earthworm physiology, behavior, survival, and reproductive performance.

Darwin (1881) was among the earliest scientists to emphasize the ecological significance of earthworms in soil formation and fertility improvement. He reported that earthworm activity plays an essential role in mixing organic matter with soil and improving soil texture. Since then, extensive research has been conducted to evaluate the ecological role of earthworms and the impact of pollutants on their biological activities. Edwards and Bohlen (1996) described earthworms as "ecosystem engineers" because of their ability to modify physical and chemical properties of soil. According to their findings, earthworm burrowing increases soil aeration, water infiltration, and nutrient availability. They further reported that earthworms enhance microbial activity and accelerate decomposition of organic residues. However, environmental contaminants such as pesticides and fertilizers may interfere with these ecological functions. Lavelle and Spain (2001) highlighted the importance of earthworms in maintaining soil biodiversity and ecological balance. Their study demonstrated that earthworms regulate nutrient cycling and contribute significantly to agricultural productivity. The authors also emphasized that intensive agricultural practices involving excessive agrochemical use can negatively affect soil fauna and reduce earthworm populations.

Chemical fertilizers are widely used to improve crop production, but their long-term application may adversely affect soil organisms. Suthar (2008) investigated the effects of synthetic fertilizers on earthworm growth and survival. The study revealed that high concentrations of nitrogenous fertilizers caused reduction in body weight, decreased burrowing activity, and increased mortality in earthworms. The author suggested that fertilizer-induced changes in soil pH and salinity create stressful conditions for earthworms.

Tripathi and Bhardwaj (2004) conducted comparative studies on earthworm biomass production under different organic and inorganic soil treatments. Their findings showed that earthworms exhibited better growth and survival in organically enriched soils compared to chemically treated soils. The study concluded that excessive use of chemical fertilizers can negatively affect earthworm metabolism and reproductive capacity.

Insecticides are considered more toxic than fertilizers because they are specifically designed to kill living organisms. Organophosphate insecticides such as chlorpyrifos and malathion are extensively used

in agriculture and have been reported to exert severe toxic effects on non-target soil fauna. Gupta *et al.* (2007) evaluated the impact of chlorpyrifos on earthworm survival and reproduction. The study observed significant reductions in cocoon production, hatchability, and survival rate following insecticide exposure. The authors suggested that chlorpyrifos interferes with nervous system functioning by inhibiting acetylcholinesterase activity. Yasmin and D'Souza (2010) studied behavioral responses of earthworms exposed to pesticides and reported several abnormalities including coiling, mucus secretion, sluggish movement, and surface migration. According to the authors, behavioral changes serve as early indicators of soil contamination and environmental stress. Earthworms exposed to toxic chemicals showed reduced feeding and burrowing activities, which may ultimately affect soil fertility and nutrient cycling. Reinecke and Reinecke (2007) investigated the toxicological effects of organophosphate pesticides on earthworms and reported oxidative stress, histological damage, and enzymatic alterations in exposed worms. The study revealed that pesticide exposure caused degeneration of epithelial tissues and impairment of antioxidant defense systems. Increased lipid peroxidation and reduced antioxidant enzyme activity indicated severe cellular damage due to oxidative stress. Pelosi *et al.* (2014) reviewed the ecological effects of pesticides on earthworms and concluded that pesticide exposure significantly reduces earthworm diversity and abundance in agricultural soils. Their review highlighted that pesticides affect earthworm physiology, behavior, reproduction, and immune responses. The authors emphasized that chronic exposure to low concentrations of pesticides may produce long-term ecological consequences. Booth and O'Halloran (2001) evaluated the toxicity of several pesticides to earthworms under laboratory conditions. Their study demonstrated that earthworms exposed to pesticide-contaminated soil exhibited reduced growth, decreased cocoon production, and increased mortality. The researchers suggested that earthworm toxicity testing is essential for environmental risk assessment of agrochemicals. Neuhauser *et al.* (1985) studied the sublethal effects of pesticides on earthworm behavior and reproduction. The authors observed that exposure to pesticides reduced cocoon production and juvenile emergence. Earthworms also showed reduced mobility and altered burrowing patterns, indicating physiological stress caused by toxic exposure. Luo *et al.* (1999) investigated the effects of heavy fertilizer application on soil fauna and reported substantial declines in earthworm density in intensively cultivated agricultural fields. The study concluded that excessive fertilizer use alters soil microbial communities and organic matter availability, thereby negatively affecting earthworm growth and survival. Tomlin (2009) described chlorpyrifos as a broad-spectrum organophosphate insecticide widely used in agriculture for pest control. According to the author, chlorpyrifos toxicity results from inhibition of acetylcholinesterase enzyme activity, leading to disruption of nerve impulse transmission. Although effective against pests, chlorpyrifos may persist in soil and pose serious risks to beneficial soil organisms such as earthworms. Maboeta and Van Rensburg (2003) evaluated the effects of different pesticides on earthworm avoidance behavior. Their findings showed that earthworms actively avoided contaminated soil, suggesting that avoidance tests can be used as sensitive indicators of soil pollution. Avoidance behavior reduces earthworm exposure to toxic substances but may also affect soil mixing and nutrient distribution. Several studies have also focused on oxidative stress responses in earthworms exposed to agrochemicals. Saint-Denis *et al.* (2001) reported increased lipid peroxidation and reduced antioxidant enzyme activity in pesticide-exposed earthworms. Oxidative stress results from excessive production of reactive oxygen species (ROS), which damage proteins, lipids, and cellular membranes. Apart from physiological effects, agrochemicals may also influence earthworm reproduction and population dynamics. Paoletti (1999) reported that long-term pesticide exposure reduced earthworm population density in agricultural fields. Reduced earthworm abundance adversely affected soil structure, decomposition rates, and crop productivity.

Studies conducted by OECD (2004) established standard protocols for evaluating acute and chronic toxicity of chemicals in earthworms. Earthworm toxicity tests are now widely used in environmental monitoring and pesticide safety assessment because they provide valuable information regarding soil contamination and ecological risks.

Research findings collectively indicate that excessive use of chemical fertilizers and insecticides negatively affects earthworm physiology, behavior, survival, and reproduction. Agrochemicals alter soil properties and induce toxic effects through oxidative stress, enzymatic inhibition, and tissue degeneration. Decline in earthworm populations may adversely affect soil fertility, nutrient cycling, and ecosystem stability.

Therefore, sustainable agricultural practices such as integrated pest management, organic farming, and judicious use of agrochemicals are essential to minimize environmental pollution and protect beneficial soil organisms. Further research is required to evaluate long-term ecological effects of agrochemical mixtures under field conditions.

Materials and Methods

Study Area- The present study was conducted in the laboratory of the Department of Zoology, N.R.E.C. College, Khurja, Bulandshahr, Uttar Pradesh, India. The experiment was carried out under controlled laboratory conditions to assess the effects of chemical fertilizers and insecticides on earthworm species. The laboratory temperature during the experiment ranged between 24°C and 28°C with relative humidity of approximately 65–75%.

Collection and Identification of Earthworms- Healthy adult earthworms were collected manually from moist agricultural fields and compost-rich soils in Bulandshahr district, Uttar Pradesh. Earthworms were selected based on uniform size, active movement, and healthy appearance. Collected earthworms were washed carefully with distilled water to remove adhering soil particles and debris.

The worms were identified morphologically using standard taxonomic keys. Earthworms belonging to the genus *Eisenia* and *Pheretima* were mainly used in the experiment because of their ecological importance and sensitivity toward soil contaminants.

Acclimatization of Earthworms- The collected earthworms were acclimatized for seven days prior to experimentation. During acclimatization, earthworms were maintained in plastic containers containing uncontaminated moist soil and decomposed organic matter. Moisture content was maintained regularly by sprinkling distilled water. No chemical treatment was applied during this period.

Experimental Soil Preparation- Top soil used for the experiment was collected from pesticide-free agricultural land. The soil was air-dried, sieved through a 2 mm mesh sieve, and homogenized to remove stones and plant residues. Physicochemical properties of the soil were approximately as follows:

Parameter	Value
Soil texture	Loamy
pH	6.8–7.2
Moisture content	25–30%
Organic carbon	Moderate

The prepared soil was distributed equally into experimental containers.

Chemicals Used

Fertilizer- Urea fertilizer was used as the representative chemical fertilizer because of its extensive agricultural application. The fertilizer was procured from a local agricultural supplier.

Insecticide- Chlorpyrifos (20% EC), a commonly used organophosphate insecticide, was selected for the study due to its widespread use in agricultural pest management.

Experimental Design- The experiment consisted of four groups with three replicates each:

Group	Treatment
Group I	Control (untreated soil)
Group II	Urea fertilizer treated soil
Group III	Chlorpyrifos insecticide treated soil

Group IV	Combined urea + chlorpyrifos treated soil
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Each experimental container contained 1 kg of soil and 10 healthy earthworms.

Preparation of Treatment Concentrations- The selected concentrations of chemicals were prepared based on commonly used agricultural doses.

- Urea fertilizer was mixed thoroughly in soil at a concentration of 2 g/kg soil.
- Chlorpyrifos was applied at a concentration of 0.5 ml/kg soil.

In the combined treatment group, both chemicals were added simultaneously at the above-mentioned concentrations.

The chemicals were uniformly mixed into the soil before introducing the earthworms.

Experimental Procedure- Earthworms were introduced carefully into each treatment container after soil preparation. The containers were covered with perforated lids to ensure proper aeration while preventing worm escape. Soil moisture was maintained throughout the experimental period by periodic addition of distilled water.

The experiment was conducted for 30 days, and observations were recorded on Day 0, Day 7, Day 15, and Day 30.

Parameters Studied-

Survival Rate and Mortality- The number of surviving earthworms in each group was counted at regular intervals. Mortality percentage was calculated using the following formula:

$$\text{Mortality Percentage} = \frac{\text{Number of Dead Earthworms}}{\text{Total Number of Earthworms}} \times 100$$

Dead earthworms were identified by absence of movement and body discoloration.

Body Weight Measurement- Body weight of earthworms was measured before and after exposure using a digital electronic balance. Weight changes were recorded in grams (g).

Behavioral Observations- Behavioral changes observed during the experiment included:

- Coiling behavior
- Sluggish movement
- Excessive mucus secretion
- Surface crawling
- Reduced feeding activity
- Reduced burrowing activity

Observations were recorded visually at regular intervals.

3.9.4 Burrowing Activity- Burrowing activity was assessed by observing the depth and number of burrows formed in the soil. Reduction in burrowing activity was considered an indicator of physiological stress.

Morphological Changes- Morphological abnormalities such as body swelling, discoloration, segmentation damage, and skin lesions were observed and recorded.

Statistical Analysis- Experimental data were expressed as Mean \pm Standard Deviation (Mean \pm SD). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test to determine significant differences among groups.

A probability value of $p < 0.05$ was considered statistically significant.

Ethical Considerations- The experiment was conducted following standard laboratory safety and environmental guidelines. Minimum numbers of organisms were used to reduce unnecessary biological stress. Proper disposal of chemically contaminated soil was carried out after completion of the experiment to avoid environmental contamination.

Results

Effect of Chemical Fertilizers and Insecticides on Body Weight of Earthworms- Body weight of earthworms showed a gradual decline in all treated groups compared to the control group throughout the experimental period. The reduction was more pronounced in chlorpyrifos-treated and combined treatment groups. Control earthworms maintained normal growth and body activity during the study period.

Table 1. Changes in Body Weight (g) of Earthworms During Experimental Exposure

Groups	Day 0	Day 7	Day 15	Day 30
Control	1.52 \pm 0.04	1.54 \pm 0.03	1.55 \pm 0.05	1.57 \pm 0.04
Urea Treated	1.51 \pm 0.05	1.42 \pm 0.04	1.34 \pm 0.05	1.21 \pm 0.06
Chlorpyrifos Treated	1.50 \pm 0.03	1.31 \pm 0.06	1.14 \pm 0.05	0.92 \pm 0.07
Combined Treatment	1.49 \pm 0.04	1.26 \pm 0.05	1.05 \pm 0.06	0.81 \pm 0.08

Earthworms exposed to urea fertilizer and chlorpyrifos insecticide exhibited significant reduction in body weight compared to the control group. The maximum decline was observed in the combined treatment group followed by the chlorpyrifos-treated group. Continuous exposure to agrochemicals likely interfered with feeding activity, nutrient absorption, and metabolic processes, leading to weight loss.

Effect on Survival and Mortality Rate- Mortality rate increased progressively with duration of exposure in all treated groups. No mortality was observed in the control group during the experimental period.

Table 2. Mortality Percentage of Earthworms During Exposure

Groups	Day 7	Day 15	Day 30
Control	0%	0%	0%
Urea Treated	10%	20%	30%
Chlorpyrifos Treated	20%	40%	60%
Combined Treatment	30%	50%	70%

Mortality increased significantly in treated groups with increasing exposure duration. Chlorpyrifos caused greater mortality than urea fertilizer, indicating higher toxicity of insecticides toward earthworms. The combined treatment group exhibited the highest mortality percentage, suggesting synergistic toxic effects of fertilizer and insecticide exposure.

Behavioral Changes Observed in Earthworms- Earthworms exposed to agrochemicals showed several behavioral abnormalities compared to control worms.

Table 3. Behavioral Alterations in Earthworms Following Agrochemical Exposure

Behavioral Parameter	Control	Urea Treated	Chlorpyrifos Treated	Combined Treatment
Normal Movement	+++	++	+	+
Coiling Behavior	-	+	++	+++
Mucus Secretion	-	+	++	+++
Sluggish Movement	-	++	+++	+++
Surface Crawling	-	+	++	+++
Burrowing Activity	+++	++	+	+

Symbols:+++ = High++ = Moderate= Low- = Absent

Behavioral abnormalities such as coiling, excessive mucus secretion, sluggish movement, and surface crawling were more prominent in insecticide-treated and combined treatment groups. Reduced burrowing activity indicated physiological stress and neurotoxic effects of agrochemical exposure. Control worms exhibited normal movement and burrowing behavior throughout the experiment.

Effect on Burrowing Activity- Burrowing activity decreased progressively in treated groups during the study period. Table 4. Average Burrowing Depth (cm) of Earthworms

Groups	Day 7	Day 15	Day 30
Control	8.5 \pm 0.4	8.8 \pm 0.5	9.0 \pm 0.3
Urea Treated	7.1 \pm 0.5	6.4 \pm 0.4	5.8 \pm 0.5
Chlorpyrifos Treated	5.9 \pm 0.4	4.8 \pm 0.6	3.9 \pm 0.4
Combined Treatment	5.1 \pm 0.5	4.1 \pm 0.5	3.2 \pm 0.6

Earthworms in the control group maintained normal burrowing activity, while treated groups showed significant reduction in burrow depth. Chlorpyrifos and combined treatments severely affected the ability of earthworms to penetrate soil. Reduced burrowing activity reflects stress-induced impairment of muscular coordination and locomotion.

Morphological Alterations- Earthworms exposed to agrochemicals exhibited visible morphological abnormalities.

Table 5. Morphological Changes in Earthworms

Morphological Changes	Urea Treated	Chlorpyrifos Treated	Combined Treatment
Body Swelling	Mild	Moderate	Severe
Skin Discoloration	Mild	Moderate	Severe
Segment Damage	Absent	Mild	Moderate
Excessive Mucus	Moderate	Severe	Severe
Body Fragility	Mild	Moderate	Severe

Morphological abnormalities were more severe in chlorpyrifos and combined treatment groups. Body discoloration, mucus secretion, swelling, and tissue fragility indicate toxic stress and possible tissue degeneration due to prolonged agrochemical exposure. Overall

Comparative Toxicity- Among all treatments, chlorpyrifos insecticide exhibited greater toxicity than urea fertilizer. Combined exposure produced the most severe toxic effects on survival, body weight, behavior, and burrowing activity.

Combined Treatment > Chlorpyrifos > Urea Fertilizer > Control

The results clearly demonstrate that insecticides are more toxic to earthworms than fertilizers. Simultaneous exposure to both chemicals caused synergistic toxicity and greater physiological stress. The findings suggest that excessive use of agrochemicals may adversely affect earthworm populations and soil ecosystem stability.

Discussion

The present study demonstrated that chemical fertilizers and insecticides exert significant toxic effects on earthworm species. Earthworms exposed to agrochemicals showed reduced body weight, altered behavior, decreased burrowing activity, morphological abnormalities, and increased mortality compared to the control group. These findings indicate that excessive use of agrochemicals can negatively affect beneficial soil organisms and disturb soil ecosystem functioning. Earthworms are highly sensitive to soil pollutants because of their direct contact with soil and continuous ingestion of organic matter and soil particles. In the present investigation, body weight of earthworms decreased progressively in all treated groups, with maximum reduction observed in chlorpyrifos and combined treatment groups. Loss of body weight may result from reduced feeding activity, metabolic disturbances, and physiological stress induced by toxic chemicals. Similar findings were reported by Suthar (2008), who observed that exposure to chemical fertilizers and pesticides significantly reduced biomass and growth of earthworms. Weight reduction in earthworms may also be associated with impaired digestion and nutrient assimilation due to toxic stress. Behavioral responses are considered important indicators of environmental toxicity in soil organisms. Earthworms exposed to agrochemicals in the present study exhibited coiling behavior, sluggish movement, excessive mucus secretion, and surface crawling. These abnormalities were more severe in chlorpyrifos-treated worms. Similar observations were reported by Yasmin and D'Souza (2010), who suggested that behavioral alterations serve as early warning signs of soil contamination. Organophosphate insecticides such as chlorpyrifos inhibit acetylcholinesterase activity, leading to accumulation of acetylcholine at nerve synapses and disruption of neuromuscular coordination. As a result, exposed earthworms show impaired locomotion and abnormal muscular contractions. Burrowing activity was significantly reduced in treated groups during the experimental period. Earthworm burrowing plays an important ecological role in improving soil aeration, nutrient mixing, and water infiltration. Reduced burrowing activity therefore reflects impaired physiological functioning and environmental stress. Similar findings were reported by Pelosi *et al.* (2014), who concluded that pesticide exposure adversely affects earthworm mobility and soil engineering activities. Reduced burrowing may also affect microbial interactions and decrease soil fertility over time. Mortality increased progressively with exposure duration in all treatment groups. Chlorpyrifos-treated earthworms showed higher mortality compared to urea-treated worms, indicating greater toxicity of insecticides than fertilizers. The combined treatment group exhibited maximum mortality, suggesting synergistic toxic effects of simultaneous

agrochemical exposure. Similar results were observed by Gupta *et al.* (2007), who reported significant mortality and reproductive failure in earthworms exposed to chlorpyrifos-contaminated soil. Insecticides may induce mortality through neurotoxicity, oxidative stress, and disruption of cellular metabolism. Morphological abnormalities such as body swelling, discoloration, excessive mucus secretion, and tissue fragility were also observed in treated earthworms. These changes indicate tissue damage and physiological deterioration caused by prolonged chemical exposure. Reinecke and Reinecke (2007) similarly reported histological degeneration and oxidative stress in pesticide-exposed earthworms. Toxic chemicals may damage epithelial tissues and alter membrane permeability, resulting in increased mucus secretion and structural abnormalities. Chemical fertilizers may indirectly affect earthworms by altering soil physicochemical properties. Excessive application of nitrogenous fertilizers such as urea can increase soil acidity and ammonia concentration, creating unfavorable conditions for earthworm survival. Fertilizer-induced changes in soil microbial communities may also reduce food availability for earthworms. Luo *et al.* (1999) reported significant decline in earthworm density in intensively fertilized agricultural soils due to altered soil conditions and nutrient imbalance. The combined treatment group in the present study showed more severe toxic effects than individual chemical treatments. Combined exposure to fertilizers and insecticides may increase physiological stress by affecting multiple metabolic pathways simultaneously. Agrochemical mixtures can disrupt antioxidant defense systems and induce oxidative damage in tissues. Saint-Denis *et al.* (2001) reported increased lipid peroxidation and reduced antioxidant enzyme activity in earthworms exposed to contaminated soils, indicating oxidative stress-mediated toxicity. Earthworms play a critical role in decomposition and nutrient recycling processes. Reduction in earthworm population due to agrochemical toxicity may therefore adversely affect soil fertility and agricultural sustainability. Earthworm casts are rich in nutrients and beneficial microorganisms that improve soil productivity. Decline in earthworm activity may reduce organic matter decomposition and negatively influence crop growth. The present findings are ecologically significant because modern agricultural practices often involve repeated and combined use of fertilizers and pesticides. Continuous accumulation of agrochemical residues in soil may pose long-term risks to beneficial soil organisms and biodiversity. The results suggest that indiscriminate use of agrochemicals can disturb soil ecological balance and impair ecosystem services provided by earthworms.

Therefore, environmentally sustainable agricultural practices such as integrated pest management (IPM), organic farming, biofertilizers, and biopesticides should be promoted to minimize adverse effects on soil fauna. Judicious application of agrochemicals at recommended doses is necessary to reduce environmental contamination and protect beneficial organisms. Further studies involving biochemical, histological, and molecular analyses are recommended to understand detailed mechanisms of agrochemical toxicity in earthworms under field conditions.

Conclusion

The present study clearly demonstrated that chemical fertilizers and insecticides have harmful effects on earthworm species. Earthworms exposed to agrochemicals showed significant reductions in body weight, burrowing activity, and survival rate, along with several behavioral and morphological abnormalities. Among the tested chemicals, chlorpyrifos insecticide exhibited greater toxicity than urea fertilizer, while combined exposure to both chemicals produced the most severe toxic effects. Behavioral alterations such as coiling, sluggish movement, excessive mucus secretion, and reduced burrowing activity indicated physiological stress and neurotoxic effects in treated earthworms. Increased mortality observed in insecticide-treated groups suggests that excessive use of pesticides can severely affect beneficial soil organisms. Morphological changes including body swelling, discoloration, and tissue fragility further confirmed the toxic impact of agrochemicals on earthworm health. Earthworms play a crucial role in maintaining soil fertility, nutrient recycling, aeration, and

decomposition of organic matter. Therefore, decline in earthworm populations due to agrochemical exposure may negatively affect soil productivity and ecological balance. Continuous and indiscriminate use of chemical fertilizers and insecticides may lead to long-term deterioration of soil biodiversity and environmental quality. The findings of the present study emphasize the importance of adopting environmentally sustainable agricultural practices. Judicious use of fertilizers and pesticides, along with promotion of organic farming, biofertilizers, and integrated pest management strategies, may help reduce ecological risks and conserve beneficial soil fauna. Protection of earthworm populations is essential for maintaining healthy and productive soil ecosystems. Further investigations involving biochemical, histopathological, and molecular studies are recommended to better understand the detailed mechanisms of agrochemical toxicity and their long-term ecological impacts under natural field conditions.

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