



Evaluating The Toxic Effects Of 2,4-D Herbicide on *Eutyphoeus waltoni* Michaelsen Earthworms

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ABSTRACT

*This research examines the biotoxicological effects of the herbicide 2,4-D in *Eutyphoeus waltoni*, with special emphasis on the soil and feed materials effects on worm survival. Thus, an experiment was designed to conduct a range of concentrations (LC10, LC50, LC90) for a period of 48 hours and also 72 hours using several types of soil; namely BD + W, silt BD + Gb, BD + Ws + Gb, loamy, clay and sandy soils. It was found that the longer the exposure to the herbicide, 2,4-D the more toxic it becomes, and the highest LC50 being recorded also in the BD+W+Gb soil mix, inferring that earthworms in that medium are more vulnerable. Nevertheless, sand frosted earth was found to have the least vulnerability level, probably because it retained the least amount of the herbicide. These results venture beyond the rationale of efficacy of herbicides in that they highlight the need for localized studies for each use of a herbicide since the composition of the soil determines toxicity and effects on the critical organisms in the soil that help sustain the balance of the ecosystem.*

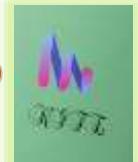
Keywords: 2,4-D Herbicide, *Eutyphoeus waltoni*, Michaelsen Earthworms, Earthworm Toxicity, Herbicide Exposure Duration, Soil Composition Impact, Site-specific herbicide application, Soil health and ecosystem.

1. INTRODUCTION

In developed areas, herbicides comprise more than 70% of all the pesticides found in the market. Between the years 1960-1985, the use of herbicides grew on average 16.7% gross annually worldwide. The largest consumer of herbicides is the United States. In Asia-Pacific region, the proportion of herbicide consumption is only 13.4%. It is expected that herbicides market will still grow at 4.5% on year basis. This is the highest growth rate so far for usable herbicides in the world. By 2002, the share of herbicides in the overall pesticide consumption in Europe was about 35% (ECPA 2003). In 2009, Phillips McDougall conducted a survey among key agrochemical players within the globe with the intention of capturing how long it takes and how much it costs to invent, formulate and get a regulatory approval for a new simple chemical-based crop protection product. Total expenses for R&D in 2007 according to these 14 leading crop protection companies exceeded 2328 million dollars, or 6.7% from their crop protection revenues. These companies also communicated how much money they expect to spend in discovery, development and regulatory approval of a new agrochemical product in 2012. In general, it was expected to be a growth of expenditures on that five-year period between them of 26.4% – 4.8 % average rate so increase each year. On the other hand, the global adoption of conservation tillage (CT) has also increased considerably in the past few decades. In contrast, the no-till systems, as well as the reduced tillage systems, may also present problems from weeds. With this tillage system, changes the pattern of weed seed banks within the uppermost layers of soil, as well as the timing on which weeds are expected to grow - emerge. Weeds especially grasses and perennial weeds may also pose a challenge looking at the no tillage systems as well as the reduced ones. There has been a growing appreciation towards the role of earthworms in the soil ecosystem prompting the need to encourage stewardship from farmers, researchers, and the scientific community.

2. LITERATURE REVIEW

Triques et al. (2021) analyzed the effects of sugarcane pesticides fipronil and 2,4-D on plants and soil organisms separately and discussed the adverse environmental consequences of agriculture. The findings indicate that the use of both pesticides is detrimental to soil health since it affects organisms like earthworms, which are essential for soil healthy functioning, and microbes found in soils and soil pores. Likewise, the research shows that the application of these pesticides can also induce toxicity to the plants where in caused adverse effects are



retardation of growth through inhibition of germination and biomass enhancement which distressed the yield of the crops. The authors also present an argument on the importance of the potential risk of the negative impact of pesticides on the aquatic and terrestrial non-target organisms when performing ecotoxicological studies. This work serves to highlight the need to implement pest control measures that do not entirely depend on chemicals and which preserve the ecological balance and as such encourages future studies on the effects of using pesticide in pest management strategies.

Boughattas et al. (2022) focused on understanding how environmental microplastics as well as the herbicide 2,4-dichlorophenoxyacetic acid, or 2,4-D, interact in the soil in relation with some earthworm species, *Eisenia andrei*, which makes them concerned in so many ways of how different poison would work in soil ecosystems. Their investigation conclusions demonstrate that flowers and plants grown on soil contaminated with microplastics and 2,4-D showed interferences in the behavior and survival rates of earthworms, and got physiological responses which resulted in changes in their growth and proportion of the young ones they produced. The study also indicates that if microplastics are present in the soil, they enhance the toxicity of the herbicide 2,4-D at detailed levels, which together with soil organisms poses a greater risk synergistically than was previously appreciated. Further, the results of the study also highlight the need to consider more than one environmental stressor in evaluating their effects on soil and biodiversity. This study enriches knowledge on the necessity of urgent regulatory frameworks on the disposal of microplastics and their coexistence with pesticides in the environment, calling for incorporation of environmental risk assessment in agro ecosystems management.

De Santo et al. (2019) conducted a thorough risk assessment of the use of metsulfuron-methyl-based herbicides particularly to the soil fauna subjected to laboratory and field trials. It is highlighted in this study that herbicide toxicity tests should include in-field assessments, instead of relying on laboratory ones, which may be less representative of the soil environment. Their results suggest that metsulfuron-methyl is extremely dangerous to a range of soil organisms, compromising both their survival and reproduction. Given the adverse effects of herbicides on soil systems, this research emphasizes the need for ecotoxicological assessment including different environmental characteristics and organisms performing both in situ and ex situ evaluation. This paper asserts that herbicide policies as well as practices associated with their use need to take into account the ecosystem, so as to mitigate the impact of their use on soil organisms and functioning.

Santo (2018) aimed to determine the ecotoxicological properties of the herbicides metsulfuron-methyl and isoxaflutole with respect to their influence on soil-living organisms. This research emphasizes the substantial negative effects which these herbicides, especially on soil species such as earthworms and other invertebrates that contribute to soil health and its ecosystem, can have. In this regard, both laboratory experiments as well as field evaluations showed the relevant need to investigate the effects of herbicides on soil biodiversity beyond the immediate exposure period. The results of Santo suggest a toxic degree for both herbicides, which in turn would affect structuring and functioning of the soil community. Such outcomes add to augmenting calls on regulating the application of herbicides, along the lines of highlighting the agricultural features related to environmental risk issues in order to protect key organisms residing in soils.

3. RESEARCH METHODOLOGY

3.1. Research Design

The method employed in this study is experimental research which aims to investigate the effect of the herbicide 2,4-D on the earthworm *Eutwyphoeus waltoni*. The study examines the impact of different soil compositions and types of feed on the herbicide lethal concentrations after certain periods of exposure (48 hours and 72 hours). The experimental layout also incorporates controlled laboratory conditions in which the results are dependable and can be contrasted with various soil types without undue variations.



3.2. Data Collection

Data collection was done through controlled exposure experiments involving deliberately exposing earthworms to particular concentrations of 2, 4, D in particular types of soil along with different feeding materials. Several units were prepared for each soil type that included BD+Ws, BD+Gb, BD+Ws+Gb, loamy soil, clay soil, and sandy soil. The Lethal concentrations (LC10, LC50, LC90) were calculated as per the percentage mortalities of the earthworms after 48 and 72 hours after administration of 2,4-D. There were also intervals between observation periods so as not to miss out on how many earthworms remained alive or dead and their general wellbeing.

3.3. Research Area

The study was carried out in Osmania University, Hyderabad, in the state of Telangana. This site was chosen because of the different types of soils present and the availability of materials for carrying out ecological and toxicological studies. Laboratory facilities provided in the university enabled the control of some atmospheric conditions which are paramount in the accuracy of the results yielded from experiments.

3.4. Data Analysis

Data analysis included the determination of lethal concentration values (LC10, LC50, LC90) based on mortality data gathered throughout the respective exposure periods. The LC50 data was analyzed to study the level of toxicity of the herbicide 2,4-D in different soils and food substrates while also drawing attention to the differential vulnerability of earthworms. Statistical procedures were applied to assess the degree of significance of the toxic concentrations assessed at 48 and 72 hours post treatment, which helped in understanding the effects of chronic exposure to the herbicide. The numerical values were also converted into tables and charts, which enabled easier comparison and understanding of the data on the effect of soil composition on the toxic effect of 2,4-D on earthworms.

4. DATA ANALYSIS

Table 1: Toxicity of herbicide 2,4-D to earthworm species *Eutwyphoeus waltoni* after 48 hr exposure to different soil types and feed materials. LC10, LC50, LC90 are lethal concentrations indicating a range of toxicity levels. LC50 is consistently recorded as 450 mg/kg for most soil types, suggesting that at this concentration, it poses a moderate risk to the earthworms. The LC90 values range from 1,100 mg/kg to 1,500 mg/kg, which is an indication of the maximum concentration at which 90% of the earthworm population may be affected. This soil combination, BD+Ws+Gb, gives the highest LC50 value at 550 mg/kg and has the lowest LC90 at 1,100 mg/kg, meaning that the earthworms are highly susceptible to 2,4-D exposure in that particular feed material. This data underlines the importance of careful herbicide application management in soils of different types to prevent harmful effects on soil-inhabiting organisms, like earthworms, that are crucial for soil health and ecosystem functioning.

Table 1. The toxicity of 48 h exposure of herbicide 2,4-D against earthworm *Eutwyphoeus waltoni*

Soil Type/Feed Material	LC10 (mg/kg)	LC50 (mg/kg)	LC90 (mg/kg)
BD+Ws	250	450	1500
BD+Gb	250	450	1300
BD+Ws+Gb	250	550	1100
Loamy Soil	250	450	1400
Clay Soil	250	450	1500
Sandy Soil	250	450	1100

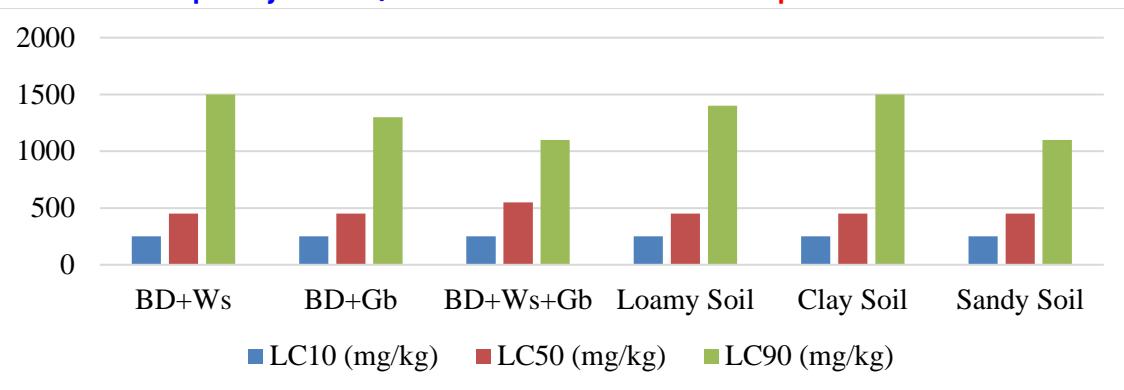
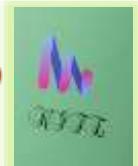


Figure 1: Graphical Representation of the toxicity of 48 h exposure of herbicide 2,4-D against earthworm *Eutypheus waltoni*

Table 2 depicts the LC10, LC50, and LC90 values for herbicide 2,4-D on the earthworm species *Eutypheus waltoni* after an exposure duration of 72 hours, from which it is evident that the lethal concentrations are not uniform across the board since the soil types and feed materials are different. The LC50 values indicate that the median lethal dose for most soil types ranges from 390 mg/kg to 520 mg/kg, which is a minor increase in toxicity considering the exposure period of 48 hours. Interestingly, the BD + Ws + Gb mixture has the highest LC50 at 520 mg/kg, meaning earthworms become more sensitive to this feed material. The LC90 is between 950 mg/kg and 1,400 mg/kg. Sandy soil recorded the highest tolerance at 1,400 mg/kg, showing that earthworms in sandy soil are not so affected by higher concentrations of the herbicide. The version points out that, upon applying 2,4-D, site-specific soil analysis is crucial, owing to the fact that differences in soil composition influence variations in the herbicide and affect earthworms living under its influence, as those little insects play a vital role for maintaining soil health and harmony.

Table 2. The toxicity of 72 h exposure of herbicide 2,4-D against earthworm *Eutypheus waltoni*

Soil Type/Feed Material	LC10 (mg/kg)	LC50 (mg/kg)	LC90 (mg/kg)
BD + Ws	180	390	950
BD + Gb	210	410	1150
BD + Ws + Gb	190	520	1250
Loamy soil	220	380	1300
Clay soil	205	420	1100
Sandy soil	195	430	1400

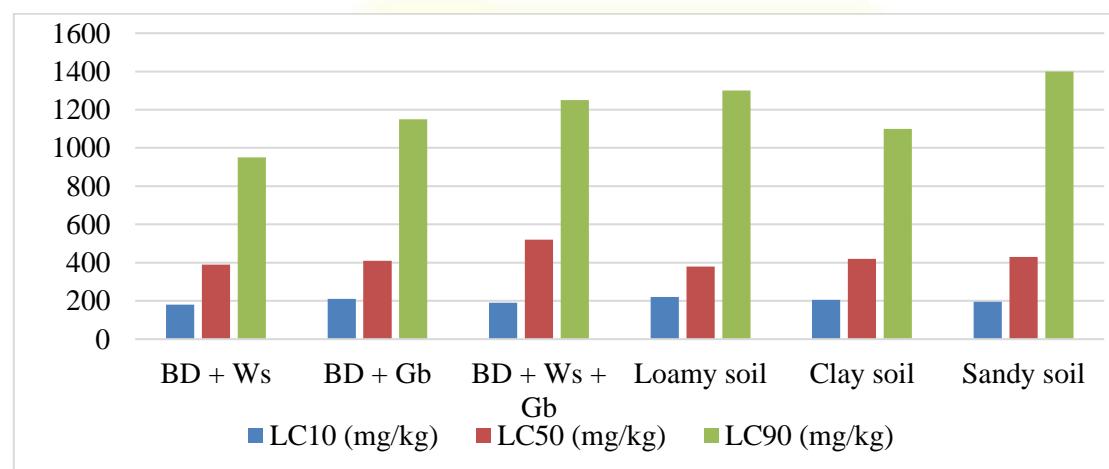


Figure 2: Graphical representation of the toxicity of 72 h exposure of herbicide 2,4-D against earthworm *Eutypheus waltoni*



5. CONCLUSION

The study shows that 2,4-D herbicide exerts different levels of toxicity on *Eutyphoeus waltoni* earthworms, whose effects depend on the type of soil and feed materials. Increased exposure was reflected in the increased toxicity values as assessed from 48 hours to 72 hours. The LC50 values suggest moderate risk to earthworms under standard exposure with marked susceptibility in some composition of soil, especially BD+Ws+Gb combination. The highest toxicity levels were indicated here in this soil/feed mixture at 72 hours after exposure, which shows some specific properties of soil to enhance the effect of herbicide on earthworms. Tolerance levels were higher for sandy soil; that is, retention or bioavailability may have been minimized, thus slowing the earthworm's absorption of these herbicides. These findings call for a specific and site-specific approach to the use of herbicides as demanded by the local characteristics. This would reduce the potential ecological disruption in key organisms in soil, and, consequently, promote soil health and stability in the ecosystem.

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