OPTIMIZATION OF TOXICOLOGY TESTING USING EARTHWORMS

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DECLARATION

I declare that this project is my original work and has not been submitted in any institution for award of degree. Any information gotten from other sources has been dully cited.

Signature……………………………… ...   Date ……………………

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Supervisor's approval

I confirm that this research project has been conducted and submitted with my approval as the student supervisor.

Signature……………………………… ...   Date ……………………

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DEDICATION

I dedicate this project to my loving parents and siblings, thank you for being there for me throughout this period. Your unending love gave me the zeal to press on to the end

ACKNOWLEDGEMENT

I wish to thank God for guiding me through the whole period, giving me strength, wisdom and provision to see this project to completion. I am forever grateful.

My deepest gratitude goes to my family. I thank you for your support, understanding and love through my study period, may God bless you abundantly.

Am truly grateful to my supervisor Dr. Michael Mungoma you were more than a lecturer and through your commitment and guidance I was able to complete this project in time. I wouldn't forget laboratory technician Elias Mandela who helped me in my laboratory studies may God bless you.

Finally, I am truly grateful to my friends for all your moral support may God bless you all.

ABSTRACT

Humans and other organisms are continually exposed to toxic substances. These may be found in the environment or introduced into our bodies through the food chain. Pesticides are important in controlling unwanted pests in the gardens or as part as large-scale farming. Handling of these chemicals can result in negative health effects. Tests to determine toxicity of chemicals have been documented however domestication of these tests can improve access to reliable results and can explain occurrences of diseases such as cancer.

The study aimed at optimizing a standard toxicity testing protocol by the Organization for Economic Cooperation and Development (OECD), establish the effectiveness of using local worms for toxicity testing and study the growth of earthworms. The study also intended to provide vital information about the toxicity profile of selected pesticides in the market using locally available organisms to carry out standard toxicity tests. The filter paper contact test was adopted in this study and two commercially available pesticides; acetamiprid (200g/kg) and imidacloprid (700g/kg).

The results from the study revealed that the two pesticides were toxic to the locally available earthworms. The exposure to the pesticide resulted into morphological abnormalities such as swelling and color changes after the 48 hours with death of the earthworms in at a higher concentration of the two pesticides being observed after 72 hours. Also, the study revealed that earthworms can be used in testing the toxic profile of the pesticides commonly used. Also, the results revealed that earthworms can be maintained on locally available fruit and potato peels.

In conclusion, it’s evident that pesticides have toxic effects on soil organisms such as earthworms and earthworms are as well suitable specimens for testing the toxicity of pesticides.

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ABBREVIATIONS

OECD Organization for Economic Cooperation and Development

CHAPTER ONE: INTRODUCTION

1.1 Background information

Pesticides have been widely used in agriculture for quite some time with the main purpose being crop protection hence higher yields. Additionally pesticides are used in both households and industries to control pests and diseases whose vector are insects and/or fungi (Hakeem et al., 2016). Many of these pesticides are usually harmful and cause serve effects to humans, non-target living organism and environment at large. The continuous application of these pesticides results into increased concentration of either common or persistent pesticides which are regarded as hazardous contaminants in soils, surface water, ground water and sediments. Pesticides used in the agricultural fields are supposed to be less or not even toxic to the non-target organisms, easily biodegradable and environmental friendly to some extent. However, this is not the case with the majority of pesticide applied in the crop fields and farms, households and industries. The reverse is usually the case, many pesticides kill or interfere with the non-target organisms that are harmless and at the same time very useful in the various levels of the ecosystems. These pesticides applied in the fields and farms get to the non-target organisms via various means such as water, wind and soil particles.

Soil borne pathogens, nematodes and weeds greatly contribute to reduced yields in protected and guided production of both vegetables and other high value added crops across the world (Huang et al., 2018). Farm chemicals that include soil fumigants such 1,3dichloropropene (1, 3-D), metam sodium (MS), and dazomet (DZ), have been used in management and control of these soil borne problems in many countries globally. Even though the outcomes have been good in terms of controlling some soil borne pathogens, the possible after use impacts and dangers have not been ruled out. The increased use of these chemicals has greatly affected the ecological indicators, more so the non-target organisms in both the applied fields and the adjacent fields to the one in which the pesticides have been applied (Mao et al., 2017). These chemical substances cause morphological, behavioral and physiological changes in reproductive, nervous, respiratory and osmoregulatory organs of many habitants of the soil. These soil habitants include the small invertebrates such as earthworms (Miglani & Bisht, 2020). Of all the various classes of pesticides insecticides have taken the lead in being most lethal toxic category of pesticides and pose a high risk to the non-target organisms (Hakeem et al., 2016).

Earthworms are small invertebrate organism that are known to thrive well in almost all the type of soil. These organisms are good indicator of soil health, toxicity that include soil pollutants and pesticides and are regarded as the supreme component of macro fauna (Miglani & Bisht, 2020). This is due to the fact that earth worms are liable to changes in the ecological factors, with much emphasis on those factors that are vital to the soil. Earthworms are responsible for both development and maintenance of the nutritive value of soil. This has made earthworms receive more attention as compared to the other invertebrates that are found in the soils. They represent an important part of soil biomass and are as well regarded as soil engineers. They are used as standard test organism for soil toxicity testing as well as an indicator in environmental pollution assessment due to heavy metals has been reported (Farrukh & Ali, 2015). Additionally, earthworms have been reported to accumulate high concentrations of pesticides, hence a very important indicator of soil healthy. Therefore, this study was designed to evaluate the optimization of the toxicology testing of pesticides available in the market using earthworms.

1.2 Problem statement and justification

Agriculture is the backbone of many countries all over the world. A well-established country with stable agriculture sector is able to sustain itself and its peoples. This sector is largely affected by pests, weeds and other pathogens that reduce the percentage of yields that are expected by farmers. To manage or control the effects of the pests and other pathogens, chemicals known as pesticides are used. Similarly, the expansion of agricultural activities and unguided use of pesticides has shown to have greatly affected the soil health status due to increased pollution and toxicity of the soil (Miglani & Bisht, 2020). These chemicals have shown to be effective to an extent that increased yields have been noticed. However, these chemicals on occasional use have shown to have adverse impacts on both humans, soil organisms and environment as well. These include the toxic effects on the non-target organisms such as the small invertebrates of their soil, aquatic organisms in water and other plants as well. These organisms are usually less harmful and very important in the various soil process that maintains the health status of soil. Therefore, there is need to have the bio indicators in soil that ate able to guide on the impact of the used pesticides on the soil.

Earthworms play a very critical role in safeguarding the soil ecosystem. Earthworms influence all the properties of soil that include, physical, chemical and biological properties. Physical properties such as hydraulic conductivity, porosity, bulk density, water infiltration ability and aggregate stability are maintained by earthworms. Nutrient availability in soil is done through the ability of the earthworms to ingest organic residues in different Carbon: Nitrogen ratios. All these excellent services done by earthworms to the ecosystem are at a high risk. Hence there is need of understanding the response of earthworms to various pesticide and different concentrations of these pesticides. This study, aimed at identifying the toxic effects of the selected pesticides using earthworms and identify the toxic dose of each pesticide.

1.3 Objectives

1.3.1 General objective

To optimize toxicity testing using earthworms

1.3.2 Specific objectives

1. To determine the effectiveness of earthworms (*Eisenia foetida*) in toxicity testing
2. To assess the toxicity effects of selected pesticides using earthworms (*Eisenia foetida*).
3. To investigate the growth and development of earthworms (*Eisenia foetida*).

1.4 Research questions

1. Do the selected pesticides have toxic effects on earthworms?
2. Are earthworms effective in toxicology testing?
3. How are earthworms maintained in the laboratory?

CHAPTER TWO: LITERATURE REVIEW

2.1 Classification of pesticides

Pesticides are grouped based on various aspects that include, the target organism, the toxicity, mode of action and the chemical composition. Based on the target organisms various groups of pesticides are identified; insecticides, fungicides, herbicides, rodenticides, nematicides, molluscicides and plant regulators (table 2.1). Each of this group of pesticide is designed to target specific organism or pest. However, this is not the case as the non-target organisms or soil habitants are affected by the undesired toxic effects of pesticides. Insecticides are the mostly known and commonly used group of pesticide. These insecticides are further grouped into different groups based on their chemical nature as per Insecticide Resistance Action Committee (IRAC) 2016 (Table 2.2).

Table 2. 1 Classes of pesticides.

NAME OF PESTICIDE	USE AND ACTION	EXAMPLE
Insecticides	A substance used to control or eliminate or to prevent the attack of the insects that destroys/kill/mitigate plant/ animal.	DDT, Methyl Parathion, Phorate, Chloropyrifos,
Herbicides	Substances which are used to control the noxious weed and other vegetation that is growing with the desired species causing poor plant growth.	Acetochlor, Butachlor, Terbis, Glyphosate, 2,4-D, and 2,4,5-T.
Fungicides	Substances used to destroy or inhibit the growth of fungi/diseases that infect plants/animal.	Carbendazim, Ampropylfos, Carboxin
Rodenticides	Chemicals used to kill rodents i.e. mice, rat etc.	Warfarin, Arsenous oxide
Nematicides	Substances used to repel or inhibit the nematodes damaging various crops.	Aldicarb, Carbofuran
Molluscicides	Substances used to inhibit the growth and kills snails and slugs and small black sans-culottes.	Gardene, Fentin, Copper sulfate

Plant growth regulators	A substance that causes the retardation or accelerates the rate of growth or rate of maturation.	Acibenzolar, Probenazole

Table 2. 2 classes of insecticides

Main group	Action	Example
Organophosphates	Inhibit AChE in nervous system of target organisms	Chloropyrifos, Dichlorovos, Triazophos, Profenofos, Parathion, Phorate, Diazinon
Organochlorides	Binds at GABA site Inhibit chloride flow in the nervous system of target organisms	Chlordane, Endosulfan
Carbamates	Inhibit AChE in nervous system of target organisms	Aldicarb, Carbaryl, Carbofuran, Isoprocarb
Pyrethroids	Acts on Nervous system which cause changes in nerve membrane permeability to sodium and potassium ions	Acrinathrin, Allethrin, Bioallethrin, Cycloprothrin, beta-Cyfluthrin
Neonicotinoids	Acts as an agonist of acetylcholine and is therefore effective on many insects	Acetamiprid, Clothianidin, Dinotefuran,

2.2 Toxicity and lethality of pesticides

The toxic effects of pesticides in biological systems is usually dose dependent. The toxicity of pesticide depends on two important concentrations; the lethal concentration and the lethal dose. The fifty percent lethal concentration (LC_{50}) which is the amount of pesticide dispersed in the air with milligrams per liter units, plays a vital role in the toxicity of pesticides. The lower the value of LC 50, the more lethal the pesticide is. On the other the fifty percent lethal dose LD_{50} is determined in controlled laboratory conditions where the specific dose is administered within a particular duration to estimate the toxicity of the pesticides to an organism. The fifty percent lethal dose is expressed in milligram per kilogram of body weight (mg/kg bw).

2.3 Pesticides toxicity on non-target organisms

The effects of pesticides on non-target organisms is a matter that is under debate by many researchers globally. Many report regarding the effects of pesticides on non-target organisms exist. The effects have been witnessed on both aquatic ecosystem, animal and plant diversity and terrestrial ecosystem as well (Tiwari et al., 2016). Estimates indicates that, of all the pesticides that is applied in the field only smaller percentage of 0.1% gets to the targeted point while the rest 99% have the potential to impact non-target organisms and percolate deep into the soil ecosystems such as water-table (Jayaraj et al., 2016). Most of the pesticide used, insecticides consumption is very high. The effects of these insecticides on non-target species are grouped as harmless (<50% Mortality), slightly harmful (50–79% mortality), moderately harmful (80–89% mortality) and harmful (>90% mortality) according to the field recommended dose. Additionally, insecticides also act as potential neuro-toxicants on the non-target species as it inhibits the essential enzyme known as acetylcholine (AChE) in the nervous system of insects and other organism. The insecticides have effects on other species in different degrees. For instance, biological controllers of insects such parasitoids and predators are prone to toxic effects of insecticides. On addition to the natural enemies, other soil arthropods are drastically affected by pesticide application. The high concentration residue of pesticides in the soils have as well be reported to affect many non-target organisms such as vital soil micro -fauna such as earthworms that are important pillar in the ecosystem.

2.4 Earthworms

Earthworms are small invertebrate organisms that are able to thrive almost in all soil types. They are best known for their soil health indication and toxicity that include various soil pollutants and pesticides. Based on their feeding habits earthworms, are categorized as detrivores and geophagous (Kumar Sinha et al., 2009). In addition, the geophagous group are further classified as polyhumic, oligohumic and mesohumic and all these groups are abundantly found in the tropical regions. Due to the susceptibility of earthworms to the pesticides such as insecticides, has placed these organisms to the forefront to their use as model organism to evaluate the effects of insecticides (Lehman et al., 2015). Various pesticides families such as neonicotinoids, strobilurins, sulfonylureas, triazoles, carbamates and organophosphates are considered harmful to earthworms.

Pesticides affects the mortality rate of earthworms in various ways such as distressing them or altering their physiology (Sabra et al., 2015).

Various earthworm species are used as models in evaluating the effects of pesticides. For example the *Eisenia Foetida* (Oligochaete) is proposed as the reference species for toxicity testing by the organization for economic cooperation and development (OECD) (OECD, 2016). This has been due to its easy of cultivation in the laboratory, faster maturity of only few weeks and high reproductive rate.

Pesticides have great impact negatively on the survival and reproduction of earthworms more so at higher concentrations greater than 25 mg/kg. The effects of pesticides on earthworms in soil as well depends upon many other factors such as the type of the contaminant and its concentration, characteristics of the soil (Cao et al., 2015).

2.5 Earthworm morphological groups and their exposure to pesticides

Four different ecological groups of earthworms exist with each ecological group being described based on its traits in the soil system that include their exposure to different types of pesticides (Pisa et al., 2014). The groups are "Epigeic", "Endogenic", "Anecic" and "compost" earthworms. The "epigeic" ecological group represented by worm species *Lumbricus rubellus*, *Dendrobaena octedra* and *Lumbricus castaneus* are usually found the upper soil layer of 10-15 cm and usually feed on the decaying organic matter that is present in litter. The earthworm species found in this ecological group are exposed to pesticides through the ingestion of the litter. On the other hand, "endogenic" worms which are represented by *Aporrectodea caliginosa*, *Allolobophora chlorotica* or *Allolobophora icterica* are bigger in size that range between 1-20 cm. these earthworms feed on organic matter that is incorporated and mixed with minerals in the soil that has already been exposure to pesticides. The "anecic" worms that include Lumricus terrestis, Aporrectodea longa are usually bigger and pigmented as well. They are characterized with strong muscles that illustrates the great burrowing activity with some species having giant size of between 0.1 to 1.1 m. the earthworms feed on the surface litter and this is mainly during the night. They as well create long sub-vertical burrows of between 1 to 6 m long. By doing so they ingest more soil that translates to more exposure to pesticides as a result of ingesting the pesticide contaminated soil. Lastly the "compost" ecological group of earthworms are mostly used in the vermicomposting practices. These earthworms are represented by species such as *Eisenia foetida* and *Dendrobaena veneta*. These compost worms are characterized by bright red color and strips hence the name "tiger worms". These earthworms are usually found in the controlled soil pits hence less exposed to soil contaminants and toxicants (Miglani & Bisht, 2020).

2.6 Effect of pesticides on earthworms

Pesticides have so many effects on earthworms as the majority of the organisms that are found in soil. Through various studies it has been illustrated how the pesticides interfere with the physiology of earth worms. The study investigating the effect of various pesticides and their sub-lethal effect on earthworms, demonstrated that the sub-lethal effects cause rupturing of cuticle, oozing out of coelomic fluid, swelling, paling of body and softening of body tissues (Zhao et al., 2017). Both higher and lower doses of pesticides result into physiological damage to earthworms. Similarly, other studies have shown the neurotoxicity of pesticides on earthworms. These include insecticides such as neonicotinoid imidacloprid, oxadiazine indoxacarb, pyrethroids alpha-cypermethrin and lambda-cyhalothrin and the combination of organophosphate chlorpyrifos and pyrethroid cypermethrin. All these pesticides in one way or the other primarily affects the nervous system of the earthworms (Miglani & Bisht, 2020).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study design

This was an experimental study involving earthworms. The study was entirely conducted in the entomology laboratory in Mount Kenya University.

3.2 Materials, apparatus and solvents

The apparatus and solvents used in this study included glass Petri dishes, falcon tubes (15 ml) forceps, distilled water, Eppendorf tubes, Whatman filter papers No. 1, analytical weighing balance and digital thermometer.

3.3 Study earthworm species

The earthworm of *Eisenia foetida* species were sourced from Jomo Kenyatta University of agriculture and technology (JKUAT) organic farm. They were transported to the entomology laboratory in Mount Kenya University in woven carrier bag under standard conditions. From this carrier bag they were transferred into a wide-open basin from where they were maintained.

3.1.1 Maintenance of the *Eisenia foetida*

The maintenance of these earthworm was done for a period of seven days. The temperature of the room was maintained at 19.0±2^{O}C. To ensure their continuity, free organic material that included fruit peelings and potatoes peelings were added by laying them under the earthworm holding container before transferring the earthworms in the same container. The earthworms were checked on three times a day, morning, noon and evening for all the seven days. In order to ensure that they were still alive, the ability of the earthworm to respond to the stimuli that included touching was assessed. The temperature of the particular laboratory was as well monitored thrice per day and noted.

3.3.2 Preparation of the *Eisenia foetida* earthworm species for the study

Adult complete earthworms were identified by locating the clitellum and the average weight of 300-400 mg was determined by weighing each and every earthworm selected. The 25 earthworms selected were then laid on clean filter papers moist with distilled water to allow then devoid the entire content of the gut for a period of one-hour. The gut devoid earthworms were then cleaned by washing them with clean and pesticide free distilled water and then kept on moist filter paper for another one-hour. After this they were rinsed, dried and they were now ready for use.

3.4 Test pesticide

Two commercially available pesticides one containing acetamiprid (200g/kg) and the other containing imidacloprid (700g/kg) were used. These chemical agents were purchased from the local agrochemical shop located in Thika town, Kiambu County and taken to the laboratory prior to the study day.

3.4.1 Preparation of the pesticides dilutions

The study pesticides; acetamiprid (200g/kg) and imidacloprid (700g/kg) were dissolved in distilled water to make the stock solutions. The imidacloprid present in the entire content of the pesticide (5g) was calculated and found to be 3.5g. To make a stock concentration of 0.001 mg/ml, exactly 2.9 g of the powder was weighed and dissolved in 10ml. This stock solution was serially diluted to obtain the other lower working concentrations of 1×10^{-3} and 1×10^{-11} mg/ml. The acetamiprid present in the powdered pesticide (20g) was 4 g. The stock solution of 0.001 mg/ml was prepared by dissolving 10 grams of powder in 10 ml of distilled water. This was serially diluted to obtain the other concentrations.

3.5 Evaluation of the toxicity of pesticides on earthworm.

3.5.1 Contact toxicity test

The toxic effect of the two selected pesticides on earthworms was conducted by the contact toxicity test as described in the OECD (1984) guidelines for testing of chemicals no. 207 (OECD, 1984). This test was carried by adapting the procedures of Jeyaprakasam et al.(2021) with minor modifications. Briefly, round glass petri-dishes with diameter of 14 cm and 2 cm height were used as the test containers. These petri-dishes were fitted with clean and sterile filter paper Whatman No.1 that had been resized prior to the study. The petri-dishes were then labeled per the respective pesticide and concentration levels. On to these Petri dishes exactly 1 ml of the respective concentration of the pesticide was added using 1ml micropipette. This solution was left to dry and then wetted by adding exactly 1 ml of sterile distilled water. Per each pesticide under test an extra Petri dishes was added and sprayed with only distilled water to serve as the control. In each plate a mature and complete earthworm of average weight 350 mg that had been washed with distilled water was carefully added and the timer set. All the plates were then kept in the dark inside the cabinets. The mortality and morphological changes on the earthworms was then monitored after a period of 24 hours, 48 hours and 72 hours then noted in the laboratory research note book.

3.6 Data management and analysis

All the experiments were conducted in four replicates. The data that consisted of the number of earthworms that died at particular dose level of the respective pesticide were tabulated in the laboratory note book. The data was then analyzed for descriptive statistics and then expressed as Mean ± Standard error of the mean. The results were then presented in form of tables and graphs.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1RESULTS

4.1.1 Growth and development of Earthworms (*Eisenia foetida*)

The growth and development of earthworms was monitored for a period of seven days.

Average temperature	19.49 ^{O}C
Food	Water melon, banana and potato peelings
Average weight	444.5 mg
Average length	7.767 cm

4.1.2 Contact toxicity test

The toxicity of acetamiprid (200g/kg) and imidacloprid (700g/kg) on earthworm, *Eisenia foetida* was done by the filter paper contact test. The results including the observations made after 24 hours. 48 hours and 48 hours are summarized in tables 4.1 and 4.2.

Serial number	Concentration of acetamiprid(mg/ml)	Changes observed after 24 hours	Changes observed after 48 hours	Changes after 72 hours
1	1×10^{-3}	Motility of the earthworms in the Petri dish had decrease but the earthworm still responded to physical stimulus that is touch	The earthworm in this dish had swellings in the middle and at the end. Still responded to physical stimulus	The swelling busted and the earth worm died

2	1×10^{-4}	Motility of the earthworms in the Petri dish had decrease but the earthworm still responded to physical stimulus that is touch	The earthworm in this dish had swellings in the middle and at the end. Still responded to physical stimulus The skin had turned pale yellow.	The swelling busted and the earth worm died
3	1×10^{-5}	Motility of the earthworms in the Petri dish had decrease but the earthworm still responded to physical stimulus that is touch	The earthworm had fewer swellings Still responded to physical stimulus The skin had turned pale yellow.	The swelling busted and the earth worm died
4	1×10^{-6}	Motility of the earthworms in the Petri dish had decrease but the earthworm still responded to physical stimulus that is touch	The earthworm had fewer swellings Still responded to physical stimulus The skin had turned pale yellow	The swelling busted and the earth worm died
5	1×10^{-7}	Motility of the earthworms in	The earthworm had swellings	The earthworm died

		the Petri dish had decrease but the earthworm still responded to physical stimulus that is touch	responded to physical stimulus and moved around the dish	
6	1×10^{-8}	Motility of the earthworms in the Petri dish had decrease but the earthworm still responded to physical stimulus that is touch	The earthworm had no swellings responded to physical stimulus and moved around the dish	The earthworm did not die and had no swellings
7	1×10^{-9}	Motility of the earthworms in the Petri dish had decrease but the earthworm still responded to physical stimulus that is touch	The earthworm had no swellings responded to physical stimulus and moved around the dish	The earthworm did not die and had no swellings
8	1×10^{-10}	Motility of the earthworms in the Petri dish had decrease but the earthworm still responded	The earthworm had no swellings responded to physical stimulus and	The earthworm did not die and had no swellings

		to physical stimulus that is touch	moved around the dish	
9	1×10^{-11}	Motility of the earthworms in the Petri dish had decrease but the earthworm still responded to physical stimulus that is touch	The earthworm had no swellings responded to physical stimulus and moved around the dish	The earthworm did not die and had no swellings
10	1×10^{-12}	Motility of the earthworms in the Petri dish had decrease but the earthworm still responded to physical stimulus that is touch	The earthworm had no swellings responded to physical stimulus and moved around the dish	The earthworm did not die and had no swellings
11	Control	Motility of the earthworm in the petri dish was normal and the earthworm responded to stimuli normally The earthworm also had no swellings	Motility of the earthworm in the petri dish was normal and the earthworm responded to stimuli normally The earthworm also had no swellings	Motility of the earthworm in the petri dish was normal and the earthworm responded to stimuli normally The earthworm also had no swellings

				The earthworm did not die

Table 4. 3 Effects of Acetamiprid (200g/kg) on Earthworms; *Eisenia foetida*

Serial number	Concentration of imidacloprid(mg/ml)	Changes observed after 24 hours	Changes observed after 48 hours	Changes after 72 hours
1	1×10^{-3}	Motility of the earthworms in the Petri dish had decrease but the earthworm still responded to physical stimulus that is touch	The earthworm in this dish had swellings in the middle and at the end. Still responded to physical stimulus	The swelling busted and the earth worm died
2	1×10^{-4}	Motility of the earthworms in the Petri dish had decrease but the earthworm still responded to physical stimulus that is touch	The earthworm in this dish had swellings in the middle and at the end. Still responded to physical stimulus The skin had turned pale yellow.	The swelling busted and the earth worm died
3	1×10^{-5}	Motility of the earthworms in the Petri dish	The earthworm had fewer swellings	The swelling busted and the earth worm died

		had decrease but the earthworm still responded to physical stimulus that is touch	Still responded to physical stimulus The skin had turned pale yellow.	
4	1×10^{-6}	Motility of the earthworms in the Petri dish had decrease but the earthworm still responded to physical stimulus that is touch	The earthworm had fewer swellings Still responded to physical stimulus The skin had turned pale yellow	The swelling busted and the earth worm died
5	1×10^{-7}	Motility of the earthworms in the Petri dish had decrease but the earthworm still responded to physical stimulus that is touch	The earthworm had swellings responded to physical stimulus and moved around the dish	The swelling busted and the earth worm died
6	1×10^{-8}	Motility of the earthworms in the Petri dish had decrease but the earthworm still responded to physical	The earthworm had swellings responded to physical stimulus and moved around the dish	The swelling busted and the earth worm died

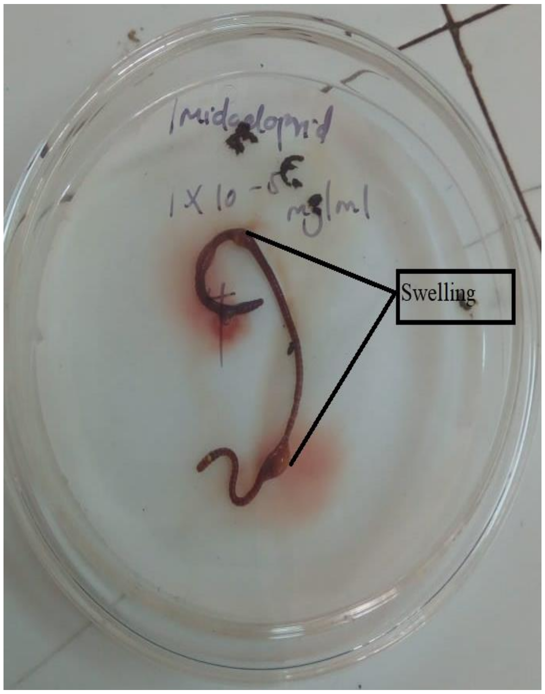
		stimulus that is touch		
7	1×10^{-9}	Motility of the earthworms in the Petri dish had decrease but the earthworm still responded to physical stimulus that is touch	The earthworm had swellings responded to physical stimulus and moved around the dish	The earthworm did not die.
8	1×10^{-10}	Motility of the earthworms in the Petri dish had decrease but the earthworm still responded to physical stimulus that is touch	The earthworm had no swellings responded to physical stimulus and moved around the dish	The earthworm did not die and had no swellings
9	1×10^{-11}	Motility of the earthworms in the Petri dish had decrease but the earthworm still responded to physical stimulus that is touch	The earthworm had no swellings responded to physical stimulus and moved around the dish	The earthworm did not die and had no swellings
10	1×10^{-12}	Motility of the earthworms in	The earthworm had no	The earthworm did not die and

		the Petri dish had decrease but the earthworm still responded to physical stimulus that is touch	swellings responded to physical stimulus and moved around the dish	had no swellings
11	Control	Motility of the earthworm in the petri dish was normal and the earthworm responded to stimuli normally The earthworm also had no swellings	Motility of the earthworm in the petri dish was normal and the earthworm responded to stimuli normally The earthworm also had no swellings	Motility of the earthworm in the petri dish was normal and the earthworm responded to stimuli normally The earthworm also had no swellings The earthworm did not die

Table 4. 4 Effects of imidacloprid (700g/kg) on Earthworms; *Eisenia foetida*

4.2 DISCUSSION

The acute toxicity test of earthworms is efficient tool that is necessary for assessing the risks of contaminants in soils. The endpoint in this assessment is usually mortality. In the present study the toxic effects of two selected pesticides acetamiprid and imidacloprid on earthworms; *Eisenia foetida* was evaluated. The 72 hours contact filter paper test method of OECD was used. The contact filter paper test is an initial test for screening the toxic effects of pesticides/chemicals to earthworms where the pesticides is absorbed into the test specimens via the skin. Pesticides results into toxic effects such as morphological abnormalities, reduced mortality and response to stimuli with the hallmark being mortality of earthworms which are usually observed after 24 hours, 48 hours and 72 hours. The results of the current study revealed that the two investigated pesticides were toxic to earthworms. In the contact filter paper test after 24 hours no motility and morphological abnormalities were recorded for both pesticides and all the earthworms were responding to stimuli. However, the motility of the earthworm was observed to have reduced compared to the control. Similarly, after 48 hours no mortality was observed in both the pesticides and earthworms were still responding to stimuli but not as much as compared to the control which was more active. However, in the serial dilution 1-5, in the acetamiprid (200g/kg) and 1-7 in the imidacloprid (700g/kg), morphological abnormalities that included swelling and change in color in the sections of the earthworm body were observed (Image 1 and 2). After 72 hours of contact test, earthworms in serial dilution 1-5 for acetamiprid (200g/kg) and 1-6 for imidacloprid (700g/kg) mortality of earthworms accompanied with morphological abnormalities pesticides was observed.



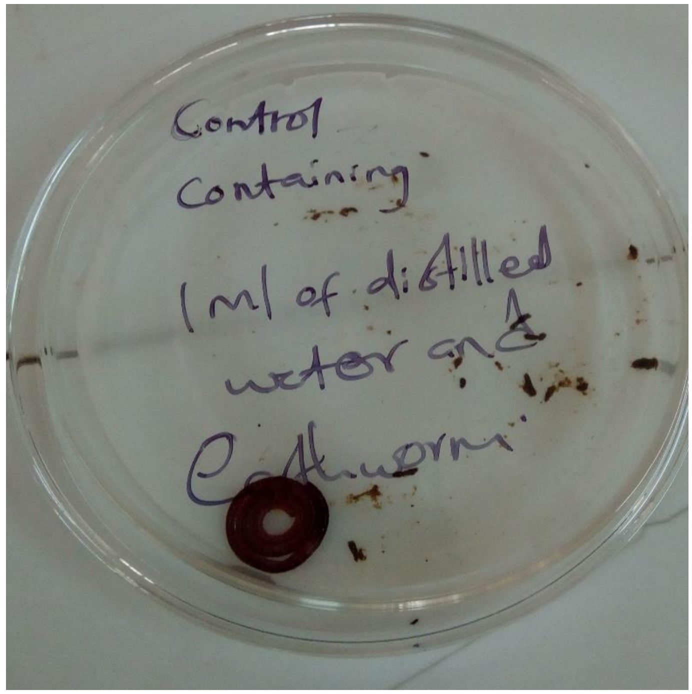
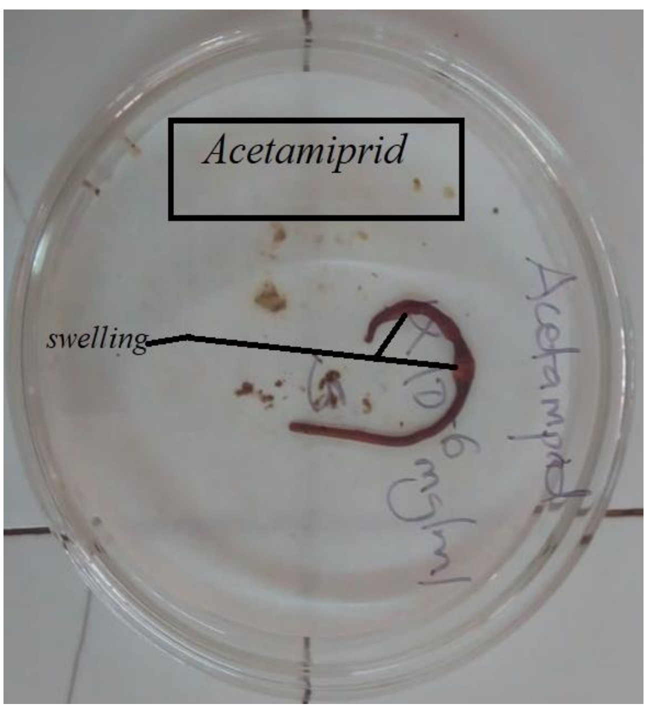


Image 1 Morphological abnormalities of Imidacloprid on earthworm

Image 2 control containing 1 ml of distilled water and earthworm for comparison



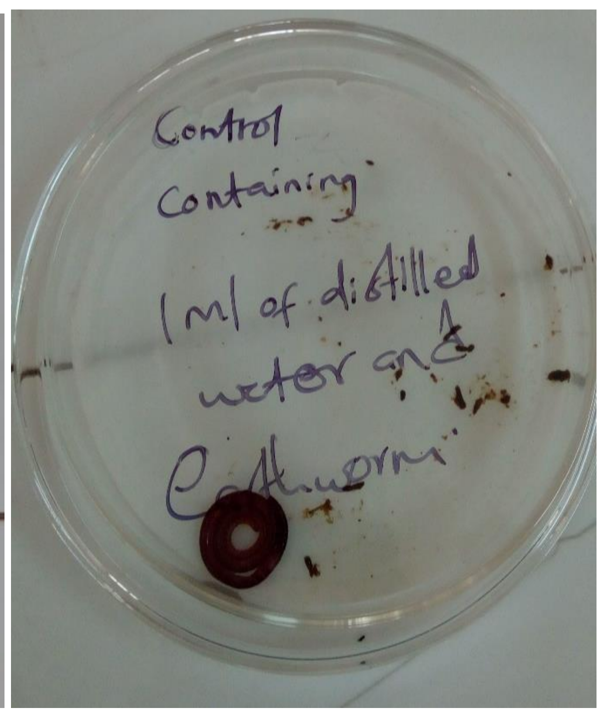


Image 3 Morphological abnormalities of acetamiprid on earthworms

Image 4 Control containing 1 ml of distilled water and earthworm for comparison

The ability of earthworms to be chosen as the specimen of analysis in testing the toxicity of pesticides was assessed in this study. The ability of the earthworms to respond positively to pesticides showed that they are suitable as specimen on assessing the toxicity of pesticides. From the outcomes of the study which included change in the color of the earthworms, swelling of the sections of the body of earthworms and reduced response to the stimuli indicated they are efficient in monitoring toxic effects of pesticides. All the noted changes both morphologically and physiologically were as the result of the pesticides.

The maintenance of earthworms in the laboratory was done by monitoring them over a period of seven days. In wide plastic container, free organic materials include fruit and Irish potatoes peelings were added at the bottom of this container prior to adding the earthworm stock on the top. During this period the temperature was maintained at $19\pm2\ ^{O}C$ and frequent scrabbling in the compost to check if they were still alive was done occasionally. The physiological parameters such as responding to stimuli was as well monitored as well as weight was monitored. Throughout this period earthworms responded positively to stimuli and their motility rate was normal. The weight monitoring revealed the majority of earthworms recorded weight range of 300-430 mg.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Optimization of this testing can be done at 19 ±2 ^{O}C. Earthworms can be maintained using locally available fruit peels such as watermelon peels, banana peels and potato peels, however, they prefer the watermelon and banana peels. The pesticides used were toxic to earthworms. Earthworms can be used for toxicity testing

5.2 Recommendation

From this study the following recommendation can be made;

1. The lethal dose of acetamiprid (200g/kg) and imidacloprid (700g/kg) be determined using the modified artificial soil.
2. Earthworms be recommended for toxicity testing of medicines used by human
3. Further tests be carried out using large populations of earthworms and at varying temperatures

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