Assessment of Toxicity of Some Agricultural Pesticides on Earthworm (Lumbricus Terrestris)


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ABSTRACT

The toxicity of some selected widely used Agricultural Pesticides (Insecticides, Herbicides and Fungicides) on earthworm (Lumbricus terrestris) were assessed using two different types of bioassay, a 48 Hours contact filter paper bioassay test and a 14 days soil bioassay test. Some selected heavy metals were also tested in the soil before and after the experiment to determine the level of their presence in the selected pesticides. The 24 hours and 48 hours LC50 values for contact filter paper test were determined by Probit Analysis using SPSS. The result of 48 hours contact filter paper test based on LC50 values indicates Insecticides; Pyrethroid, Neonicotinoids and Organophosphates to be highly toxic to earthworm (Lumbricus terrestris) with LC50 values ranges from 0.000ml – 0.001ml. Herbicides and Fungicides were relatively low toxic with LC50 values ranges from 0.002 ml for Butachlor and 0.006 ml for Mancozeb respectively. The result of 14 days soil bioassay test revealed different pattern of toxicity, Insecticides; Pyrethroid and Neonicotinoids still shows high level of toxicity under soil condition. Herbicides and Fungicides do not pose a serious threat to earthworms’ survival under soil condition. The result of heavy metals test revealed an increased level of these toxic heavy metals (Zn, Cu, Cr and Cd) in the soil after the experiment. It was concluded that insecticides Pyrethroid and Neonicotinoids are highly toxic to earthworms in the soil based on agricultural recommended dose/rate of application. Determination for suitable application rate for these insecticides to farmers that would reduce these effects was also recommended.

KEY WORDS
Bioassay, Butachlor, Earthworms, Mancozeb, Pesticides, Probit, Toxicity

INTRODUCTION

Pesticides are substances or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. Pesticides are a special kind of products for crop protection (Kellongg et al., 2000, Rocket 2007, Zhu et al., 2008). Crop protection products in general protect plants from damaging influences such as weeds, diseases or insects. A pesticide is generally a chemical or biological agent (such as a virus, bacterium, antimicrobial or disinfectant) that through its effect deters, incapacitates, kills or otherwise discourages pests (Miller 2004). Target pests can include insects, plant pathogens, weeds, molluscs, birds, mammals, fish, nematodes (roundworms), and microbes that destroy property, cause nuisance, spread disease or are vectors for disease. Although there are human benefits to the use of pesticides, some also have drawbacks, such as potential toxicity to humans and other animals (Kuniuку et al., 2001). According to the Stockholm Convention on Persistent Organic Pollutants, 9 of the 12 most dangerous and persistent organic chemicals are pesticides. Pesticides are categorized into four main substituent chemicals: herbicides; fungicides; insecticides and bactericides (Stockholm Convention, 1960).
Herbicides and pesticides have become an inseparable part of modern agricultural production and technology (Croteau, 1990, Usmani & Knowles, 2001, Willrich & Boethel, 2001, Stephen et al., 2011, Pimentel 2011). Unfortunately, more attention is paid to their production and use than to their adverse effects on agro ecosystems, above all environmental contamination (Gunnell et al., 2007)

Earthworms are common soil organisms in most environments that are adversely affected by agrochemicals. They play an important role in improving texture, structure, and soil aggregation, physical and chemical properties of the soil with improved fertility (Delahaut and Koval, 1989, Lavelle and Spain, 2001, Wang et al., 2004, Patrick et al., 1995, Bartlett et al., 2010). They represent up to 60–80% of the total animal biomass in soil (Ouellet et al., 2008; Jouquet et al., 2010). Earthworms are sensitive and thus susceptible to soil chemicals especially agrochemicals because they lack hard cuticle around their body (Lanno et al., 2004; Nahmani et al., 2007). This makes them suitable bio indicators of soil contamination, and can be used to provide thresholds for insecticide applications (Suthar et al., 2008; Lourenco et al., 2011). Earthworms are highly susceptible to changes of ecological factors, particularly those intrinsic to the soil, and earthworm behavior can therefore reflect soil contamination. It is known that earthworms reflect changes taking place in the soil, particularly changes of soil physical, chemical, and biological properties, as well as changes in the water-air and thermal regimes. (Suther et al., 2008).

Figure 1: Earthworm (Lumbricus terrestris) (normal body form).

Even when damage is not caused to the earthworms directly, bioaccumulation may produce serious damages to higher trophic levels (Darling and Thomas, 2005; Hobbelen et al., 2006; van Gestel et al., 2011). They have long been used as a key index of eco toxicological diagnosis although they are becoming extinct in many agriculture soils due to the lethal effects of long term application of agrochemicals (Diercxsens et al., 1985, Xiao et al., 2004; Zhu et al., 2008.; Kamitani and Kaneko, 2007; Reinecke and Reinecke, 2007; Hackenberger et al., 2008). The use of insecticides in agriculture especially in developing nations is one of the most important factors contributing to the massive increase in food production worldwide (Kranthi et al., 2002). Nonetheless overuse and indiscriminate application causes adaptation or evolution of resistance in target pests and thereby a diminishing of the effectiveness of these chemicals (Van Driesche & Bellows, 1996, Wang et al., 2008, Tomberlin et al., 2002). This, couple with the lethal effects on non-target organisms (Suther et al., 2008, Santos et al., 2010; Walker et al., 2010).

Widespread use of insecticides in agricultural areas worldwide, has cause for concern about soil contamination (Tillman & Mulrooney, 2000, Nath et al., 1997; Suh et al., 2000, Zhou et al., 2006; Reinecke and Reinecke, 2007; De Silva and van Gestel, 2009; De Silva et al., 2010; Garcia et al., 2011; Santos et al., 2011). Furthermore, the security of soil ecosystems is threatened by insecticides, which reduce earthworm population and lead to the build-up of waste materials (Edwards and Bohlen, 1992). The relationship between death and diminution of earth- worms and the application of pesticides are still elusive, and little is known about the impact of novel pesticides on earthworms using the standard test method as described in the guidelines of the OECD (1984, 2004).

2. Objectives:
The main aim of this study is to get a more comprehensive understanding on the toxic effects of pesticides on earthworms, and to provide informative and baseline data for use in ecological risk assessment on soil ecosystem

MATERIALS AND METHODS

3.1. Earthworms:
Sexually mature earthworms (Lumbricus terrestris) from soil with no history of any chemical application or agricultural activity in the savanna ecological zone of Nigeria were used for this study. The study site is within the Abubakar Tafawa Balewa University (ATBU) Yelwa campus Bauchi, Nigeria. The sampling site is located at latitude 10°.31 and longitude 9°.84. Worms were carefully collected from the soil, transferred into fresh plastic
pots containing native soil and transported to the laboratory for the experiment. The earthworms were acclimatized with the laboratory conditions for the period of 3 weeks by maintaining their optimal condition for survival to rule out any possible interference or influence on the experimental set up by the laboratory conditions.

2. Experimental Chemicals:
The agrochemicals used in this study were Atrazine, Miafrun, Butachlor, Orizor plus, Paraquat, Glyphosate, Cypermethrin, Lamdacyhalothrin, Dimethoate, Imidacot, Chlophyrisphos (Termicot), and Mancozeb. These cut across Insecticides, Herbicides and Fungicides.

3. Toxicity Test Methods:
3.3.1. 48 Hours Contact Filter Paper Test:
Pesticides were diluted with distilled water, different range of the test concentrations (dose) were based on farmers’ rate of application obtained from the study area which is similar to most agricultural areas in northern Nigeria. A piece of filter paper was treated with the test substances (Pesticides) and placed in a petri dish. Earthworms were placed on top of filter papers impregnated with chemical concentration on a covered Petri-dish. The set-up was replicated three times for each of the selected pesticides and a similar design was set-up using distilled water as a control. The dish was incubated in the dark at 20 ± 1 ºC for 48 hours and mortality was recorded at time intervals.

3.3.2. Fourteen Days Soil test:
The natural soil was obtained from the same field from which the earthworms were collected. Homogenized and air dried soil were sieved through 2 mm mesh. Adult earthworms were exposed to a soil mixed with different concentration of pesticides. Six earthworms were placed on 600 g of natural soil in a plastic container of 3.5 litre capacity and allowed to borrow. The earthworms were fed with urine-free dried and grinded cattle manure (cow dung’s) throughout the period of the experiment. These set up was repeated in three replicate for all the selected pesticides and control were also prepared using distilled water. Mortality of earthworms was evaluated on daily basis to determine the LCT of each pesticide. To check the mortality, the test containers were emptied onto a clean tray and earthworms were separated from the soil. Earthworms were judged to be dead when they fail to respond to gentle mechanical stimulations with a blunt probe.

3.4. Statistical Analysis:
Probit analysis had been used to determine the LC\textsubscript{50} value at 95 % Confidence level using SPSS.

4. Results:
4.1. 48 Hour Filter Paper Contact Test:
The result of filter paper contact test is presented in Tables (1 and 2). The result showed that different insecticides, herbicides and fungicides varied in their contact toxicities. Earthworm were more susceptible to all pesticides of the insecticides category viz: with mortality ranges from 68 % to 100 % after 24 hours of exposure. Lamda Cyhalothrin, Immidacot, Cypermethrin, Dimethoate and Termicot (Chlopyrifsos) with \textit{LC}_{50} values ranging from 1 x 10^{-3} ml – 2 x 10^{-4} ml. Pesticides within the herbicide category inflicted the worms after 24 hours of exposure with increasing to decreasing magnitudes of mortality. For example; Butaclor, Orizor Plus and Triazine (Atrazine) recorded high mortality on earthworms with 88 %, 82 % and 61 % respectively. Other herbicides had relatively low mortality effects on the worms. Paraquate had 49 % mortality on the worms, Glyphosate and Miafrun caused the least mortality (38 % and 27 %). While Insecticides Lamda Cyhalothrin, Cypermethrin, Immidacot and Termicot tend to be highly toxic to earthworms (\textit{limbricus terrestris}) after 48 hours exposure with \textit{LC}_{50} values ranging from (0.000 ml – 0.002 ml) These chemicals also presents the highest number of mortality in all the concentration/dilution in the treatment group used for all the experiments including the preliminary test after 24 hours and 48 hours exposure time.

Insecticide Dimethoate of chemical class Organophosphate and Herbicide Butachlor of chemical class Acetanilide present similar toxicity level after 24 hours of exposure with \textit{LC}_{50} value of (0.003 ml). Moreover Butachlor present similar toxicity level after 48 hours of exposure with \textit{LC}_{50} value of (0.002 ml).

Dimethoate and Termicot (Chlopyrifsos) Insecticides of chemical class Organophosphate present similar toxic level to those of Pyrethroid and Neonicotinoids chemical class but they tend to show relatively little lower toxic level in terms of mortality at lower concentration/dilution of the treatment group used for all the experiment after 48 hours of exposure far below the farmer’s rate of application.

The selected Herbicides, Atrazine of chemical class Carbamate (Dithiocarbamate) triazine, and Orizor Plus slightly differ in toxicity level to earthworms (\textit{limbricus terrestris}) after 24 hours of exposure with \textit{LC}_{50} values of 0.007 ml and 0.005 ml respectively and present similar toxicity level after 48 hours of exposure each presenting \textit{LC}_{50} value of 0.003 ml. But in terms of mortality they equally shows less toxic level at lower concentrations/dilutions far below the farmer’s rates of application after 48 hours.
Paraquate of chemical class Quaternary Nitrogen Compound, Mancozeb of chemical class Carbamate (Dithiocarbamate), Glyphosate of chemical class Phosphates, and Miafrun present different pattern of toxicity level to earthworm (*lumbricus terrestris*) after 24 hours exposure with LC50 values 0.016 ml, 0.020 ml, 0.024 ml and 0.033 ml respectively and they equally shows similar pattern of toxicity level after 48 hours exposure with LC50 values of: Glyphosate 0.005 ml, Paraquate 0.005 ml, Miafrun 0.006 ml and Mancozeb 0.006 ml respectively. They also show high toxic level by presenting ninety four percent mortality at concentrations/dilutions equivalent to farmers’ rate of pesticides application and relatively low toxic level at lower concentrations/dilutions.

The results of 14 days soil test was presented in Table (3). The data exhibited a clear similarity to the result of contact toxicity (48 hours filter paper contact test), each of the pesticides displayed different degree of toxicity to earthworm (*lumbricus terrestris*) even those of the same chemical class.

Cypermethrin and Lamdacyhalothrin insecticides of chemical class pyrethroid present the highest toxic level to earthworm (*lumbricus terrestris*) with total mortality of the entire test organism presenting 100% mortality.

Immidacot insecticides of chemical class Neonicotinoid present the highest toxic level to earthworm (*lumbricus terrestris*) after Cypermethrin and Lamdacyhalothrin with total mortality of the entire test organism also presenting 100% mortality within five days after the inception of the test.

### Table 1: Contact filter paper test toxic effect (LC50) of twelve pesticides against Earthworm (*lumbricus terrestris*) for 24Hrs

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Slope (SE)</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>Mortality after treatment exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miafrun</td>
<td>1.76(0.54)</td>
<td>0.019 – 0.172</td>
<td>11.43(22)</td>
<td>27</td>
</tr>
<tr>
<td>Orizor Plus</td>
<td>4.92(1.06)</td>
<td>0.004 – 0.007</td>
<td>7.86(22)</td>
<td>82</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>2.73(0.57)</td>
<td>0.003 – 0.010</td>
<td>3.68(22)</td>
<td>98</td>
</tr>
<tr>
<td>Paraquate</td>
<td>1.78(0.48)</td>
<td>0.000 – 0.001</td>
<td>11.43(22)</td>
<td>92</td>
</tr>
<tr>
<td>Mancozeb</td>
<td>4.75(1.03)</td>
<td>0.003 – 0.008</td>
<td>8.55(22)</td>
<td>82</td>
</tr>
<tr>
<td>Orizor Plus</td>
<td>6.84(2.32)</td>
<td>0.002 – 0.003</td>
<td>4.57(21)</td>
<td>92</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>2.46(0.55)</td>
<td>0.005 – 0.007</td>
<td>7.86(22)</td>
<td>98</td>
</tr>
<tr>
<td>Butoxide</td>
<td>3.00(30)</td>
<td>0.003 – 0.004</td>
<td>17.06(25)</td>
<td>100</td>
</tr>
<tr>
<td>Quaternary Nitrogen Compound</td>
<td>3.52(0.76)</td>
<td>0.000 – 0.001</td>
<td>13.38(22)</td>
<td>92</td>
</tr>
</tbody>
</table>

Abbreviations: x2(d.f); degree of freedom; %maturity after 24 hours: percentage of earthworms dead/affected after 24 hours of treatment exposure.

### Table 2: Contact filter paper test toxic effect (LC50) of twelve pesticides against Earthworm (*lumbricus terrestris*) for 48Hrs

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Slope (SE)</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>Mortality after treatment exposure</th>
</tr>
</thead>
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<tr>
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</tr>
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</tr>
</tbody>
</table>

Abbreviations: x2(d.f); degree of freedom; %maturity after 24 hours: percentage of earthworms dead/affected after 24 hours of treatment exposure.
Herbicides Orizor plus, Insecticides Dimethoate and Termicot (Chlopyrifos) of chemical class organophosphate, and Atrazine herbicide of chemical class Carbamate (Dithiocarbamate) triazine exhibit some high level of toxicity to earthworms \((lumbricus terrestris)\) with high number of mortality, just few survived the fourteen days soil test.

Paraquate and Glyphosate herbicides of chemical class Quaternary Nitrogen Compound and Phosphates respectively, exhibit similar pattern of toxicity level to earthworm \((lumbricus terrestris)\) with 39% mortality for each pesticide after fourteen day.

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>NO of Exposed EW</th>
<th>Mortality</th>
<th>%Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonicotinoids</td>
<td>18</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>Pyrethroid</td>
<td>18</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>Lambda Cyhalothrin</td>
<td>18</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>Dithiocarbamate (Carbamates)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mancozeb</td>
<td>18</td>
<td>5</td>
<td>28</td>
</tr>
<tr>
<td>Triazine (Atrazine)</td>
<td>18</td>
<td>9</td>
<td>50</td>
</tr>
<tr>
<td>Organophosphates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimethoate</td>
<td>18</td>
<td>2</td>
<td>67</td>
</tr>
<tr>
<td>Termicot (Chlopyrifos)</td>
<td>18</td>
<td>11</td>
<td>61</td>
</tr>
<tr>
<td>Acetanilide</td>
<td>18</td>
<td>6</td>
<td>33</td>
</tr>
<tr>
<td>Quaternary Nitrogen Compound</td>
<td>18</td>
<td>7</td>
<td>39</td>
</tr>
<tr>
<td>Phosphates</td>
<td>18</td>
<td>6</td>
<td>33</td>
</tr>
<tr>
<td>Miafrun</td>
<td>18</td>
<td>13</td>
<td>72</td>
</tr>
<tr>
<td>Orizor Plus</td>
<td>18</td>
<td>13</td>
<td>72</td>
</tr>
</tbody>
</table>

Table: 3 Percentage (%) Mortality for Earthworms \((lumbricus terrestris)\) in 14 Days Soil Toxicity Bioassay Test

Abbreviations: EW: earthworms, %mortality percentage rate of dead/infected earthworms due to the treatment

Butachlor of chemical class Acetanilide and Miafrun herbicides present similar pattern of toxic effect level to earthworms \((lumbricus terrestris)\) with the same number of mortality after fourteen days presenting only 33% mortality.

Mancozeb Herbicides of chemical class Carbamate (Dithiocarbamate) present the lowest number of mortality with only 28% mortality after fourteen days but it equally present some level of toxic effect.

The result of mean concentration of heavy metals in soil before and after the fourteen days soil test is presented in Table (4). The result reveals the presence of considerable amount of Zn, Cu, Cd and Cr in the soil after the experiment. This justified the presence of some heavy metals in the pesticides used during the experiment.

Cu (Copper) indicated the highest mean value in all the parameters tested except in Lambda Cyhalothrin and Mancozeb where Zn (Zinc) is the highest.

The mean values also present Cd (Cadmium) with lowest concentrations in all the soil samples.

5. Discussion:

Acute toxicity of earthworm is an efficient tool in assessing ecological risks of contaminated soils (Lukkari et al., 2005; Hamibach, 1985) and end point is mortality (Karnok and Hemilink, 1982; Dean-Rrss, 1983, Ellisetal 2007). Assessment on the toxic effect of some selected widely used Agricultural pesticides on earthworms \((lumbricus terrestris)\) was conducted in the present study using two different test methods of OECD (48 hours contact filter paper test and fourteen days soil test). The result of this study reveals all the pesticides were toxic to earthworms \((lumbricus terrestris)\) based on \(LC_{50}\) values from 48 hours contact filter paper test, while they show different degree of toxicity profile in fourteen days soil test based on pesticides classes.

Contact filter paper test is an initial screening technique to assess the relative toxicity of chemicals to earthworms, where pesticides are mainly absorbed by the skin, however it fail to present the situation in the soil ecosystems (Miyazaki et al., 2002; Grumianx et al., 2010; Tripathi et al., 2010).

Soil test is a more representation of natural environment of earthworms and the pesticides are mainly absorbed by gut in this method (De Silva and Van Gestel, 2009, Udovic and Lestan, 2010). Therefore the soil test is more adequate when toxicity of pesticides to earthworms is evaluated (Wang et al., 2011).

The result of this study revealed that insecticides are more toxic to earthworms than herbicides and fungicides. 48 hours \(LC_{50}\) of filter paper contact test reveals that insecticides; Pyrethroid, Neonicotinoid and Organophosphates are highly toxic to earthworms \((lumbricus terrestris)\) with \(LC_{20}\) values of these chemicals;
Cypermethrin, Lambda Cyhalothrin, Imidacloprid, Dimethoate and Termicot (chlorpyrifos) ranges between 0.000 ml to 0.001 ml, the toxic nature of these insecticides based on 48 hours filter paper contact test is similar to that of (Wang, et al., 2012), who reported the acute toxicity of Neonicotinoid (Imidacloprid) as super toxic to earthworms (Eisenia fetida), Pyrethroid (Cypermethrin and Lambda Cyhalothrin) and Organophosphate (Termicot) as very toxic.

<table>
<thead>
<tr>
<th>Table 4: The Mean Concentrations of Some Heavy Metals Detected in Soil Samples Before (Control) and After the Soil Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pesticides</strong></td>
</tr>
<tr>
<td>Neonicotinoids</td>
</tr>
<tr>
<td>Imidacot</td>
</tr>
<tr>
<td>Pyrethroid</td>
</tr>
<tr>
<td>Cypermethrin</td>
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<tr>
<td>L. Cyhalothrin</td>
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<tr>
<td>Dithiocarbamate (Carbamates)</td>
</tr>
<tr>
<td>Mancozeb</td>
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<td>Triazine (Atrazine)</td>
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<tr>
<td>Organophosphates</td>
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<tr>
<td>Dimethoate</td>
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<tr>
<td>Termicot (Chlorpyrifos)</td>
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<tr>
<td>Acetanilide</td>
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<tr>
<td>Quaternary Nitrogen Compound</td>
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<tr>
<td>Parquat</td>
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<tr>
<td>Phosphates</td>
</tr>
<tr>
<td>Glyphosate</td>
</tr>
<tr>
<td>Miafrun</td>
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<tr>
<td>Orizor Plus</td>
</tr>
<tr>
<td>Control</td>
</tr>
</tbody>
</table>

Neonicotinoids are among the most effective insecticides for the control of sucking and chewing insect, they act as competitive inhibitors on nicotinic acetylcholine receptors (nAChR) in the central nervous system (Elbert et al., 2008). Ishaaya and Degheele 1998) also reported, Neonicotinoids may potentially endanger soil organism including earthworms, because they are composed of compound systems.

Imidacloprid a neonicotinoid chemical, in comparison with carbaryl, cyfluthrin, chlorpyrifos and fipronil, had more negative effect on earthworm Aporrectodea traperoida (Mostert et al., 2002). Similarly, Muhammad et al., (2010) reported Imidacloprid as toxic to earthworm p. posthuma with LC<sub>50</sub> of 0.011 ppm also with symptoms of poisoning including tiredness, twitching, cramps, and muscles weakness. Moreover Dittbrenner et al., (2011) reported that increasing Imidacloprid (neonicotinoid) concentration causes significant linear decrease in burrow volume and burrowing behaviour in two earthworm species Aporrectodea caliginosa and lumbricus terrestris.

The toxicity of Pyrethroid (Cypermethrin and Lambda Cyhalothrin) in this study is very high, revealing the lowest 48 hours LC<sub>50</sub> of 0.000 ml and 100 % mortality of all the test organism in just four days from the inception of fourteen days soil test these finding is similar to that of Gupta et al., (2010) that reported Cypermethrin as highly toxic to earthworm (perionyx axcavatus) and it’s also similar to the findings of Wang et al., (2012) that shows pyrethroid as a very toxic to Eisenia fetida.

The toxicity of Organophosphates to earthworms depends on the assessed parameter (Wang, et al., 2012), certainly they will show high toxicity if one assesses AChE inhibition and the consequent physiological damage (RaO et al., 2003; Reddy and RaO, 2008).

In this study Organophosphates (Dimethoate and Termicot “Chlorpyrifos”) display different degree of toxicity level to earthworm (lumbricus terrestris) with very toxic level base on 48 hours contact test with LC<sub>50</sub> of 0.001 ml. The toxicity of these chemicals based on fourteen days soil test differ with more than 60 % mortality of the test organism (lumbricus terrestris) these finding is in accordance with the previous report of Martikaine (1996) that indicate Dimethoate as highly toxic to earthworms, therefore continuous application of such
pesticides for higher crop production should be considered seriously. He also showed that Dimethoate can change the behavior of earthworms by acting as an acetyl cholinesterase inhibitor. Conversely Anindita et al., (2013) reported Dimethoate as not toxic to earthworms at recommended agricultural dose but studies found some sub lethal effects on the growth, reproduction and metabolism of earthworm even at the recommended dose (Dolby et al., 1995; Neuhauser and Callahan 1990). Moreover Zhou et al., (2006) finding was similar to our finding on Termicot (chlorpyriphos) in his result of chronic toxicity test demonstrated that chlorpyriphos have adverse impact on growth and reproduction in earthworms, but this is largely dependent on pesticides concentration and exposure period. Earthworm growth decreased with increased concentration of pesticides. In his result of acute toxicity (Zhou et al., 2006) report in all exposure period a clear demonstration – response relationship was observed, that earthworm mortality increased with increasing concentration of each contaminant (pesticides).

The result of Herbicides and Fungicide from this study displays varying degree of toxic effect to earthworm (lumbricus terrestris) base on 48hour LC$_{50}$ and 14 Days soil test.

Earthworm was not affected by herbicides application unless maximum recommended field rate were exceeded (Annemike, 1998).

Butachlor, Atrazine and Orizor Plus tend to be relatively toxic to earthworm (lumbricus terrestris) base on 48hours contact filter paper test with LC$_{50}$ values of (Butachlor 0.002ml, Atrazin 0.003ml and Orizor Plus0.003ml respectively). These herbicides also show different pattern of toxic effect base on 14 days soil test, with Orizor plus tend to be very toxic presenting 72% mortality of the test organism, then Atrazine 50% mortality and Butachlor presenting less toxicity in soil test with 33% mortality. This result is in line with the findings of Muthukaruppan and Gunasekara (2009) that Butachlor does not cause fatal effect to earthworm but can retard growth and cocoon production and cause damage to epithelial tissue. Tanja (2007) reports that atrazin and Butachlor has a strong depressive effect on the number of individual earthworms, in addition, the herbicides also had a strong negative impacts earthworm’s biomass.

Oluiah et al., (2010) report that Atrazine had a significant effect on earthworm (N. mbae) also reported 38 – 80% mortality of earthworm (N. mbae) when expose to atrazin. Similarly, Xiao et al., (2010) reported low survival rate of earthworms when treated with atrazine. In another report, Ahmed, S. T. (2013) showed the animals’ progressive change in signs and symptoms due to the level of toxicity ranging from visibly undetectable marks to coiling, curling, extrusion of coelomic fluid, segmental constriction and swelling. In several animals the swollen portion burst causing bloody lesions, limp and ultimately death (Ahmed, S. T. (2013)).

In this study the toxicity of fungicides (Mancozeb) base on 48 hours LC$_{50}$ and 14 days soil test reveals the least toxic effect in comparison with other selected pesticides with 48 hours LC$_{50}$ of 0.006 ml and 28 % mortality in 14 days soil test. This finding is in conformity with smith (1992) who reported that most fungicides have relatively narrow spectrum of toxicity to soil inhabitant and aquatic organism. Their greatest environmental impact is toxicity to soil microorganism but these effects are short term.

Herbicides; Paraquate, Glyphosate and Miafrun Present similar toxicity pattern to earthworms (lumbricus terrestris) with 48 hours LC$_{50}$ of 0.005 ml for all the herbicides. They equally shows similar toxicity pattern base on 14 days soil test with percentage mortality of 39 % for Paraquate and 33 % mortality for Miafrun and Glyphosate. These finding is similar to the previous studies that reveals with normal agricultural use Paraquate does not pose a risk to earthworm, soil microbes and beneficial insect. The residues of Paraquate and diquat have no detrimental effects on soil organism including earthworms, micro anthropods, microorganism and soil nitrification and respiration. However Nathan (2005) reported that Glyphosate have no fatal effect on earthworms but has a negative effect on the biomass. Dolby et al., (1995) also reported that a single application at recommended field rate of Glyphosate had no effect on the growth or survival of earthworms’ species (Aporrectodea trapezoids, A. rosea, A. caliginosa and A. longa) application of Glyphosate at recommended rate will not harm earthworm. Ware (1994) reported that contact herbicides, which kill weeds through foliage application are slightly toxic to soil organism and moderately to aquatic organism and are non-persistent in the soil except for few ones like triazene which can persist in the soil for several years.

Pelosi C., et al., 2013 suggested on experiments assessing the effects of the same pesticides on the same earthworm species at different organisation levels carried out to derive the links between the responses of pesticides at different developmental stages of earthworm.

Earthworms are one of the first receptors affected by soil contamination. They are more susceptible to metal pollution than many other groups of soil invertebrates, and toxicity data on earthworms are important in determining “safe levels” for metals and other contaminants in soil. (Jurate et al., 2010)

Results of heavy metals analysis in the selected pesticides revealed the presence of all the metals studied: Copper (Cu), Zinc (Zn), Chromium (Cr) and Cadmium (Cd) whose contents between one pesticide to another differ in concentrations.

Findings of heavy metals investigation in the pesticides provides preliminary information of presence of dangerous substances that are absolutely undisclosed as components of the pesticides by the manufacturers. This therefore questions the authenticity of manufacturer’s information regarding the actual ingredient(s) and
chemical composition of the pesticides. Nevertheless, the presence of heavy metals in the pesticides is of significant concern because they are additive contaminants of soil which add up to the quality deterioration of the ecosystem.

Heavy metals have been shown to cause lysosomal membrane instability, changes in gene expression, oxidative stress to earthworms (Spurgeon et al., 2004a; Berthelot et al., 2008; van Gestel et al., 2009), to reduce growth (Spurgeon et al., 1994), to slow sexual development (Spurgeon, Hopkin, 1996; Spurgeon et al., 2004b), to reduce cocoon production and hatchability (Reinecke et al., 2001; Davies et al., 2003; Spurgeon et al., 2004a), juvenile viability (Bengtsson et al., 1986; van Gestel et al., 1992), to cause mortality (Neuhauser et al., 1985; Spurgeon et al., 1994; Davies et al., 2003) and affect the population size, abundance and species diversity of earthworms (Spurgeon et al., 2005). The effects are dependent on metal and soil parameters and on species. Earthworms, to a certain degree, can regulate the uptake of essential metals (such as Cu, Zn) from soil, although the regulation of non-essential metals (e.g., Cd, Pb) is probably less, if any (Morgan, Morgan, 1988; van Gestel et al., 1993; Nahmani et al., 2009).

Cadmium and Lead does not cause significant acute toxicity to earthworms, although the impact on reproduction and growth was rather significant and suggested that earthworms under such heavy metal pollution level are at risk enough to cause reproduction disturbances leading to changes in population growth rate and size. Jurate et al., (2010). Despite the high toxicity of cadmium, the earthworm (eisenia fetida) is able to accumulate this metal in its body tissue Conder et al., (2003)

Earthworm, Eisenia fetida have the capability to accumulate heavy metals in its body (Macki Aleagha et al., 2009). The availability of metals in soil is affected by soil pH (Jordao et al., 2006).

Conclusion:
The result of this study has shown the toxicity of the selected pesticides to Lumbricus terrestris with variation in the severity with the category of chemical classes. Insecticides induced high toxic effect (based on rate of application) by revealing high level of mortality under the two bioassay procedures employed. Herbicides and fungicides demonstrated relatively less toxic effect.

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Professor A. Abdulhameed and Dr.A.J. Nayaya developed the idea and had an important role in the result and material section. Yuguda,A.U., Zainab Abubakar And Jibo AU performed the statistical analysis, the discussion and the abstract submission. The whole team had a proof read of the manuscript before final submission for publication

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