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Effect of atrazine and glyphosate on the activity and survivability of earthworms (*Lumbricus terrestris*) on farmland in Demsa Benue Trough, Adamawa State, Nigeria

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ABSTRACT
Herbicides are toxic agrochemicals being used to manage weeds in agricultural farms and gardens. These herbicides are rampantly used by farmers in Nigeria without cognizance to the long or short term effects on non-target organisms such as earthworms. The effect of two herbicides, Atrazine and Glyphosate on the activity and survivability of earthworm (*Lumbricus terrestris*) were studied under field conditions in 10 mesocosms over a period of 4 weeks. Five mesocosms each containing 40 earthworms were exposed to different concentrations of Atrazine (1000, 2000, 3000, 3500 and 4000 g/h). The other five mesocosms each containing 40 individual earthworms were exposed to different concentrations of Glyphosate (2.0, 2.4, 3.0, 3.5 and 4.0L/h). Data collected before and after treatments with Atrazine and Glyphosate were subjected to ordinary least square (OLS) regression analysis to address the first objective which is to determine the effect of atrazine and glyphosate on the activity and survivability of earthworms and analysis of variance (ANOVA) was employed to solve the second and third objectives respectively which were to determine the difference between survivability of earthworm and different doses of atrazine and glyphosate and to determine the difference between mortality of earthworm before and after treatment with atrazine and glyphosate The result of the study showed that the effect on activity and survivability was significant (P<0.05) in which survivability was significantly reduced with increase in doses of Atrazine and Glyphosate. Mortality was also significant with increase in doses of Atrazine and Glyphosate after treatments (P<0.05). Knowledge about these effects is therefore relevant for successful agricultural practices and the preservation of soil organisms' diversity.

Keywords: Atrazine, Glyphosate, Activity, Survivability, *Lumbricus terresteris*

INTRODUCTION
Ecological soil environment is a multifunctional area that requires some knowledge about the various components of hazardous particles relevant to the proportions of the biological organisms' affected (Usman, 2013). Herbicides are toxic agrochemicals which have been used to manage weeds in agricultural farms and gardens., these herbicides are wildly used by farmers in Nigeria without considering the long or short term effects in soil medium (Usman *et al*., 2017).

The use of herbicides in agriculture has over the years contributed enormously to both food and cash crop production all over the world of which Nigeria is not left out (Ayansina and Oso, 2006). One of the challenges farming system have to deal with in recent times has been the injudicious use of herbicides against the manufacturers' recommended rate to facilitate management of weeds. As a result of this, manufacturers have embraced production and supply into the market all kinds of herbicides that are meant for the elimination of different kinds of weeds at different stages of their growth (Sebiomo *et al*., 2011). Perhaps, the adulteration and non-efficacy of some herbicides in controlling the target weeds has resulted in the application of these chemicals by most farmers at not recommended rates.

A large number of farmers in Nigeria cannot read and understand herbicide label. This has resulted in the contamination of streams, rivers and ground water which are important natural resources (Mesiol, *et al*., 2018). These contaminations do not pose danger to only the non-target organisms that could be beneficial and the environment, but exposes human beings to many health challenges. Hence, the need to study the effects of some of these herbicides which are commonly used in Nigeria in order to assess their repressive effects on some of the beneficial microorganisms in the soil.

The implications of increased herbicide use for soil biology are being questioned, but a comprehensive review on this topic is lacking (Rose *et al*., 2016). The increasing use of herbicides generally call for caution due to contamination caused by these chemicals to soil and possible toxic effect on the soil organisms which includes earthworm species (Zarea and Karimi, 2012). The modern agricultural production, maintained that herbicide application should be on a regular practice primarily to minimize weeds problems in crop production (Usman *et al*., 2017). When soil is treated with herbicides possibilities exist that the chemical may exert certain effect on non-target organisms (Munoz-Leoz *et al*., 2013).

Earthworms are beneficial in terms of plant growth, mycorrhizal colonization rate, and nitrogenase activity of free-living N-fixing microoganisms, and soil microbial biomass (Zarea, *et al*., 2009; Zarea, 2010). The exclusive role of earthworms in agricultural activities is through mixing of the soil particles during digesting, depositing their casts throughout the soil column, and improving aeration and drainage of agricultural soils (Kavitha *et al*., 2011). Earthworms are also important contributors to the recycling of carbon and nitrogen in the ecosystem hence, they are used as bio indicators of soil quality (Bartz *et al*., 2013). Michael *et al*. (2016) had pointed out that glyphosate and atrazine may disrupt the feeding behavior and ecology of certain groups of earthworms. Similarly, Sebiomo *et al*., (2011) highlighted that herbicides treatment on earthworm species resulted in a significant drop in dehydrogenase activity. Therefore. This study was carried out with a view to determine the effect of atrazine and glyphosate on the activity and survivability of earthworms, to determine the difference between survivability of earthworms and different doses of atrazine and glyphosate and to determine the different between mortality of earthworms before and after treatment with atrazine and glyphosate.

MATERIALS AND METHODS

Study Site

This research work (mesocosm base) was conducted between May and June 2018 on farmlands in Demsa Local Government Area located in southern part of Adamawa state of Nigeria with Coordinates: 9°25′N 12°8′E (Demsa, 2019).

Herbicides:
The commercially available herbicides namely

i. Atrazine(2-chloro-ethylamino-6-isopropylamino-s-triazine)

Type: selective herbicide recommended for weeds control of pre- and post-emergence broadleaf weeds in crops such as maize (corn) and sugarcane. Recommended Dose: 3kg per ha.

ii. Glyphosate (Glyphosate 41% w/w)

Type: Broad Spectrum non-selective post emergence herbicide recommended for post emergence of weeds in the field and irrigation canals.

Recommended Doses:2.4 ltr per ha.

Collection of Soil

Soil containing sandy-loamy was collected from the first 30cm depth of the farmland by digging with a hoe while measuring the depth using centimeter ruler. This soil was sieved using 2 mm net to ensure that there were no earthworms' juveniles or their cocoon present then, filled into the wooden containers (mesocosms) of dimension 30 x 30cm

Experimental Setup and Collection of Earthworms

We established a two-factorial experiment using the factors Earthworms and herbicides. The anecic earthworm species, *Lumbricus terristris* are large worms feeding on soil surface litter that form deep vertical burrows into which organic fragments were incorporated are commonly found in sandy-loamy soil in Demsa Local Government Area, four hundred earthworms used in this study were collected from a farmland behind GDSS Demsa in Demsa LGA by digging carefully through the soil at 20 cm-30cm level using hoe. In order to prevent the earthworms from escaping, neat were fixed at the bottom of every treated box. These earthworms were acclimatized for 24hrs covered in a local gourd before transferring into 10 mesocosms which were divided into five treatment groups labelled A, B, C, D and E to be expose to different concentration of atrazine and glyphosate. Each group contain 40 individual earthworms (Oluah *et al*., 2010) Five mesocosms were exposed to 2.0, 2.4, 3.0, 3.5 and 4.0 L/h of Glyphosate herbicide respectively While, 1000, 2000, 3000, 3500 and 4000 g/h respectively were exposed to Atrazine herbicide after 21days.

Treatment and Application of Herbicides

Experimental treatment (A)

200, 250, 300, 350, and 400 mls respectively of Glyphosate 41% w/w were dispensed in 20 litres water capacity knapsack sprayer and sprayed on mesocosms labelled A, B, C, D and E, respectively where, treatment containing 250mls served as the control experiment which is also the recommended dose given by the manufacturers.

Experimental setup (B)

1000, 2000, 3000, 3500, and 4000 grams respectively of Atrazine (2-chloro-ethylamino-6-isopropylamino-s-triazine) were prepared and dispensed in 20 litres water capacity knapsack sprayer and sprayed on five mesocosms A, B, C, D and E, respectively where, treatment containing 3000 grams served as control.

Data Collection

The cast produced by the earthworms were counted and recorded daily for five days by placing a stick of matches in every noticed position of cast to indicate continuous activity and life in the earthworm, number of earthworms identified after 3 weeks indicates survivors.

Data Analysis

The data collected from the experiment was analyzed using various statistical techniques in line with the objectives of the study. The ordinary least square (OLS) regression analysis (simple regression) was employed in order to achieve objective (i), which is to determine the effect of different doses of atrazine and glyphosate on earthworm's activity and survivability. Analysis of variance (ANOVA) was used to achieve objective (ii) and objective (iii) which are to determine if there is any significant difference in survivability of earthworm on different doses of atrazine and glyphosate and to test if there is any significant difference in mortality of earthworms before and after treatment with various doses of atrazine and glyphosate.

Regression Analysis

The regression analysis was used to test the effect of various herbicides (Atrazine and Glyphosate) on earthworm activity and survivability. The choice of this tool of analysis is because there is need to determine the average impact of the various herbicides on earthworm activity and survivability. Therefore, the Ordinary Least Square (OLS) estimation technique was used in estimating the impact of each of the herbicides on earthworm activity and survivability.

Model specification:

The following model was used to examine the impact of various doses of herbicides on earthworm activity and survivability.
The models were express as:
Model I:
EWAS = βo +βiADA + Ut -------- eqn.1
Where:
EWAS = Earthworm activity and survivability
ADA = Average dose of Atrazine
βo – βi = Regression Parameters
Ut = Random Error Term
Model II:
EWAS = βo +βiADG + Ut --------- eqn. 2
Where:
EWAS = Earthworm activity and survivability
ADG = Average dose of Glyphosate
βo – βi = Regression Parameters
Ut = Random Error Term
Apriori Expectation

$\beta o < 0$ $\beta i < 0$

The apriori expectation suggests that we are expecting an inverse relationship between ADA and EWAS. Similarly, ADG is also expected to have same inverse relationship with EWAS which means that if average doses are increased earthworms survivability and activity decreases and vice-versa.

RESULTS

Effect of various herbicides on the activity and survivability of earthworms

Model I: Impact of Atrazine on Earthworm Activity and Survivability.
EWAS = 15.65632 – 0.208779 ADA
(Se) = (26.4072) (0.803563)
t* = 0.592879* -0.259817*
R2 = 0.02
D.W. = 1.35
Note: Standard errors in parenthesis, t-statistics in asterisk.

Based on the estimated result of the relationship between earthworm activity and survivability, and average dosage of Atrazine, there was an inverse relationship between earthworm activity and survivability, and average dosage of Atrazine. This means that as the average dosage of Atrazine increases, the earthworm activity and survivability decrease. This was in line with the apriori expectation of the model. It is generally expected that increases in dosage of Atrazine herbicide above the conventional or company recommended rate will increase mortality of earthworms, thus decreasing activity and survivability of earthworms. The coefficient of Average Dosage of Atrazine (ADA) being – 0.209 suggests that if average dose of Atrazine (ADA) should increase by 1 g, earthworm activity and survivability (EWAS) will decrease by 0.209 units. However, the standard error and t-statistic of the model suggests that ADA is not statistically significant in explaining earthworm activity and survivability. This therefore suggests that Glyphosate is more efficient in treating activity and survivability of earthworms than Atrazine. The R2 of the model being 0.02 (2%) is very low, and it suggests that only 2% of variation in earthworm activity and survivability is explained by dosages of Atrazine.

However, the low R2 value is one of the typical features of cross-sectional data analysis in regression. Finally, the Durbin Watson (D.W) statistics of the model which is 1.35 is far from 2, which suggests the presence of positive serial correlation among the residuals of the model.

Table 1: Regression Analysis

Impact of Atrazine	**Impact of Glyphosate**
EWAS = 15.656	*EWAS = 158.637*
ADA= – 0.209	*ADG= – 4.485*
(Se) = (26.407)	*(Se) = (65.032)*
(0.804)	*(1.974)*
t* = 0.593*	*t* = 2.439**
-0.259*	*-2.272**
R2= 0.02	$R^2 = 0.63$
D.W. =1.35	*D.W. = 1.69*

Model II: Impact of Glyphosate on earthworm survivability and activity

EWAS = 158.6374 – 4.484733 ADG

(Se) = (65.03223) (1.974317)

t* = 2.439366* -2.271536*

R2 = 0.63

D.W. = 1.69

Note: Standard errors in parenthesis, t-statistics in asterisk.

Based on the estimated result of the relationship between earthworm activity and survivability, and average dosage of Glyphosate, there was an inverse relationship between earthworm activity and survivability, and average dosage of Glyphosate. This means that as the average dosage of glyphosate increases, the earthworm activity and survivability decrease. This is in line with the apriori expectation of the model, as it is generally expected that increases in dosage of herbicides will increase mortality of earthworms, thus decreasing activity and survivability. The coefficient of Average dosage of Glyphosate (ADG) being –4.485 suggests that if ADG should increase by 1 Litre, EWAS will decrease by 4.485 population.

The standard errors and t-statistics of the model suggest that the variable ADG is statistically significant in explaining EWAS. The R2 of the model, being 0.63 suggests that about 63% of earthworm activity and survivability is explained by the application of dosages of Glyphosate, while the remaining 37% of variation in earthworm activity and survivability is explained by all other variables that are captured in the model such as soil type, temperature, humidity, etc. The Durbin Watson statistics of the model being 1.69 is not very far away from 2, and it suggests the presence of slight serial correlation among the residuals of the variables.

From table 2, the result of the one-way Analysis of Variance is presented on the survivability of earthworm on different doses of various herbicides. The null hypothesis that being tested, is that there is no significant difference in the survivability of earthworm on different doses of various herbicides. The value of the F- calculated was found to be (3.769) which is greater than the critical value of the F being (3.056). Therefore, we have to reject the null hypothesis and accept the alternate hypothesis. We therefore conclude that there is a significant difference in survivability of earthworm on different doses of various herbicides. This conclusion suggests that not just any dose of herbicide will provide good result on the survivability of earthworms, but at least a dose that is not arbitrary from what the producers of the herbicide recommendation.

Table 2: Survivability of Earthworms on different dose of herbicides

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	92.7	4	23.175	3.768	0.026	3.055568
Within Groups	92.25	15	6.15			
Total	184	19				

Note: the value of Fcrit (3.056) shows that there was significant different in survivability of earthworms when treated with different dose of atrazine and glyphosate.

Table 3 shows the result of the one-way Analysis of Variance on the mortality of earthworm before and after treatment with various doses of atrazine and glyphosate. The null hypothesis being tested here is that there is no significant difference in the mortality of earthworm before and after treatment with various doses of herbicides, suggesting indirectly, that herbicides do not have impact on mortality of earthworms. The value of the F-calculated was found to be 69.420, while the F-critical (Fα, k-1, n-1 = F0.05) is 5.987.

Based on the decision rule, the null hypothesis (Ho) was rejected while, the alternate hypothesis (H1) accepted. This shows that there is a significant difference in the mortality of earthworm before and after treatment with various herbicides. the outcomes of the results revealed that various herbicides have positive impact on the mortality of earthworms.

Table 3: Mortality of Earthworms before and after treatment with various doses of herbicides

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	162	1	162	69.429	0.000	5.987
Within Groups	14	6	2.333			
Total	176	7				

Note: the value of Fcrit (5.987) shows that there was significant mortality of earthworms after treatment with different dose of atrazine and glyphosate.

DISCUSSION

The result of this study showed that Atrazine herbicide used had significant effect on earthworm activity and survivability. This was similar to the finding of Oluah *et al*. (2010), who found that atrazine had significant effect on the earthworm species, Nsukkadrilus mbae studied under laboratory condition. The outcome of this study was also in agreement with the work of Lydy and Linck (2003) that Atrazine was more toxic than Chlorpoyrifs on soil organisms. However, Chelinho *et al*. (2010) had reported that atrazine applied at recommended rates had no significant impact on earthworm population but, began to inhibit earthworm reproduction in one soil when it was applied at 20.7 mg/kg, which is equivalent to a conventional application rate distributed only in the top 1cm of soil (Svendsen *et al*., 2008).

Therefore, indiscriminate use of atrazine herbicide may affect non-target organisms such as earthworms in the soil and can cause serious damage to the ecosystem (Reinecke and Reinecke, 2007) Despite some adverse effects of herbicides reported by various experimental systems in different research works, it implies that the magnitude and duration of any effects may not be easily predictable. Furthermore, even if adverse effects are observed, it is difficult to determine the exact cause of the effect. Pelosi *et al*. (2013) found that an increase in herbicide usage corresponded with decreased numbers of three different earthworms, but they could not determine whether the effects were direct, or whether they were an indirect result of the decreased organic matter inputs caused by conventional versus organic farming practices. On the contrary, Cheng *et al*. (2008) had found that multiple herbicide treatments in a long-term (15 year) field experiment did not significantly affect nematode communities under turf-grass compared with equivalent controls without herbicide treatments; whereas high N-fertilizer treatment did alter nematode community structure. This showed that the effects of other management practices or experimental treatments have the potential to be misattributed to the effects of herbicides though. However, biological function of non-target organisms in the soil particularly the earthworms that are critical in evaluation of soil fertility could be enhanced if farmers would adhere to herbicides application rates.

Based on the results obtained from the regression test (model II) using average dose of glyphosate, it was clear that the effect of glyphosate on earthworm activity and survivability was dose dependent. Increase in concentration of glyphosate particularly above the recommended rate indicated significant impact on earthworm activity and survivability. Therefore, the decrease in activity and survivability is in consonant with the work of Bon *et al*. (2006) had observed that earthworms (Esenia fetida) have been affected by the application of glyphosate. Gaupp-Berghausen *et al*. (2015) have noted that not only did exposure to herbicide reduce the number of surface casts produced, it also reduced the mean mass of individual casts (546 ± 202 mg per cast vs. 1,408 ± 140 mg per cast). This indicates that glyphosate has a harmful effect on the normal functioning of earthworms and their population in a given ecosystem. Haney *et al*. (2002) also showed that glyphosate significantly stimulated soil microbial activity in a dose-dependent manner as measured by Carbon (C) and Nitrogen (N) mineralization, but did not affect soil microbial biomass at any rates. Although it was not clear whether higher C mineralization resulted from the breakdown of the herbicide or the native soil organic matter. Indiscriminate use of herbicides may affect non-target organisms in the soil and can cause serious damage to ecosystem (Reinecke and Reinecke, 2007).

In another study, the impact of glyphosate at 10, 100, 500, and 1000 mg/kg on the earthworm, E. fetida was a gradual and significant reduction in mean weight (50%) at all test concentrations (Correia and Moreira, 2010). In the same study, 2, 4-D at 500 and 1000 mg/kg caused 100% mortality, while after 14 days, 30–40% mortality levels were observed at 1, 10, and 100 mg/kg. These rates are very high and the experiments were designed to assess the thresholds of adverse effects. However, E. fetida exhibited strong avoidance behavior in field soil treated with glyphosate at 1.44 kg/ha (Casabe´ *et al*., 2007). Avoidance behavior was also demonstrated when E. fetida were exposed to a formulation containing 5% glyphosate by mass (as isopropylamine salt) but the exact concentration was unclear (Verrell and Van-Buskirk, 2004). Also, Gaupp-Berghausan *et al*. (2015) have reported that significant decrease of hatching rate of cocoon (17% to 43% for *Lumbricus territris* and 32% to 71% for *Apporrectodea caliginosa*) after glyphosate herbicide was used.

Though studies have shown that earthworms possess chemoreceptors and sensory tubercles which give them the ability to detect chemicals in the soil and therefore move away from the affected area to minimize the hazardous impact of the chemical ingredient (Casabe *et al*., 2007). This ability could only be exercised at concentration or application rate not exceeding the manufacturers' recommendation. According to Zaller *et al*. (2014), glyphosate also has the potential to alter ecological interactions between earthworms, mycorrhizal fungi, and above-ground plants, leading to reduced mycorrhizal plant colonization and modified earthworm feeding behavior. Similarly, Okiwelu *et al*. (2011) reported that Galumna spp., Schelonbates spp., and Criptophagus spp. were susceptible to the coactive mortality effect of both herbicides and noted that, there was drastic reduction of mesofauna group in the upper 10 cm of the soil after the application of Atrazine.

On the other hand, Liphadzi *et al*. (2005) investigated the direct effects of five different glyphosate rates ranging from 0.56–4.48 kg/ha on soil nematode communities in a controlled growth-chamber experiment and found that total nematode density and densities of individual populations (herbivores, fungivores, microbivores, omnivores) were unaffected by all of the tested application rates. Similarly, application of glyphosate (0.9 kg/ha) had no significant impact on the number of Collembola in a maize-turnip rotation up to 40 days after application (Lins *et al*., 2007).

Significant differences were observed for treatments with Atrazine and Glyphosate herbicides indicating that survivability was not enhanced by their application. This also suggested that comparison between earthworms and survivability in soil were confirmed by differences in soil herbicide concentration. It therefore also shows that dose of various herbicides applied at rate greater than manufacturers' recommendation for instance 3.5 to 4.0 kg/h for Atrazine while, up-to 3 L/h for Glyphosate reduced the survivability of earthworms. There is little evidence to suggest that atrazine herbicides significantly inhibit microbial activity or C-cycling when applied at recommended rates. Moreno *et al*. (2007), found that Atrazine only affected microbial activity (respiration, dehydrogenase activity) at levels greater than 100 mg/kg, wherein increases, rather than decreases were observed. Furthermore, Atrazine applied at a conventional rate (5 mg/kg) to five different soils had no significant effect on β-glucosidase activity (Mahı´a *et a*l., 2011).

There was a significant shift from the stated null hypothesis. Mortality of earthworms were observed to be significant. Griffiths *et al*. (2008) have reported that herbicides can influence soil community structure and functions both directly through effect on soil organisms and indirectly through effects on supporting plant communities. Zarea and Karimi (2012) also showed that growth, reproduction and health of earthworms are being threatened by excessive application of herbicides to soil. The mortality of earthworms is also dependent on time they are expose to herbicides (Zarea and Karimi, 2012). The influence of other factors such as temperature and humidity could also play a critical role in the mortality of earthworms.

CONCLUSION

It is well understood that the effect of atrazine and glyphosate on the activity and survivability of earthworms is a complex subject. Although, the use of herbicides in conventional farming have become more frequent in Nigeria. Farmers are more interested on increase yield of farm produce at the expense of earthworm survivability. Mortality of earthworms was significantly recorded at higher doses of atrazine and glyphosate, therefore possibility also exist that the impact of earthworms on physical, chemical and biological properties of soil could as well be affected. Similarly, the role of earthworms in the soil ecosystem has become of great interest as researchers, scientist and educated farmers promotes management practices that encourage earthworm activity and survivability.

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