European Journal of Science, Innovation and Technology

ISSN 2786-4936

Vol. 2 No. 1 (2022)

High Mortality and Impaired Locomotor Response of Organophosphates Herbicide, Glyphosate on the African Mound Termite, *Macrotermes bellicosus* Workers

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Abstract. Glyphosate is one of the most common and widely used herbicide in sub-Saharan Africa including Nigeria and other parts of the world. Therefore, this study investigated the effects of glyphosate-based herbicide, Vinash® on the survival and locomotion of ability of worker termites *Macrotermes bellicosus*. Worker termites were exposed to six concentrations of glyphosate viz 2.34 ml, 4.68 ml, 9.36 ml, 14.04 ml, 18.72 ml and 23.4 ml per 500ml of water. Percentage mortality was recorded every 12 hours over a 48-hour period. For the locomotion performance trial, termites were exposed to similar concentrations (as above) by topical application after which the termites were acclimated for two (2) minutes then allowed to walk on a stage for 15 seconds and the distance travelled by each termite was recorded. Glyphosate exhibited a degree of mortality against worker termites but was observed to be concentration and exposure time dependent. After a 48-hour exposure, the higher concentrations tested exhibited 100% mortality in termites. Based on the LC_{50} values estimated, glyphosate is slightly toxic to worker termites. Termites' locomotion ability was also significantly affected by exposure to glyphosate but was dependent on concentration as termites exposed to lower concentrations moved faster and travelled longer distances than termites exposed to higher concentrations. Results from this study suggests that glyphosate is capable of causing high mortality levels and reductions in locomotion ability in worker termites and this may consequently affect the ecosystem services rendered by termites. Therefore, manufacturing, storage, use and disposal of glyphosate-based herbicides should be effectively controlled.

Keywords: Mortality, *Macrotermes, bellicosus*, Organophosphates herbicide, Glyphosate

Introduction

Weed control management has a vital role in increasing crop yield and yield components in agriculture. Cultural practices of weeding are laborious, tedious; time consuming and expensive in contrast chemical weed control method is easy, time saving and effective (Hameed *et al*., 2017). Most farmers use herbicides such as Organophosphate herbicide, Glyphosate for weed control in Nigeria and other farming countries (Abraham *et al*., 2018). Glyphosate is a non-selective, broad spectrum systemic herbicide that can control most annual and perennial plants (Tu *et al*., 2001; Benbrook, 2016). It is applied to plant to kill or suppress both broadleaf plants such as forbs, vines, shrubs, trees and grasses (Tu *et al*., 2001; NPIC, 2015). It controls weeds by inhibiting the synthesis of aromatic amino acids necessary for protein formation in susceptible plants (Tu *et al*., 2001).

Glyphosate is a phosphonic acid resulting from the formal oxidative coupling of the methyl group of methylphosphonic acid with the amino group of glycine. Glyphosate has the empirical formula $C_3H_8NO_5P$ and a molecular weight of 169.1. The solubility of glyphosate in water is 1.2% at 25 °C. It is not generally soluble in organic solvents (Sousa, *et al*., 2019)). The scientific literature reported the movement and residues of glyphosate and its breakdown product Aminomethyl phosphonic acid (AMPA) in soil and water, their toxicity to macro- and microorganisms, their effects on microbial compositions and potential indirect effects on plant,

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animal, and human health (Van Bruggen *et al*., 2018). Glyphosate may contaminate soils in and around treated areas. Glyphosate adsorbs to clay and organic matter, slowing its degradation by soil microorganisms and leading to accumulation in soils over time (Sidoli *et al.*, 2016).

Glyphosate is applied to leaves of plant to kill both broadleaf plant and grasses (NPIC, 2015). Several factors contribute to the success and widespread use of this active ingredient, including: (i) ability to translocate throughout treated plants and control re-sprouting in perennial weeds; (ii) generally favourable environmental profile including strong binding and immobility in soils and rapid biodegradation in most soils, water and sediments; (iii) plant-specific mechanism of action (5-enolpyruvyl-shikimate-3-phosphate synthetase or EPSPS enzyme of the shikimic acid pathway of plants) and (iv) innately low toxicity to animals and minimal ecological impact in forest ecosystems (Rolando *et al*., 2017). Glyphosate is applied in a variety of forms including isopropylamine salt, ammonium salt, diammonium salt, dimethylammonium salt, and potassium salt (Benbrook, 2016).

Glyphosate is a systemic herbicide that is phloem mobile and is readily translocated throughout the plant (Perez *et al*., 2011; Rolando *et al*., 2017). From the leaf surface, glyphosate molecules are absorbed into the plant cells where they are translocated to the meristems of growing plants. Glyphosate kills plants by inhibiting the activity of the enzyme 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSP), by binding to it, forming a complex (Sammons & Gaines, 2014) which is necessary for the formation of the aromatic amino acids' tyrosine, tryptophan, and phenylalanine (Tu *et al*., 2001; Perez *et al*., 2011). This inhibition prevents the production of chorismate, which is the last common precursor in the biosynthesis of numerous aromatic compounds in bacteria, fungi and plants.

For years glyphosate has been thought to be of relatively low toxicity to birds and mammals but moderately toxic to fish (Tu *et al*., 2001; Benbrook, 2016). After some reviews, however, glyphosate was classified in 2015 as a "probable human carcinogen" by the International Agency for Research on Cancer, based on increased prevalence of rare liver and kidney tumors in chronic animal feeding studies, epidemiological studies reporting positive associations with non-Hodgkin lymphoma, and strong mechanistic evidence of genotoxicity and ability to trigger oxidative stress (Benbrook, 2016).

Glyphosate herbicides have both direct and indirect effects on animals including beneficial insect such as biocontrol agents (Katembo *et al.*, 2013) and at varying concentrations. Terrestrial fauna residing in forested areas treated with glyphosate are potentially at risk of exposure to glyphosate via direct spray, spray drift or wash-off following rainfall events, and uptake via inhalation and absorption. Secondary exposure is also possible through the ingestion of flora and fauna food sources containing glyphosate residues (Rolando *et al*., 2017). Indirect effects of glyphosate to both terrestrial and aquatic fauna have been associated with changes in plant community composition, habitat structure, cover, and food sources and are primarily a consequence of glyphosate's phytotoxic effects rather than a result of ecotoxic qualities unique to the active ingredient (Rolando *et al*., 2017).

Glyphosate have been shown to have effects on bacteria, protozoa, invertebrates such as insects, fish and amphibians e.g. frogs at a much lower toxicity but show significant effect in growth, reproduction and metabolism of snails and worms at concentrations of < 1 mg/L of glyphosate (Perez *et al*., 2011; Abraham *et al*., 2018). Glyphosate doses between 0.1 and 10 mg acid equivalent l^{-1} have been found to reduce growth in the earthworm *Aporrectoden caliginom* (Haplotaxida: Lumbricidae) (Savigny) and affect reproduction and development in the freshwater snail *Pseudosuccinea columella* (Hygrophila: Lymnaeidae) (Say). Despite these findings there are others that report negative and lethal effects on invertebrates such as amphipods (see Balbuena *et al*., 2017 and references therein).

Termites form an essential component of soil ecology having successfully coevolved for millions of years (Khan *et al*., 2018). They are dominant invertebrates in tropical soils and represent as much as 10% of all animal biomass and up to 95% of soil insect biomass (Khan *et al*., 2018). Termites can be used as food for both humans and chickens (van Huis, 2017). Termites act as herbivores as well as decomposers, feeding on a wide range of living, dead or decaying plant material including and turnover of large volumes of soil rich in organic matter and fungi (Freymann *et al*., 2008). Termite mounds have been used for geochemical prospecting, building, fertilizer, hunting small mammals and eaten a practice called geophagy (practice of eating earth or soils) (van Huis, 2017). Mushrooms (basidiomes) of Termitomyces species are commonly observed on termite mounds and are collected by humans as food (De Fine Licht *et al*., 2005). Through their mound-building activities and impact on plant growth, termites enhance the heterogeneity of their ecosystems (Khan *et al*., 2018). Tropical rainforest is often associated with low-fertility soils and termite cycling of organic matter efficiently contributes to the return of nutrients to the vegetation (Khan *et al*., 2018). Termites are also the main agents for primary breakdown of surface mulches under conservation agriculture (Khan *et al*., 2018). They also perforate soil surfaces resulting in increased water infiltration rates.

Higher termites are gathered into a single family, the Termitidae, which comprises approximately 75% of all modern termite species (Krishna *et al*., 2013). Despite the obvious damage done by this class of insects, termites play a key role in ecosystem functions and are called ecosystem engineers or keystone species (Joquet *et al*., 2015). Through bioturbation they incorporate plant litter and crop residues into the soil, thereby modifying biological, chemical, and physical soil processes that affect the flow of energy and material resulting in the modification of the habitat of other soil biota (Joquet *et al*., 2016; Khan *et al*., 2018).

In spite of this call, the European Union, including Germany, has given approval for the use of glyphosate for the period 16 December 2017 to 15 December 2022 under strict conditions including ‘paying particular attention to the risk to diversity and abundance of non-target terrestrial arthropods and vertebrates via trophic interactions (Abraham *et al*., 2018). Therefore, this study evaluates the effects of organophosphate herbicide, glyphosate on the survival and mobility of termites, *Macrotermes bellicosus*.

Materials and Methods

Pesticide & Herbicide preparation

Vinash® (Glyphosate), one of the most used products for controlling weeds in Edo State, Nigeria, was chosen for this study. The chemical was purchased from the market at Ring Road Benin City, Edo State. A range of concentrations were tested from below to far above manufacturers recommended values for usage in weed control. The full rate 2.34ml/500ml of water was obtained based on the manufacturers recommended values (75ml/ 16litres of water). The concentrations used were 2.34ml (full rate) per 500ml of water, 4.68 (2x full rate) per 500ml of water, 9.36ml (4x full rate) per 500ml of water, 14.04ml (6x full rate) per 500ml of water, 18.72ml (8x full rate) and 23.4ml (10x full rate) (Plate 2). The concentrations of herbicide were measured and mixed with 500 ml of water in separate bottles. The mixture was shaken thoroughly to ensure a level solution.

Animals & Treatment

Macrotermes bellicosus worker termites were collected from a termitarium at Faculty of Life Sciences, (6°23’55”N and 5°36’54”E), University of Benin, Benin City, Edo state, Nigeria. The termitarium was dug up using a shovel and soil containing termites were put into a plastic container and taken to the laboratory of the Department of Animal and Environmental

Biology, and kept in a cool dark place until needed for the experiment (not more than 12 hours). When needed the termites were removed from the soil using a fine camel brush.

Mortality bioassay

To perform the mortality bioassay of worker termites, a drop of the test chemicals at different concentrations 2.34 ml, 4.68 ml, 9.36 ml, 14.04 ml, 18.72 ml and 23.4 ml per 500ml of water was applied dorsally to each insect in a petri-dish already lined with filter paper (Whatman No. 1) with 9 cm diameter. Since worker termites are the caste of termites which go out often to scout for food, they might be sprayed on by herbicide users or weed controllers. A control (herbicide-free) was set up where a drop of distilled water was applied to each insect. Five replicates of five insects were used for each of treatment. The experiment was laid out following the randomized design (Plate 4). All Petri dishes having termites (treated with the herbicide or untreated) placed inside were kept in darkness by covering with a black plastic sheet to imitate the dark environment of the termite mounds at a temperature of 24.03 ± 2.78˚C and relative humidity of 60 – 70%. Insect mortality was monitored every 12 hours for up to 48 hours after application of the herbicide. A termite was considered dead if it showed no signs of movement when touched lightly with a soft camel hair brush. Percentage mortality was calculated using the equation:

$$\text{Mortality (\%)}=\frac{\text{Number of dead termites}}{\text{Total number of termites}}\text{ x }100$$

Locomotion trials

Termites were obtained for the locomotion trials as previously described. After collection the insects were placed in Petri dishes in preparation for the experiment. The effect of herbicide exposure to termite locomotor ability was determined by recording the ability to walk and the distance when exposed to a range of concentrations (2.34 ml, 4.68 ml, 9.36 ml, 14.04 ml, 18.72 ml and 23.4 ml per 500ml of water). During spraying of glyphosate herbicide termites might be sprayed upon since termite workers go out to forage. Five termite worker were exposed singularly to a particular concentration by applying one drop per chemical dorsally to the insect and kept in an open Petri dish for 2 minutes to acclimatize after which the insect was allowed to walk on a stage of 83x60 cm for 15 seconds and the total distance walked measured. An insect was recorded as not able to move when it is dead, no sign of movement detected when righted and probed with a soft brush or unable to move a significant distance i.e. moving and falling on its back. A total of five replicates were used for each concentration of the test chemicals. A control treatment was also set up using drop of distilled water. The speed of individual termite was calculated by dividing the distance covered (in cm) by the total time the termite was exposed for (15 seconds).

Statistical Analysis

Following arcsine square root transformation of the mortality data, the effects of different concentrations of glyphosate and exposure time on mortality was analysed using a Generalized Linear Model (GLZ) (assuming normal distribution with an identity link function). When the overall results were significant in the GLZ analysis, the difference among the treatments was compared using the sequential Bonferroni test. Probit regression was used to estimate the concentrations of the herbicide estimated to cause 50 and 90% mortality (LC_{50} and LC_{90}), the concentrations causing 50% and 90% of tested individuals to die in a given period (i.e. 24, 36 and 48 hours). The effect of the herbicide on the distance and speed of *Macrotermes bellicosus* was evaluated using General Linear Model Analysis of Variance (GLM ANOVA). When the overall results were significant in the GLM ANOVA, the difference among the treatments was compared using Tukey’s Honest Significant Difference (HSD) test. All analyses were performed using SPSS Statistical software, version 22.0 (IBM SPSS, Chicago, IL, USA).

Results

Mortality Tests

Macrotermes bellicosus exhibited varying levels of mortality when exposed to different concentrations of glyphosate (Figures 1-4). Percentage mortality varied significantly (GLZ: Wald $\chi^2_6 = 15.658$; P=0.016) following a 12-hour exposure (Figure 1). There was no significant difference in percentage mortality between termites exposed to 2.34ml, 4.68ml, 9.36ml and 14.04ml. The highest percentage mortality recorded was due to exposure to 23.4ml and the least percentage mortality was control [0.0ml (water)]. Following a 24-hour exposure percentage mortality of termites varied significantly (Wald $\chi^2_6 = 109.44$; P=0.0001) with 23.4ml causing the highest percentage mortality and the control the least percentage mortality (Figure 2). Percentage mortality varied significantly (Wald $\chi^2_6 = 276.54$; P=0.0001) following a 36-hour exposure with 18.72ml and 23.4ml highest percentage mortality and the least percentage mortality was in the control treatment (Figure 3). Following a 48-hour exposure, percentage mortality varied significantly (Wald $\chi^2_6 = 276.54$; P=0.0001) with no significant difference in mortality between 4.68ml, 9.36ml, 14.04ml, 18.72ml and 23.4ml of glyphosate (Figure 4). The concentrations used caused between 68 and 100% mortality after 48-hour exposure. Percentage mortality increased as a function of exposure time (Figure 5). Based on the mortality tests, concentrations estimated to cause 50% (LC_{50}) and 90% (LC_{90}) were calculated. LC_{50} and LC_{90} decreased with increase in exposure time (Table 1). When termites were exposed for 24 hours LC_{50} was 20.10ml while LC_{90} was 102.16ml. Following a 36-hour exposure of termites to different concentrations of glyphosate LC_{50} was 4.14ml and LC_{90} was 13.31ml. Finally, when termites were exposed for 48hours LC_{50} was 2.15ml and LC_{90} was 8.19ml.

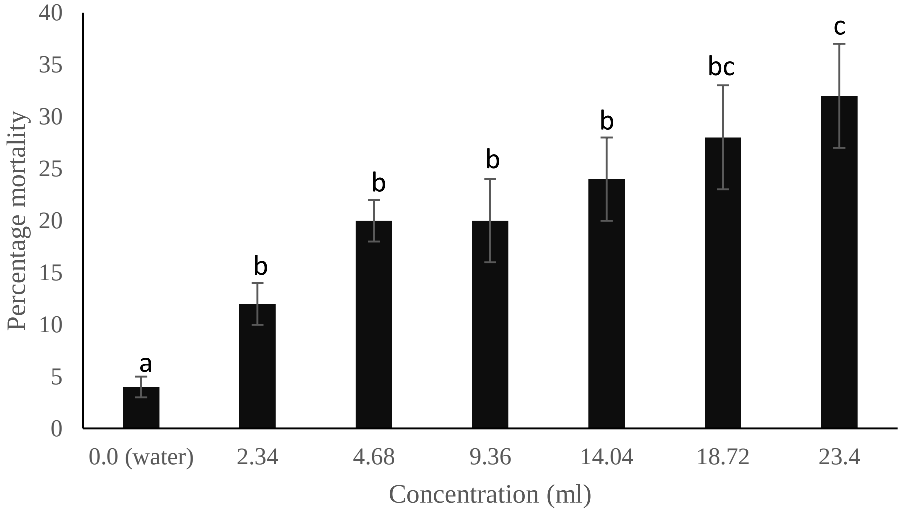

Figure 1: Percentage mean (±SE) mortality of *Macrotermes bellicosus* following a 12-hour exposure to different concentrations of glyphosate. Means capped with different letters are significantly (sequential Bonferroni test: P<0.05)

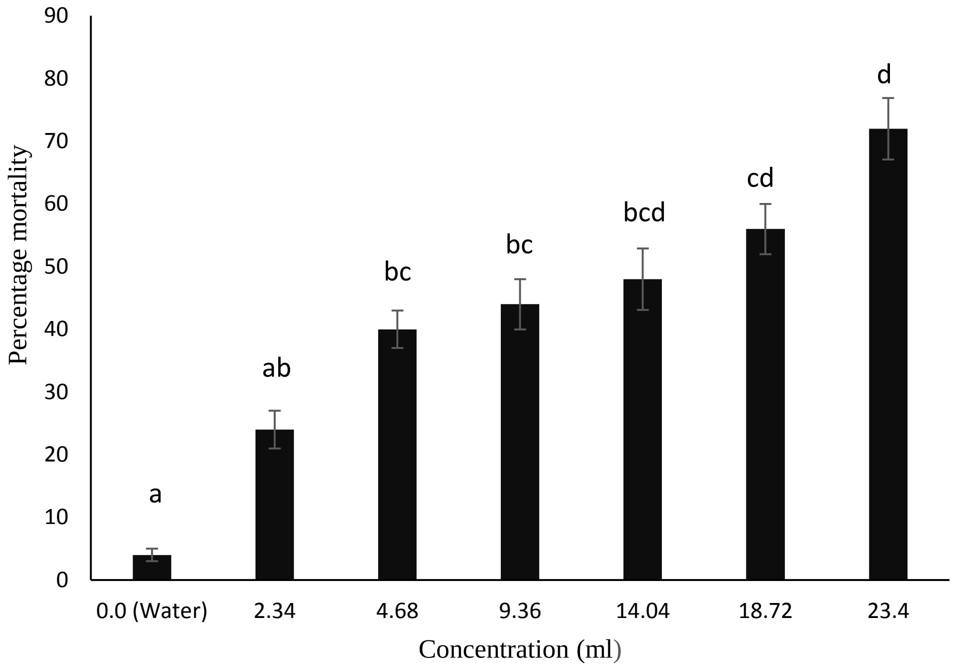

Figure 2: Percentage mean (±SE) mortality of *Macrotermes bellicosus* following a 24-hour exposure to different concentrations of glyphosate. Means capped with different letters are significantly (sequential Bonferroni test: P<0.05)

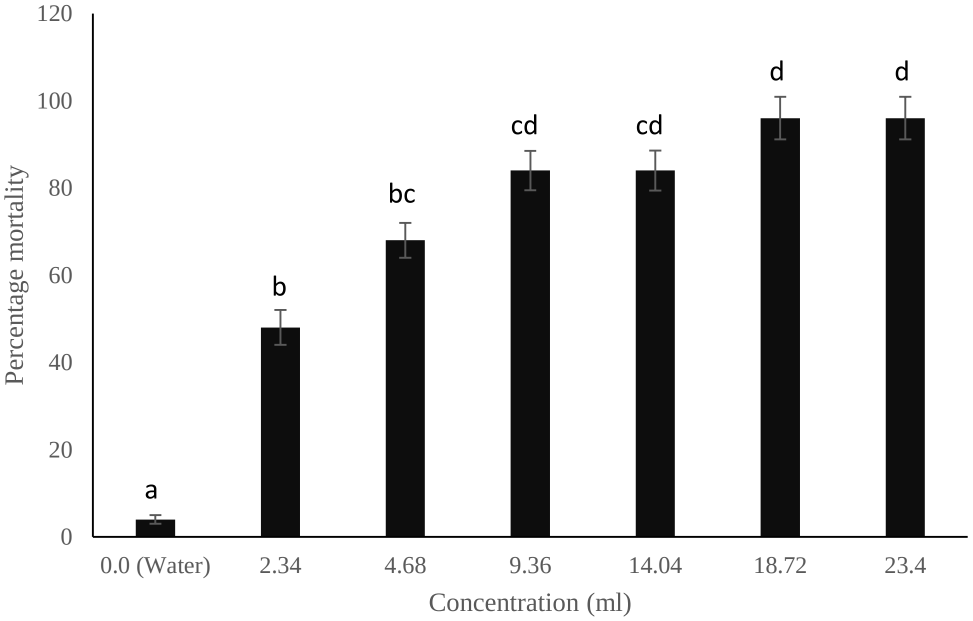

Figure 3: Percentage mean (±SE) mortality of *Macrotermes bellicosus* following a 36-hour exposure to different concentrations of glyphosate. Means capped with different letters are significantly (sequential Bonferroni test: P<0.05)

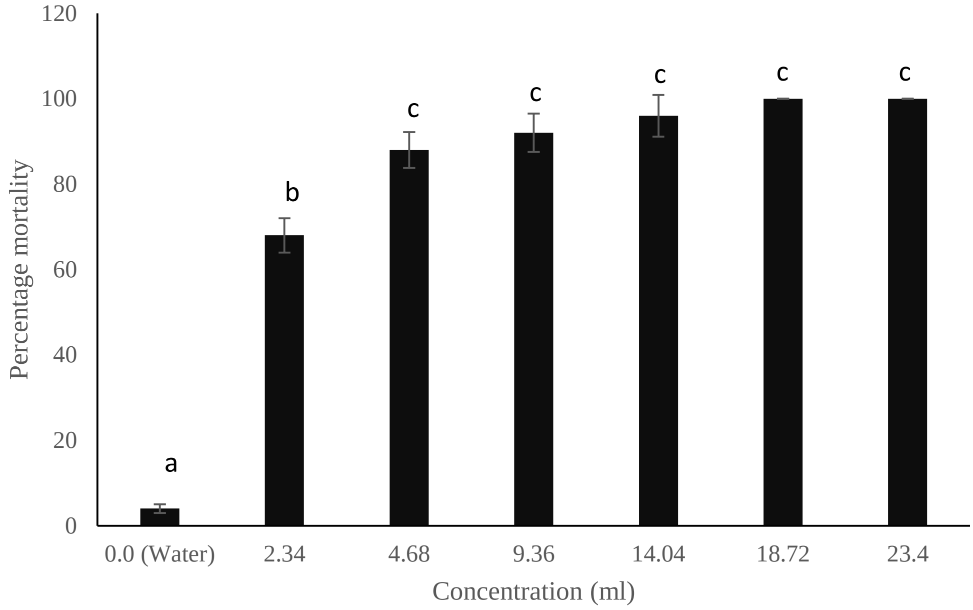

Figure 4: Percentage mean (±SE) mortality of *Macrotermes bellicosus* following a 48-hour exposure to different concentrations of glyphosate. Means capped with different letters are significantly (sequential Bonferroni test: P<0.05)

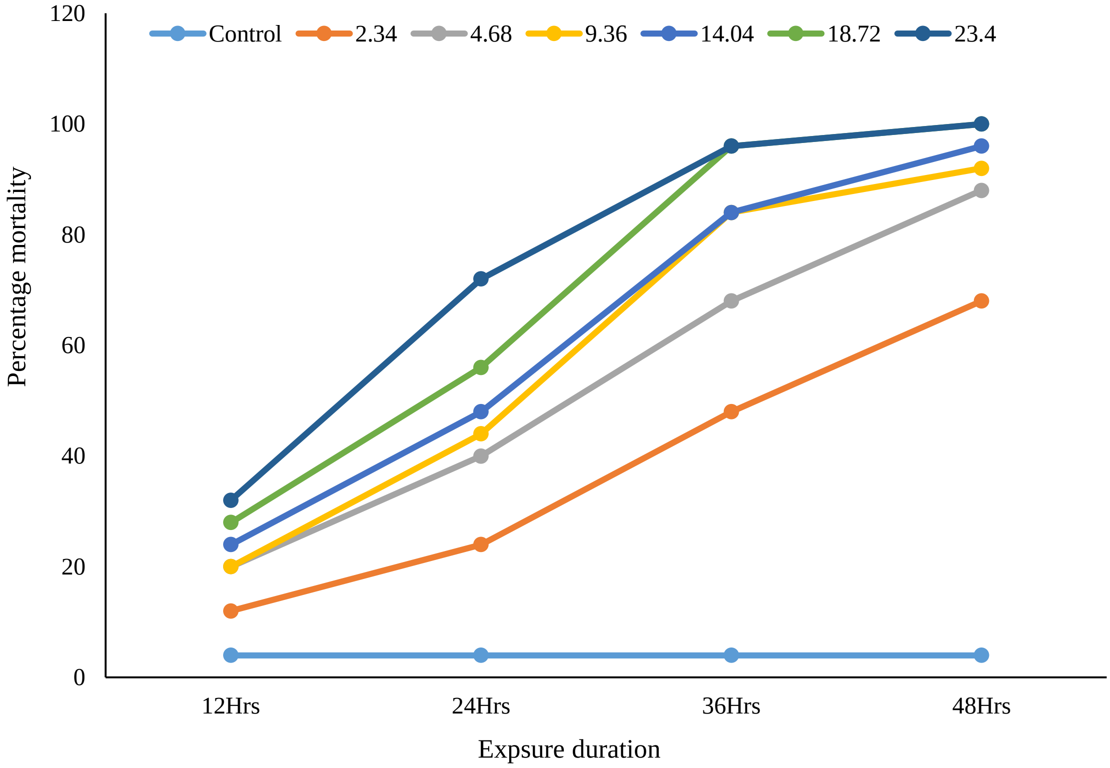

Figure 5: Percentage mortality of *Macrotermes bellicosus* following exposure to four different time periods

Table 1. Index of toxicity (LC_{50} and LC_{90}) of *Macrotermes bellicosus* when to different concentrations of paraquat for various durations

Exposure time (hours)	Index of toxicity		95% confidence interval	
	LC_{50} (ml)	LC_{90} (ml)	LC_{50} (ml)	LC_{90} (ml)
24	20.068	102.16	18.27-23-15	102.11-107.42
36	4.14	13.31	3.26-4.91	12.27-14.93
48	2.148	8.192	1.99-2.27	7.12-9.10

Locomotion Performance

Locomotion performance of *Macrotermes bellicosus* varied with an increase in the concentrations of glyphosate. Distance covered by termites varied significantly ($F_{6,34}$= 3.64; P = 0.011) with longest distance covered by termites treated with 14.0ml and the shortest distance covered by termites exposed to 18.72ml and 23.4ml (Figure 6). Speed of termites also varied significantly ($F_{6,34}$= 3.64; P= 0.011) with termites treated with 14.04ml of glyphosate having the highest speed and termites treated with 18.72ml and 23.4ml having the least speed (Figure 7). There was no significant difference in distance covered and speed in termites treated with water (control), 2.34ml, 4.68ml and 9.36ml of glyphosate.

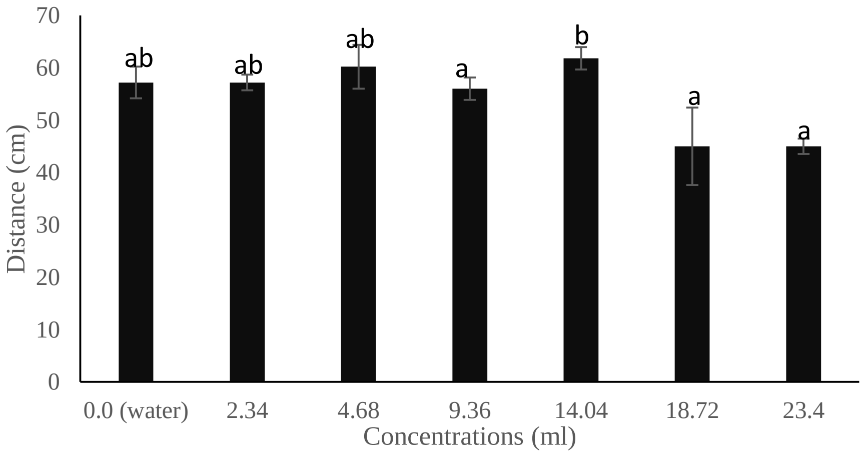

Figure 6: Mean (±SE) distance covered by *Macrotermes bellicosus* after exposure to different concentrations of glyphosate herbicide. Means capped with same letters are not significantly different [after Tukey's Honest Significant Difference (HSD) test: P>0.05]

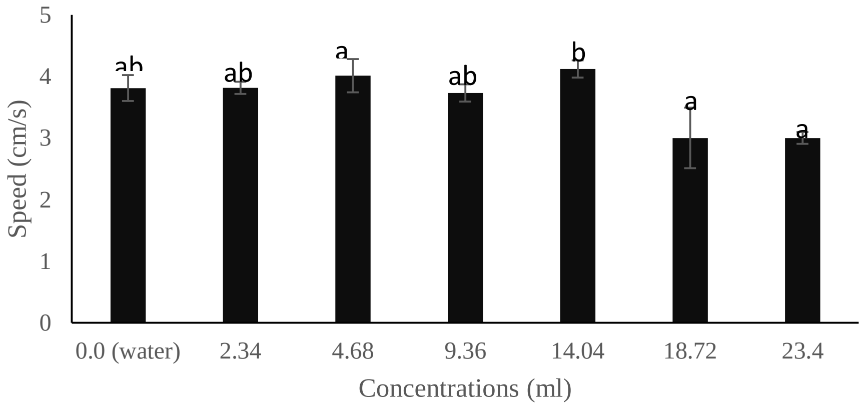

Figure 7: Mean (±SE) speed of *Macrotermes bellicosus* after exposure to different concentrations of paraquat herbicide. Means capped with same letters are not significantly different [after Tukey's Honest Significant Difference (HSD) test: P>0.05]

Discussion

High demands for food in growing world population has led to an increase in the use of pesticides (herbicides) to enhance crop yield. In many sub-Saharan countries including Nigeria, agricultural land preparation increasingly depends on the use of glyphosate-based herbicides which in turn leads to an increase in production of these herbicides (Abraham *et al*., 2018). This study reveals that glyphosate-based herbicide can kill termites both at the recommended concentration and as well as above the manufacturers recommended concentrations, and significantly impair the locomotion performance of the termites used in this study.

This study also demonstrates that exposure to glyphosate-based herbicide caused significant mortality and affected locomotive abilities in termites but the results were dependent on duration of exposure and concentration tested. Although there are several reports on the toxicity of glyphosate on other animals such as small mammals, birds, fishes, amphibian, snails, crustaceans and insects especially bees (e.g. Rolando *et al*., 2017; Abraham *et al*., 2018 and references therein), reports on termites are virtually nonexistent. Affeld *et al*. (2003) reported that glyphosate caused significant mortality to the psyllid *Arytainilla spartiophila* Förster (Psyllidae), the twig mining moth *Leucoptera spartifoliella* Hübner (Lyonetiidae) and the broom seed beetle *Bruchidius villosus* F. (Chrysomelidae) at a concentration equivalent to the recommended value and below recommended values. This study is the first to report the toxicity of glyphosate-based herbicide on worker termites. Although the toxicity of glyphosate is not directed at insects, this study shows that glyphosate-based herbicide can cause up to 100% mortality in termites at a longer duration (48 hours) and at high concentrations above recommended concentration but other reports on other insect species have stated otherwise (Hill *et al*., 2012). The study also showed that toxicity increased at higher concentrations and these results are consistent with previous reports (Hill *et al*., 2012; Abraham *et al*., 2018). Also, Lough (2003) reported decrease in ant community over a period of two years after glyphosate application in a forest. Extrapolation of these results from laboratory to the field must take into consideration the behavior of the insect species. Worker termites' forages during the day when herbicide application occurs and is therefore likely to come in contact with droplets of the herbicide.

LC_{50} values were generally decreased with an increased in exposure time thereby implying that toxicity increased with time and this corresponds to the reports of Hill *et al*. (2012). Concentrations estimated to cause 50% mortality could not be estimated as mortality was low after 12-hour exposure. Based on the LC_{50} values and the recommended concentration for use glyphosate-based herbicide is slightly harmful to termites.

Reports on the effect of glyphosate on termite locomotion is non-existent, therefore, this study is the first to report the effect of glyphosate-based herbicide on termite locomotion. This study showed that at low concentrations of glyphosate herbicide termites move faster and covered more distance which could be due to secondary consequences of behavioural modification such as disruption in the detection of kairomones that result in an increase of angular speed due to higher arrestment of kariomone patches and a repellent or irritant effect of pesticides (Desneux *et al*., 2007). Also, this study showed that at higher concentrations termite mobility was greatly reduced which can be caused by direct intoxication resulting in knock-down effect, trembling and tumbling. Increase in mobility of termites might result in greater activity but in contrast reduced mobility can result lead to predation and increased exposure to harsh environmental conditions. Irritant effect may induce movement of termites away from treated areas and might result in reduced foraging area leading to reduction in food supply (Desneux *et al*., 2007). The reports of this study contradict a similar study by Balbuena *et al*. (2015) on honeybee *Apis melifera*. The authors reported no effect in locomotive abilities of foraging bees fed with sucrose solution contaminated with glyphosate herbicide but found that glyphosate affects navigation in honey bees which was not tested in this study. Mortality and reduced mobility in foraging termites might lead to a reduced work force which might result in starvation of the colony as other termite's caste depends on the food brought back by foraging worker termites. Also, this might negatively affect their functions as ecosystem engineers (Sahu, 2017).

In conclusion, this study showed that glyphosate can kill beneficial arthropods such as termites especially at concentrations above the recommended rates. The recent rise in the use of glyphosate-based herbicide is alarming therefore, relevant authorities should regulate the use of glyphosate and glyphosate-based herbicides to prevent potential damages to the environment. Government authorities should be effectively encouraged to control the licensing, manufacturing, storage, import, method of use, delivery, disposal of glyphosate-based herbicides. A decline in the population of termites (which are important as ecosystem engineers or keystone species play a key role as detritivores and have positive effect on soil structure and nutrient richness) may lead to reduction in the ecosystem services rendered by this insect species or may result in the loss of ecosystem integrity.

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