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Earthworms' response to Pendimethalin in a maize- based cropping system and *in situ* toxicity testing in Southern Guinea Savannah, Nigeria

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Abstract

Pendimethalin at 1.0, 1.5, 2.0 l/ha, hoe weeded, Weedy Check and mancozeb (2 kg a. i. /ha) were applied to maize plots. Soil and worm cast were randomly sampled for physico-chemical analyses Before Spraying (BS) and worm cast alone at 90 Days After Spraying (DAS). Earthworm density and species were determined using formalin extraction method at planting and 30 DAS. Lethal Concentrations for 50% (LC_{50}) mortality on two earthworm species were determined by Contact Filter Paper (CFP) and Soil Test (ST). Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.The mortality data from the lethal toxicity tests were evaluated using probit analysis (Finney, 1971) with the help of a computer programme. The sub lethal toxicity values were compared with Chi square (X^2) test. Densities of *Lumbricus terrestris* (0.6±0.3*), Eisenia fetida* (0.9±0.3), and *Libyodrilus violaceus* (0.9±0.2) BP were lower at 30 DAP (0.8±0.2, 2.3±0.5 and 0.9±0.1 respectively). *E. fetida* was more acutely affected by Pendimethalin in CPF-test than *L. violaceus* as indicated by their LC_{50} values of 1.93 and 2.56 l/ha respectively. *L. violaceus* suffered negative growth in the range 2.6% in the control to 39.4% at 2 l/ha for *L. violaceus.* The toxicity varied with earthworm species.

Keywords: Pendimethalin, LC_{50}, *Eisenia fetida, Libyodrilus violaceus Lumbricus terrestris.*

1.0. Introduction

Earthworms react to herbicides due to an even distribution of sensitive receptors all over the body. In soil, they may escape into deeper layers and the toxic effect of herbicide on them may be partly reduced. Pizl (1988) reported that Zeatin 50 was moderately toxic to earthworms and the degree of toxicity varied with species in the laboratory tests. The LC_{50} test (Lethal concentration that is expected to produce death in 50% of the tested organisms) is the most common way of estimating the toxicity of herbicides but it is difficult to conclude from such mortality test what kind of ecological effects a pesticide might have when it is used under field conditions. According to him, sub-lethal effects such as retarded growth or development, low fertility, etc. might cause population changes in the field although the animals do not suffer from high acute toxicity (Pizl, 1988).

Earthworms are found in a wide range of habitats throughout the world, being adapted to many different soil types as well as to lakes and streams. Earthworms are often called night crawlers, garden worms, red worms or, simply, worms (Karen and Koval, 2014).Worm excrement is commonly called worm casts or castings. These soil clusters are glued together when excreted by the earthworm and are quite resistant to erosive forces. Their castings contain many more microorganisms than food sources because their intestines inoculate the casts with microorganisms (Van Gestel and Van Dis, 1988).A greater proportion (80%) of biomass of terrestrial invertebrates is represented by earthworms which play an important role in structuring and increasing the nutrient content of the soil. Therefore, they can be suitable bio-indicators of chemical contamination of the soil in terrestrial ecosystems providing an early warning of deterioration in soil quality (Sorourand Larink, 2001; Bustos-Obregón and Goicochea,2002).*Eisenia fetida* is also known as the red worm, red wiggler, brandling worm, dung worm, and the tiger worm. Itbelongs to family lumbricidae, phylum annelida, class oligocheata. Each of these names including its scientific name *Eisenia fetida* relate to some it its physical characteristics. *Eisenia fetida* can regenerate the anterior segments of its body from segments 23 and 24 and the posterior segments of its body from segments 20 and 21. The first (head) segment contains a pair of cerebral ganglia which are nerve cords that act like eyes for the worm. Presumably these pairs of cerebral ganglia are responsible for *Eisenia fetida*'s great sensitivity to light. Some estimate that the *Eisenia fetida* can consume up to its entire body weight in food each day.

Eisenia fetida usually create cocoons at a rate of about one per 14 to 21days. These cocoons usually contain 8-20 eggs, however, usually only about two of these actually hatch. Cocoons are very small and also change color as they age, beginning very light and becoming darker with a reddish tinge before baby worms emerge. The average incubation period for *E. fetida* is between 32 and 73 days. These worms usually live around 1.5 to 2 years, though they have been known to live for up to 5 years (*http://bioweb.uwlax.edu/bio203/2010/yard_jose/*)
L. violaceus is a West Africa earthworm species belongingto the family Eudrilidae, phylum annelida, and classoligocheata. It is described to varying extent by (Bamgbose *et al*, 2000). It is an endogeic (soil dwelling), limicolousspecies, that is, unlike most friable soil earthworms, available all year round. The species is widely distributed in the middle belt down south of Nigeria and in Cameroon. It makes a major contribution to the productivity of wetland soils in Nigeria (Owa *et al*, 2010). It is un-pigmented with round segmentation. It has an annular (ring form) and pinkish clitellum between segments 13 and 18. The female pore appears as a pair of humid clear zones between segments 13 and 14, while the male pore is unpaired and pinkish between segments 17 and 18. It has no dorsal pore (Ebenezer *et al*, 2013).Moreover, studies have shown that earthworm skin is a significant route of contaminant uptake and thus investigation of earthworm biomarkers in the ecological risk assessment (ERA) can be helpful (Lord *et al*, 1980).There are no relevant international standards for earthworms' toxicity testing. There are many methods of testing toxicity of chemicals to earthworms, including spot application, forced feeding and immersion tests (OECD, 1984).Most herbicides are nontoxic to earthworms, although some, such as 2,4-D, pendimethalin (N-(1-ethylpropyl)-3, 4-dimethy1-2, 6-dinitrobenzea-amine), and simazine, are toxic at high exposure rates. Earthworms that crawl on the soil surface (such as night crawlers) have a higher exposure to surface-applied pesticides than those feeding and burrowing below the soil surface. On the other hand, pesticides injected in a small slot in the soil (such as the seed slot) may not come in contact with many earthworms and therefore will not pose a significant threat for the population at large.

Objective

i. To evaluate the impact of pendimethalin on earthworms as index of soil pollution on the.

ii. To determine the LC_{50} and sub-lethal concentrations of pendimethalin for earthworms.

2.0. Materials and Methods

This research was conducted in two planting seasons at Ladoke Akintola University of Technology (LAUTECH), Teaching and Research Farm (LTRF), Ogbomoso and Ogbomoso farm settlement (OFS), located on (Lat. 80^{o} $10^{'}$; Long $4^{o}10^{'}$E; Altitude 700 m), in the Southern Guinea Savanna (SGS) vegetation zone in the South Western Nigeria.

2.1 Initial soil and worm cast sampling for Physico-chemical analysis

Systematic soil sampling, 0-15 cm deep (top soil) was carried out on the field using soil auger. This was done across each of the field (2,059 m^2) in diagonal systematic sampling on thirty spots before land preparation started. The soil samples were mixed, sub-sampled and analyzed for pH, cation exchange capacity, particle size and organic matter (OM). Also, 0.25 m x 0.25 mquadrat was used to sample worm cast following the same diagonal method. Worm casts within the quadrats were collected, weighed and analyzed for exchangeable cations such as Potassium, and exchangeable cation exchange capacity. Particle size and pH were also determined. All these samples were analyzed at International Institute of Tropical Agriculture (IITA) soil laboratory, Ibadan.

2.2 Land preparation and experimental design

The site in each of the two locations was disc ploughed twice and manually levelled.

The experiment was laid out in a Randomized Complete Block Design (RCBD) with six treatments in a block and replicated thrice. Each block contained nine (9) beds of 5 m by 5 m. There were 2 m alley between blocks and beds. There were three levels of Pendimethalin; two controls of hand weeding alone and zero weeding (weedy check); and a toxic standard chemical (fungicide: Mancozeb) to cater for earthworm toxicity testing (Hogger, and Ammon, 1994). The treatments were as follows:

P3 = (50% = 1.0l/ha)
W_0 = (zero weed control) .
P2 = (75% = 1.5l/ha)
Wh = (hand weeding alone)
P1 = (100% = 2.0l/ha)
P = Pendimethalin
T. = (100% = 2.0kgai/ha)

The spray volume for pendimethalin was 200 l/ha. Maize (*Zea mays* L.), Oba super variety bought from IITA (7^o 23' 47'' N 3^o 550'' E / 7.39639^{o}N), Ibadan, was planted in August as late maize at LAUTECH Teaching and Research Farm (LTRF) and Ogbomoso Farm Settlement (OFS) (Southern Guinea Savannah).The prepared plots were sowed with the maize grains spaced at 0.1 m x 0.3 m at 2 grains per stand which was thinned down to one at two weeks after planting (2WAP) given a population of 40,800 plants/ha.

2.3 Estimation of earthworm abundance in the field

Earthworm populations were estimated three times in the course of the experiment. The first estimation took place before the herbicide application. The second and third estimation were respectively carried out at one, and three months after herbicide application (Kula, 1992). Extraction of the earthworms was done with three applications of five litres each of 0.1% formaldehyde solution in a metal ring of 0.25 metre square (0.56 m diameter) and 0.15m height. The metal ring was always pressed into the soil to about 0.05 m with strong wood (Hogger, 1993). Two samples per plot were taken and the expelled earthworms were collected in water, identified to species level, counted and weighed using a digital scale of capacity 0.1 – 120 g.

2.4 Data analysis

All data collected were analyzed using analysis of variance (ANOVA) after square root ($\sqrt{}$ (x+0.5), and log ($\log_e$(x+1) transformations, where x is the number and weight of earthworm in the respective equation. The treatment means were separated using Duncan Multiple Range Test (DMRT) where F-values were significant.

2.5 Determination of LC_{50} and sub lethal toxicity of pendimethalinon *Eisenia fetida* (Sp. A) and *Libyodrilus violaceus* (Sp. B)

The two species of earthworms were collected at LAUTECH environment under Teak/Gmelina woodlot which is about 1.26 ha.

2.5.1 Extraction of earthworms from the soil

Earthworm collection was done by digging the soil to about 0.3 m, the worms were collected into small quantity of soil in polyethylene bags and kept according to species in labelled plastic containers containing soil and decaying plant materials. The worms were kept for only seven days before use.

2.5.2 Treatments applied for earthworms' toxicity testing

The following treatments were applied to *E. fetida* (Sp. A) and *L. violaceus* (Sp. B) to test the toxicity of the Pendimethalin using the spray volume of 200 l/ha distilled water:

Pendimethalin (P)

50% (1 l/ha)	125% (2.5 l/ha)
75% (1.5 l/ha)	150% (3 l/ha)
100% (2 l/ha)	Control (C)

2.5.3 Earthworms' lethal toxicity testing

Two test methods were used to evaluate the LC_{50} of pendimethalin for earthworms: the contact filter paper and the soil tests.

2.5.3.1. Contact Filter Paper Test (CFP- test)

The herbicides were dispersed in water at the indicated concentrations and shaken properly for two minutes. 1 ml of the solution was then transferred to each filter paper-lined Petri dish. The solution was allowed to evaporate to dryness and the paper rewetted with 2ml distilled water. One earthworm was added to each dish. The dishes were then covered with lid and placed under canopy in the shade of Teak/Gmelina woodlot for 48 hours (Pizl, 1988) and average minimum and maximum daily temperature range measured; four replicates were prepared for each herbicide concentration and earthworm species and a control of no herbicide. Earthworms were considered dead if they fail to respond to gentle mechanical stimulus to the epilobium.

2.5.3.2 Soil Test (S-Test)

Natural soil was collected under the Teak/Gmelina woodlot at LAUTECH where earthworms were collected and used as test substrate (Pizl, 1988). The soil was air dried, sieved through a 4 mm sieve, and 750 g was transferred to each plastic container (OECD, 1984). The container was one liter capacity with respective top and bottom diameter of 13 and 11 cm and a height of 8 cm. Moisture content of the dried soil was determined, and then moist to 25% moisture content. The pH of the soil was measured at the beginning, and moisture content of the soil at the end was determined. Ten earthworm specimens were added to each container, covered with perforated lid and arranged on the ground under canopy in the Teak/Gmelina woodlot in a Completely Randomized Design (CRD). The temperature was measured as in CFP-test. After acclimatization for seven days, 10 ml of each herbicide solution was added onto the soil in each container. Four replicates were maintained. Earthworm mortality was assessed after 7 days on the basis of response to mechanical stimuli.

2.5.3.3 Earthworms' sub-lethal toxicity testing

Change in growth rate of worms cultured in soil was selected as the criterion for sub-lethal toxicity evaluation. 100 g of the dried soil was placed into each container and 25 cm^3 distilled water was added to bring moisture level to 25%. Earthworms were washed with distilled water, gently blotted with tissue paper and weighed immediately using A & GULF Digital Scale of 600 g maximum capacity and can weigh to an accuracy of 0.01 g. A single worm was placed on the soil in each container. After 5 hours, to allow the earthworms to penetrate into the substrate, 5 ml of the herbicide solution was measured onto the soil surface at the stated concentrations. A control of no herbicide was included. The treatment was in four replicates. The containers were covered with perforated lid and treated as for the soil test. After 7 days, the earthworms were sorted out of the soil and weighed. Dead worms were not considered (Pizl, 1988). The growth rate of the earthworms in each treatment was calculated as:

$$R = \text{Log}_e \frac{\sum \text{mass day 7}}{\sum \text{mass day 0}} \qquad \text{(Martin, 1982)}$$

Where, ∑mass day 7 is the sum of mass at day 7, and ∑mass day 0 is the sum of mass at day zero (The first day of introduction of treatment).

Percentage weight loss was also calculated for the two of species of earthworms by expressing the initial weight as a percentage of the final weight.

2.5.3.4 Data analysis

The mortality data from the lethal toxicity tests were evaluated using probit analysis (Finney, 1971) with the help of a computer programme. The sub lethal toxicity values were compared with Chi square (X^2) test.

3.0. Results

3.1 Worm cast weight per unit area before land preparation

Table 1 showed the weight of worm cast per unit area estimated from the two sites before land preparation. Ogbomoso farm settlement had significantly higher worm cast weight (6.93 ton/ha) than LAUTECH farm (6.33 ton/ha) ($\alpha_{0.05}$) in Year A. In Year B, no significant difference was observed in worm cast weight at the two sites. Higher worm cast was recorded in Year B had than Year A.

Table1: Worm cast weight (ton/ha) at the two sites during Year A and Year B growing seasons

	Year A	Year B
LAUTECH farm	6.33	10.13
Ogbomoso farm settlement	6.93	9.60
LSD	0.45	ns

3.2 Physico-chemical properties of worm cast and soil

Tables 2 and 3showed worm casts analysis three months after spraying of the herbicide and before land preparation. Though, the textural classes of the soil and worm cast were sandy loam; worm cast contained significantly higher silts and clay and less sand when compared with the ordinary soil from the sites. This was observed during the two seasons of the experiment. The pH of the soil indicated slightly acidic nature (6.3-6.9) of the ordinary soil while worm cast varied from slightly acidic to slightly alkaline (6.7-7.4). Apart from pH, virtually all the measured parameters were higher in worm cast when compared with the soil sample taken before land clearing e. g. OC, N, P, Mg, K, and ECEC, were significantly greater in cast than soil in Year A and Year B except K in Year B when soil had K greater than cast at $\alpha_{0.05}$. Farm settlement recorded higher P and ECEC than Lautech farm in Year A. On the average, the nutrient contents in worm cast and soil were respectively 16.4 and 6.7 mg/kg C, 1.33 and 0.5 mg/kg N, 15.6 and 5.9 mg/kg P, 0.33 and 0.19 c mol+/kg K, 210 and 80mg/kg Silt, and 210 mg/kg sand and 130 mg/kg Clay. Only phosphorus was significantly higher in worm cast collected before (20.6mg/kg) land preparation than 3 months after spraying (15.6 mg/kg).In Year A, the following parameters were not significantly different in cast before land preparation and after 3 months: pH, OC, %N, P, K,ECEC, silt and clay. Also in Year A in Lautech farm OC, %N, and P were greater in cast after spraying than before land clearing. In Lautech farm in Year A, the cast before and 3 months after treatment had all the parameters statistically the same at $\alpha_{0.05}$. Farm settlement, in Year B had only %N, P, Exchangeable acidity, and silt statistically different at $\alpha_{0.05}$between the cast b and cast while others were statistically the same.

Table 2 Physico-chemical properties of worm casts and soil samples taken before land preparation at the two experimental sites during Year A and Year A cropping seasons.

		LTRF		OFS	
		Year A	Year B	Year A	Year B
pH(water)1:1	Cast	6.7[a]	7.00[a]	7.4[a]	6.7[a]
	Soil	6.3[a]	6.70[a]	6.9[a]	6.4[a]
% OC	Cast	1.81[a]	1.90[a]	0.73[a]	2.13[a]
	Soil	0.66[b]	0.80[b]	0.65[a]	0.57[b]
% N	Cast	0.12[a]	0.13[a]	0.13[a]	0.15[a]
	Soil	0.05[b]	0.06[b]	0.06[b]	0.04[b]
Mehlich P(µg/g)	Cast	12.25[a]	15.06[a]	18.69[a]	16.28[a]
	Soil	3.29[b]	11.11[b]	5.69[b]	3.36[b]
K (c mol+/kg)	Cast	0.25[a]	0.27[b]	0.50[a]	0.29[a]
	Soil	0.13[b]	0.41[a]	0.13[b]	0.07[b]
ECEC	Cast	10.08[a]	12.44[a]	12.17[a]	13.36[a]
	Soil	17.83[a]	6.96[b]	6.38[b]	4.10[b]
Sand	Cast	6.40[b]	79.0[a]	62.0[b]	60.0[b]
	Soil	79.0[a]	64.0[b]	79.0[a]	79.0[a]
Silt	Cast	21.0[a]	21.0[a]	19.0[a]	23.0[a]
	Soil	8.0[b]	8.0[b]	8.0[b]	8.00[b]
Clay	Cast	15.0[a]	15.0[a]	19.0[a]	17.00[a]
	Soil	13.0[a]	13.0[b]	13.0[b]	13.00[a]

Table 3 Physico-chemical properties of worm casts taken before land preparation and 3months after planting at the two experimental sites during Year Aand Year B cropping seasons.

		LTRF		OFS	
		Year A	**Year B**	**Year A**	**Year B**
pH(water)1:1	Cast b	6.70 a	7.00 a	7.40 a	6.7 a
	Cast	6.40 a	7.87a	6.70 a	6.3 a
% OC	Cast b	1.81 a	1.90 a	0.73 b	2.13a
	Cast	1.90 a	1.95 a	1.87 a	2.52 a
% N	Cast b	0.12 a	0.13 a	0.13 a	0.15 b
	cast	0.14 a	0.13 a	0.13 a	0.17 a
Mehlich P(µg/g)	Cast b	12.25 a	15.06 b	18.69 a	16.28 b
	Cast	16.45 a	19.72 a	18.02 a	27.71 a
K (c mol+/kg)	Cast b	0.25 a	0.27 b	0.5 a	0.29 a
	Cast	0.20 a	0.53 a	0.31 a	0.46 a
ECEC	Cast b	10.00 a	12.44 a	12.17 a	13.36 a
	Cast	8.94 a	12.46 b	11.98 a	12.81 a
Sand	Cast b	64.0 a	64.0 a	62.0 b	68.00 a
	Cast	64.0 a	64.0 a	64.0 a	64.0 a
Silt	Cast b	21.0 a	21.0 a	19.0 a	23.0 a
	Cast	19.0 a	19.0 a	19.0 a	19.0 b
Clay	Cast b	15.0 a	15.0 a	19.0 a	17.0 a
	Cast	17.0 a	17.0 a	17.0 b	17.0 a

Cast b = worm cast sampled before land preparation; Cast = worm cast 3 month after spraying

3.5. Herbicides effects on worm cast weight (ton/ha)

Worm cast weight per unit area was significantly higher at weedy plot than all other treatments at sites LTRF and OFS during both planting seasons (Tables 4 & 5). The toxic chemical, Mancozeb used also recorded low worm cast like the herbicides and the hoe weeded plots. Earthworms were scarce on the ploughed and hoe weeded plots thus little or no exposure to chemicals.

Table 4: Effect of pendimethalin on worm cast weight (ton/ha) per unit area at LAUTECH Teaching and Research Farm (LTRF) and Ogbomoso Farm Settlement (OFS) (Year A**)**

	Percentage	Rate (%)		
Treatments	**50**	**75**	**100**	**Treatment Mean**
	LTRF			
Pendimethalin	1.447±0.5	1.063±0.7	1.55±207	1.353
Hoe Weeding	0.480±0.8	0.480±0.8	1.480±0.8	0.480
Weedy Check	4.607±4.1	4.607±4.1	4.607±4.1	4.607
Mancozeb	1.223±1.3	1.223±1.3	1.223±1.3	1.223
Rate Mean	2.03	1.72	1.76	
LSD(Trt.)	3.739			
LSD(Rate)	ns			
LSD(TxR)	ns			
	OFS			
Pendimethalin	0.690±0.6	1.330±1.3	0.093±0.1	0.7044
Hoe Weeding	0.017±0.0	0.017±0.0	0.017±0.0	0.017
Weedy Check	1.260±1.2	1.260±1.2	1.260±1.2	1.26
Mancozeb	0.057±0.1	0.057±0.1	0.057±0.1	0.067
Rate Mean	0.53	0.634	0.148	
LSD(Trt.)	1.101			
LSD(Rate)	0.234			
LSD(TxR)	ns			

ns = not significant at 5% level of probability

The mean values for worm cast weight per unit area were insignificant at LTRF during the Year A and Year B planting seasons. At OFS in Year A, the mean value for worm cast was significantly lower for 100% rate when compared with 50% and 75%. The same trend occurred during the Year B growing season at OFS.

Table 5: Effect of pendimethalin on worm cast weight (ton/ha) per unit area at LAUTECH Teaching and Research Farm (LTRF) and Ogbomoso Farm Settlement (OFS) (Year B**)**

Treatments	Percentage Rate (%) 50	75	100	Treatment Mean
	LTRF			
Pendimethalin	2.571±2.231	1.973±1.779	3.040±3.212	2.528
Hoe Weeding	1.050±9.466	1.050±9.466	1.050±9.466	1.050
Weedy Check	10.87±3.844	10.87±3.844	10.87±3.844	10.87
Mancozeb	2.144±2.028	2.144±2.028	2.144±2.028	2.144
Rate Mean	4.342	3.819	3.901	
LSD(Trt.)	4.018			
LSD(Rate)	ns			
LSD(TxR)	ns			
	OFS			
Pendimethalin	4.165±5.043	1.589±1.206	3.040±3.212	2.932
Hoe Weeding	3.163±2.848	3.163±2.848	3.163±2.848	3.163
Weedy Check	2.352±2.615	2.352±2.615	2.352±2.615	2.352
Mancozeb	2.587±2.576	2.587±2.756	2.587±2.756	2.587
Rate Mean	2.739	2.314	5.289	
LSD(Trt.)	ns			
LSD(Rate)	1.287			
LSD(TxR)	ns			

ns = not significant at $\alpha_{0.05}$level of probability.

3.6 Response of *Eisenia fetida* (SAV) to pendimethalin

Earthworm sampling done on the plots before pendimethalin application revealed that there were low populations of *E. fetida*. No significant difference was observed among all the plots. The surrounding bush gave higher number of *E. fetida* which was significantly different from records got from the ploughed plots. This observation was true for both LTRF andOFS, and the two growing seasons. The herbicide rates have no significant effects on the population and weight of *E. fetida* at both LTRF and OFS one month after spraying. The same observation was true for the two growing seasons of Year A and Year B.

The noticeable and significant difference at 5% level of probability was observed between the surrounding bush and herbicide treated plots with the surrounding bush containing more earthworms. The sites and the growing seasons followed the same trend one month after spraying pendimethalin(Table 6).

The mean population estimated during the field work were 0, 0.80±0.1and 0 for 1.0, 1.5 and 2.0 l/ha pendimethalin; mancozeb had 0.76±0.43 and weedy check 0.85±0.28 before herbicide application. Population estimated one month after application were 0, 0.83±0.14 and 0.78±0.15 for 1.0, 1.5 and 2.0 l/ha Pendimethalin; mancozeb had 0.81±0.2; hoe weeding and weedy check had 0.75±0.09 and 0.86±0.17 respectively.

Earthworms were not encountered on the treated plots, hoe weeding, weedy check and the surrounding bush three months after spraying the herbicide.

4.6.1 Pendimethalin effects on *Lumbricus terrestris*

After ploughing and pegging, just before herbicide application, the plots were devoid of *L. terrestris* at both sites and throughout the two growing seasons except 2.0 l/ha pendimethalin which recorded 0.57±0.29 and 0.78±0.15 before herbicide application and one month after application respectively, but significantly higher ($p \leq 0.05$) numbers were obtained from the surrounding vegetation (Table 7). Throughout the two growing seasons and from LTRF and OFS, *L. terrestris* were not recorded on the plots including the control one month after herbicides application except a small one (0.75±0.09) recorded from the hoe weeded plot. The surrounding vegetation gave significantly higher ($\alpha_{0.05}$) number of *L. terrestris* compared to the ploughed plots one month after spraying(Table 7).Just like *E. fetida*, no *L. terrestris* was recorded from the two sites and surrounding bush and the two seasons three months after spraying the herbicides.

4.6.2 Effect of pendimethalin on *Libyodrilus violaceus*

Before herbicide application (BHA), surrounding bush gave significantly higher population with variable weights of *L. violaceus* than all the plots including control ones (Table 8). *Libyodrilus violaceus* was recorded at a very low rate during Year A survey (Table 8) from both sites, surrounding bush gave significantly higher population when compared with treated and control plots. The same observation was recorded in Year B at the two sites one month after spraying. *L. violaceus* was also not encountered three months after spraying the herbicides. The average populations of the worms encountered during the experiment were 0, 0.80±0.1 and 0 for 1.0, 1.5 and 2.0 l/ha respectively. Mancozeb had 0.83±0.14. One month after herbicide application, the following average populations were recorded: 0, 0.83±0.14 and 0.87±0.12 for 1.0, 1.5 and 2.0 l/ha respectively. Hoe weeding and weedy check had 0.81±0.2 and 0.94±0.07.

Table 6: *Eisenia fetida* population and weight (g) estimated before and 1month after herbicide spraying at LAUTECH Teaching and Research Farm (LTRF) and Ogbomoso Farm Settlement (OFS) during the Year A and Year B growing seasons

	LAUTECH TEACHING & RESEARCH FARM								OGBOMOSO FARM SETTLEMENT								
	Year A				Year B				Year A				Year B				
	BHS		1MAS		BHS		1MAS		BHS		1MAS		BHS		1MAS		
TRT	No.	wt.	No.	wt.	No.	wt.	No.	wt.	No.	wt.	No.	wt.	No.	wt.	No.	wt.	LSD ($\alpha_{0.05}$)
Pendimethalin:																	
100%	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	1.00	0.38	0.71	0.00	0.71	0.00	ns
75%	0.88	0.38	0.71	0.00	0.71	0.00	1.00	0.15	0.88	0.55	0.71	0.00	0.71	0.00	0.88	0.08	ns
50%	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	ns
Manc.	0.71	0.00	0.71	0.00	0.50	0.13	0.71	0.00	0.71	0.00	0.71	0.00	1.10	0.14	1.10	0.15	ns
HW	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.88	0.09	ns
H0	0.71	0.00	0.71	0.00	0.71	0.00	1.00	1.73	1.26	1.02	0.71	0.00	0.71	0.00	1.00	0.12	ns
Sur.Vg.	1.86	7.86	2.11	7.23	1.76	0.26	1.56	0.49	2.03	7.86	2.11	7.24	1.68	0.52	1.77	0.55	ns
LSD	0.24	1.14	0.20	0.55	0.47	0.19	0.52	0.29	0.23	0.78	2.29	0.62	0.53	0.34	0.74	0.32	

HW = Hoe weeding
H0 = Weedy check
Sur. Vg. = Surrounding Vegetation
Manc = Mancozeb
BHS = Before herbicide spraying
1MAS = One month after spraying

BHS = Before herbicide spraying
1MAS = One month after spraying

Table 7:*Lumbricus terrestris* population and weight (g) estimated before and 1month after pendimethalin application at the two sites during the Year A and Year B growing seasons

	LAUTECH TEACHING & RESEARCH FARM								OGBOMOSO FARM SETTLEMENT								
	Year A				Year B				Year A				Year B				
	BHS		1MAS		BHS		1MAS		BHS		1MAS		BHS		1MAS		
TRT	**No.**	**wt.**	**No.**	**wt.**	**No.**	**wt.**	**No.**	**wt.**	**No.**	**wt.**	**No.**	**wt.**	**No.**	**wt.**	**No.**	**wt.**	**LSD ($\alpha_{0.05}$)**
100%	0.71	0.00	0.71	0.00	0.71	0.00	1.00	0.00	0.14	0.71	0.71	0.00	0.71	0.00	0.71	0.00	ns
75%	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	ns
50%	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	ns
Manc.	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	ns
HW	0.71	0.00	0.71	0.00	0.71	0.00	0.88	0.08	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	ns
H0	0.71	0.00	0.71	0.0 0	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	ns
Sur.Vg.	1.34	6.90	1.68	7.21	1.46	0.52	1.17	0.39	1.46	6.90	1.77	7.2 1	1.77	0.62	1.39	0.52	ns
LSD	0.15	0.63	0.12	0.47	0.15	0.09	0.45	0.31	0.15	0.63	0.12	0.47	0.12	0.04	0.45	0.34	

HW = Hoe weeding
H0 = Weedy check
Sur. Vg. = Surrounding Vegetation
BHS = Before herbicide spraying

BHS = Before herbicide spraying
1MAS = One month after spraying
Manc = Mancozeb
1MAS = One month after spraying

Table 8: *Libyodrilus violaceus* population and weight (g) estimated before and 1month after pendimethalin application at the two sites during the Year A and Year B growing seasons

	LAUTECH TEACHING & RESEARCH FARM								OGBOMOSO FARM SETTLEMENT								
	Year A				Year B				Year A				Year B				
	BHS		1MAS		BHS		1MAS		BHS		1MAS		BHS		1MAS		
TRT	**No.**	**wt.**	**No.**	**wt.**	**No.**	**wt.**	**No.**	**wt.**	**No.**	**wt.**	**No.**	**wt.**	**No.**	**wt.**	**No.**	**wt**	**LSD ($\alpha_{0.05}$)**
100%	0.71	0.00	0.71	0.00	0.71	0.00	0.88	0.05	0.71	0.00	1.00	0.13	0.71	0.00	0.88	0.07	ns
75%	0.71	0.00	1.00	1.14	0.88	0.09	0.71	0.00	0.71	0.00	0.88	0.07	0.88	0.08	0.71	0.00	ns
50%	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	ns
Manc.	0.88	0.59	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	1.00	0.13	0.71	0.00	ns
HW	0.71	0.00	0.71	0.00	0.71	0.00	1.10	0.19	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	ns
W0	0.71	0.00	0.88	1.18	0.71	0.00	1.00	0.15	0.71	0.00	0.88	0.77	0.71	0.00	1.00	0.14	ns
Sur.Vg.	1.95	5.78	1.86	5.70	1.68	0.48	1.65	0.37	1.68	5.78	1.95	5.70	1.76	0.53	1.95	0.62	ns
LSD	0.21	0.83	0.34	1.15	0.21	0.13	0.61	0.29	0.13	0.88	0.27	1.19	0.46	0.20	0.40	1.15	

HW = Hoe weeding
Ho = Weedy check
Sur. Vg. = Surrounding Vegetation
BHS = Before herbicide spraying

BHS = Before herbicide spraying
1MAS = One month after spraying
Manc = Mancozeb
1MAS = One month after spraying

4.8 Lethal and sub-lethal toxicity tests

The average daily temperature range under the shade of the woodlot through the duration of the experiment was (22.4 – 28.5^{o}C). The average moisture content of the air-dried soil was 1.47% while the average moisture content of the soil after the experiment was 13.39%. The average pH of the soil was 7.2.

The results from lethal toxicity test of Pendimethalin were shown in Table 9.The LC_{50} of Pendimethalin for *E. fetida* in CPF-test was 1.93 l/ha while the value is 1.77 l/ha for soil test. Based on their respective 95%confidential interval(CI) around the LC_{50} values, CPF-test indicated relatively lower acute toxicity to *E. fetida* of Pendimethalin than the soil test.

The LC_{50} values of pendimethalin for *Libyodrilus violaceus* in CPF-test was 2.56l/ha which portrayed it as being more toxic in the soil (1.83 L/ha).

The overlapping confidential intervals (CI) for the soil tests and the two earthworms' species indicated that there was no significant difference between the LC_{50}values of Pendimethalin. *E. fetida* was more acutely affected by Pendimethalin in CPF-test than *L. violaceus* as indicated by their LC_{50} values of 1.93 l/ha and 2.56 l/ha respectively.

All the application rates of pendimethalin influenced the growth rates of the two earthworms' species negatively (Table 10).The weight of worms in the control treatments did not change significantly at 7 day of incubation and was always greater than the initial weight, indicating that the experimental conditions were satisfactory. *E. fetida* recorded highest growth rate reduction of 0.98 at 2.0 l/ha Pendimethalin. *L. violaceus* had 0.5 growth rate reduction at 2.0 l/ha Pendimethalin. There was no significant difference ($\alpha_{0.05}$) in the growth rates reduction of the two earthworms' species in all the concentrations of the herbicide considering the common chi square values although; there were significant differences when all the rates were compared with the control.

Table 9: Toxicity of Pendimethalin to earthworms as determined by two different methods

	LC_{50} (l/ha) (95% CI) PENDIMETHALIN	
Earthworm sp.	**CFP- test**	**Soil-test**
E. fetida	1.93(0.39-2.40)	1.77(1.48-2.05)
L. violaceus	2.56 (n/a)	1.83(1.51-2.16)

CI = Confidence interval.

Table 10: Effect of Pendimethalin on the Growth rate (r*) of earthworms in the soil test

Herbicide	**Conc. (L/ha)**	**Earthworm spp.** Growth rate (r*)		% Weight loss	
		E. fetida	***L. violaceus***	***E. fetida***	***L. violaceus***
Pendimethalin	1.0	0.16	0.33	13.7	28.1
	1.5	0.5	0.36	35.4	29.9
	2.0	0.98	0.5	62.5	39.4
	2.5	0.34	0.1	11.4	9.4
	3.0	0.63	-	46.7	-
Control	0.0	0.00	0.03	0.3	2.96
χ^2 0.42 0.42					
P 0.23	0.23				

\- = Dead earthworm P = probability

$$r^* = \log_e \frac{\sum \text{mass day 7}}{\sum \text{mass day 0}}$$

χ^2 = Chi square

Discussion

Percentage organic carbon, nitrogen, phosphorus and potassium were higher in worm cast compared with the ordinary soil which was very low. Worm casts had been referred to as finely divided peat-like material with excellent structure, porosity, aeration, drainage and moisture holding capacity (Dominguez, 1997). They are very water soluble, making their nutrient immediately available as plant food. Worm casts rival chemical fertilizers in their nutrient composition, providing a concentrated source of calcium, magnesium, nitrogen, phosphates and potash (http://louisvllehydroponics.com/organics.html). With the worm cast weight recorded from the two sites during the two growing seasons, it can be said that the two sites were moderately fertile.

The results of worm cast weight taken at 3 months after spraying indicated that only the weedy plots gave significantly higher weight than all other plots at both seasons and the two sites. This indicated that the weedy plots which were minimally disturbed by tillage operation showed some earthworm activities. This could be as a result of weediness of the plots providing cover for the earthworms. Edward and Bohlem (1996) reported that although most herbicides are considered to exert little direct impact on earthworms, the reduced weed cover resulting from their application may render the habitats less hospitable to earthworms.

The haphazard numbers of the three earthworm species, *E. fetida*, *L. terrestris*, and *L. violaceus* encountered on the plots before and after herbicides' treatment, with the surrounding unperturbed vegetation suggested that Pendimethalin played little or no role in the variations observed in the earthworm population. Edwards and Brown (1982) stated that herbicides tend to have low toxicity for earthworms, but can cause population reduction by decreasing organic matter input and cover from weed plants. The third months after spraying of both seasons of Year A and Year B fell within dry season and harmattan period (November) which was presumed to be too dry for earthworm activities. Sims and Gerard (1985) stated that during dry periods, worms are at the resting phase or period of quiescence and remain there until the rain fall and conditions become more favourable which cause the worms to become active again.

A number of laboratory tests for the assessment of the toxicity of pesticides to earthworms have been described (Stringer and Wright, 1973;Lord *et al*., 1980; Stenersen, 1981; Bostrom and Lops-Holmin, 1982; Heimbach, 1984; Pizl, 1988), but most of them were found to be unsatisfactory for various reasons. Contact tests in which the test compound is deposited on filter paper over which the test earthworms move (Goats, 1981) are comparatively simple to conduct and their results showed good reproducibility, but they are difficult to interpret and to apply to field practice. Thus, if the results of these types of experiments are to be useful for predicting the field situation, natural test medium and conditions and relevant modes of pesticide application should be used in the tests (Pizl, 1988).

The results of the tests indicated as confirmed by others (e.g. Pizl, 1988) that herbicides are directly toxic to earthworms in contrast to Edwards (1980) who reported that herbicides are not directly toxic to earthworms. The toxicity varied with earthworm species and type of pesticides. Discrepancies between the LC_{50} values obtained by the two test procedures could be due to a number of factors, and it may be concluded that CFP-tests did not reflect the hazards of herbicides to earthworms in soil. The degree of adsorption of the herbicides to natural soil must be considered as well as the possible degradation of herbicides by microorganisms in the non-sterile substrate of the soil test. Earthworms react to herbicides due to an even distribution of sensitive receptors all over the body (Pizl, 1988). Various kinds of behaviour may strongly influence the degree of contact with herbicide such as foraging and escape behaviour. In the soil, they may escape into deeper layers and the toxic effect of herbicide on them may be partly reduced. For example, the escape behaviour was demonstrated by some of the earthworms in the soil test in that, the worms that were already acclimatized and remained in the soil for seven days came to the soil surface and some even attached to the lid when the herbicides were sprayed on the soil. This behaviour partly explained the reason for the sparse population of, and lowered activities as indicated by worm cast production by the earthworms recorded in the field studies.

These tests, especially the CFP-test, indicated differences in susceptibility of the two earthworm species to pendimethalin which means that the toxicity of one herbicide cannot be simply extrapolated from one earthworm species to the other.

E. fetida has been suggested by many authors for toxicity testing since it is easy to rear in large numbers (Goats, 1981; Stenersen, 1981; Heimbach, 1985). Since this test, particularly the soil test showed that the results with *E. fetida* are comparable with those obtained with *L. violaceus;* this earthworm species can also provide solid information about potential toxicity of chemicals to earthworms as well as indicating the presence of toxicant in the soil if studies were conducted on its biology and cultural requirements.

In conclusion, earthworms were not affected by the sprayed chemicals but probably by the tillage operation and herbicidal removal of vegetation cover and pendimethalin was directly toxic to *L. violaceus and E. fetida*. The toxicity varied with earthworm species.

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