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AN ASSESSMENT OF THE EFFECTS OF HERBICIDES ON THE POPULATION DENSITY OF EARTHWORMS (*LUMBRICUS TERRESTRIS*) IN SOIL

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ABSTRACT

Continuous burrowing, ingesting, turning, mixing, aeration and the improvement of the drainage of soil are the major roles played by earthworms in renewing soil fertility. Larger amounts of herbicides are applied to the soil continuously by farmers as they realize the importance of these herbicides. These herbicides could then accumulate to toxic levels in the soil and become lethal to microorganisms, plant, wild life and man. In this study, we sought to assess the effects of formulated products of glyphosate, atrazine and 2, 4-dichlorophenoxyacetic acid (2, 4-D) on the survival and reproduction of the tropical earthworm (*Lumbricus terrestris*). The highest number of deaths of adult worms was recorded for 2, 4-D formulation at 0.5 mg/kg and the lowest in glyphosate formulation at 0.2 mg/kg. The general trend was that, the number of cocoons reduced with increasing concentration across all the formulations of the herbicides.

***Key words*:** Population density, Earthworms, effects of herbicides, *Lumbricus terrestris*,

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1. INTRODUCTION

In Ghana, weeds have been effectively controlled in farms by the use of herbicides. These herbicides contain a number of compounds including atrazine, triazine, paraquat and 2, 4-Dichlorophenoxyacetic acid. As the usefulness of herbicides are realized by farmers, larger amounts are applied to the soil. The fate of these compounds in the soils however is becoming a concern since they could be leached; in which case groundwater is contaminated or are immobilized, and persists on the top soil [1]. These herbicides could then concentrate to toxic levels in the soil and become lethal to microorganisms, plant, wild life, and man [2]. The concern that herbicides do not only affect weeds but also the microbial communities in soils, as well as soil organisms is increasing. Critical soil functions such as organic matter degradation, the nitrogen cycle and methane oxidation may be reduced because of these non-target effects [3].

Several research work on the effect of herbicides have been carried out on the standard worm *Eisenia fetida/andrei.* This includes the application of butachlor to *Eisenia fetida* which resulted in the reduction in growth of the worms, cocoon production and clitellum development [4]. The application of glyphosate to *Eisenia fetida* showed a catastrophic effect on the viability of cocoons and biodiversity of earthworm population [5]. Atrazine drastically reduced growth and cocoon production in *E. fatida* when applied at 100 parts per million (ppm) and completely stopped growth and reproduction at 200 ppm. Paraquate also slowed down weight gain and cocoon production at 200 ppm [6].

However, not too many research works have focused on the effects of these agro-chemicals on the earthworm species that are more common to agro ecosystems such as farmlands and gardens. In the temperate regions especially, *Eisenia fetida* and *Eisenia andrei* have been the standard species in earthworm ecotoxicology [7]. However, De Silva asserts that, the ecological role of the species belonging to the genus *Eisenia* in natural soil ecosystems is rather questionable since they are naturally vermicomposting worms [8]. Therefore, linking the observed effects in *Eisenia* sp. to ecologically relevant species in agro ecosystems is a concern. Thus, the use of indigenous tropical species for toxicity testing is ideal.

Preliminary investigations have revealed that increased use of agro-chemicals could lead to great loss of beneficial soil microbes and earthworms which help to renew the natural fertility of the soil [9,10,13]. Serious health problems can occur when many agrochemicals are absorbed into human tissues. An extensive list of epidemiological studies with the herbicide atrazine, has been discussed by Gammon et al. In their study, the authors alluded that, there existed a correlation between exposure to atrazine and high risk of prostate cancer development [11]. In this present study however, the effects of glyphosate, atrazine and 2, 4-D formulated herbicides on the population density of earthworms was investigated. Earthworm reproduction test is considered ecologically very relevant [12]. Thus; reproduction parameters such as cocoon production and hatchability of cocoons were also investigated. The effects of three of the commonly used herbicides in the Nanumba North district of Northern Ghana were studied using *Lumbricus terrestris* as model organisms.

2. RESULTS AND DISCUSSION

2.1 Mortality of adult *Lumbricus terrestris* on day 28 in response to herbicides concentrations

No deaths were recorded in the controls. However, there was a general increase in number of deaths of the worms as the concentration of the herbicides increased, except for 0.2 mg/kg of glyphosate where mortality was zero (0) (Fig. 1). For all the three herbicides studied, 2, 4-Dichlorophenoxyacetic acid recorded the highest deaths for all the five concentrations (0.05 mg/kg; 3 ± 1.61, 0.07 mg/kg; 6± 1.61, 0.1 mg/kg; 5±1.61, 0.2 mg/kg; 7±1. 61 and 0.5 mg/kg; 8±1.61) as shown in Fig. 1. Toxicity decreased in the order of glyphosate > atrazine > 2, 4-D. Unexpected observations were noted in 0.2 and 0.5 mg/kg of atrazine and 2, 4-D. That is, the examination of the expired adult worms showed that 77 (64.17%) worms had abnormal swelling and constriction of body segments. Also, Forty-three (43) live worms on the surface of the soil exhibited abnormal movement and coiling.

In this study, the recording of at least one death at the end of the 28th day of experimenting with the test herbicides implied that, each herbicide was potentially lethal to *Lumbricus terrestris.* Also, the observation that, mortality generally increased with increasing concentration of the herbicides was attributed to the inability of the worms to resist the lethal effects of the herbicides at higher doses. This assertion is in line with the findings of Bayer and Foy, who stated that, there is a downturn in earthworm populations in response to large amounts of organic chemical, even though, earthworm species vary in their tolerance to chemicals [13].

The reason associated with glyphosate treatment recording the highest number of cocoons was that, the glyphosate formulations possibly did not present adverse effects to the worms. This possibly could have resulted from glyphosate not binding strongly to soil particles and thus cocoons would not have been severely exposed to it. Also, in terrestrial or aquatic animals, glyphosate does not bioaccumulate [14, 15] and also dissipates rapidly from surface waters. It has also been reported that, soil microflora quickly biodegrade glyphosate into Aminomethylphosphoric acid (AMPA) and CO_2 [16].

However, in the case of 2, 4 - Dichlorophenoxyacetic acid and atrazine, their chemical structures are benzonoid and that might have presented higher negative effects that hindered the survival of the adult worms and consequently, their ability to lay eggs to produce higher numbers of cocoons. Also, these herbicides probably bounded very strongly to soil particles, thus prolonging their exposure time to the adult worms and cocoons. The half- life's of 2, 4-D and atrazine are 297 days and 100 days respectively thus would have been available in the soil medium long enough to have detrimental effects on the number of cocoons on day 28. The Northwest Coalition for Alternatives to Pesticides (NCAP) found that, atrazine was persistent in soil with a half-life of over 100 days in surface layers and below the surface, atrazine could persist for years [17]. They also noticed the persistence of 2, 4-D in soils was variable with half-life as short as 2 days and as long as 297 days**.**

5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), is an essential enzyme in plants for the manufacture of aromatic amino acids. Inhibition of this enzyme is the main mode of action of glyphosate. Since this enzyme is absent in animals, this herbicide would be relatively nontoxic to animals [18]. This could be the reason for its lower effects on the test worms. This does not guarantee safety to humans and it should be noted that, herbicides can be toxic to humans at both high and low doses [19]. Introduction of diseases such as cancer and disorders such as neurodegenerative reproduction and developmental changes, and respiratory effects can occur in humans as a result of prolonged exposure [20-23]. For example, irritation, itching, burning sensation of skin, and shortness of breath and burning sensation in the upper lung can occur due to 2,4-Dichlorophenoxyacetic acid pollution in the air. Drinking 2, 4-Dichlorophenoxyacetic acid can cause vomiting, diarrhea, headache, skeletal muscle injury, irritation and hypertension [24]. Also, 2, 4-D can prevent the function of spermatozoa immediately after ejaculation in to the reproductive track [25].

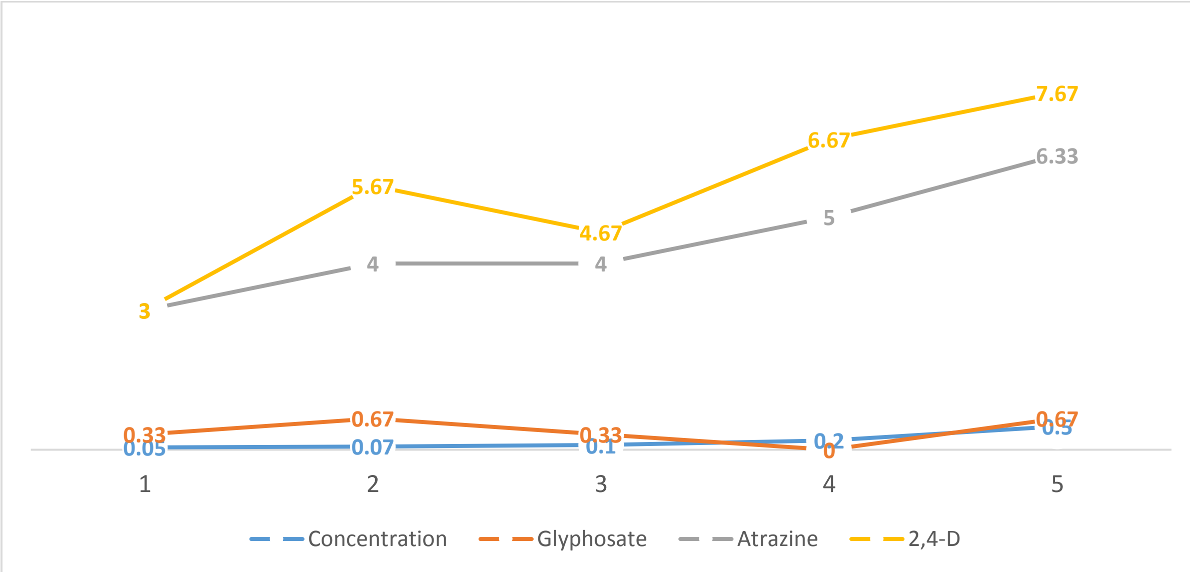

Fig.1. Number of deaths per concentration of herbicide formulation on day 28

2.2 Number of Cocoons produced on Day 28 in response to herbicides concentrations

In the control experiment, the total number of cocoons produced from the worms was 13. Figure 2 shows the mean number of cocoons for the various concentrations and type of herbicide formulation. For all the three herbicides studied, the glyphosate treatment recorded the highest numbers (11, 9, and 4 respectively) of developed cocoons for all the five different treatments followed by 2, 4-Dichlorophenoxyacetic acid while atrazine recorded the least number of cocoons for all the concentrations investigated. Across all herbicides, the lowest mean number of cocoons was 1 ± 0.80, recorded in the atrazine treatment at 0.5 mg/kg and the highest 11 ± 0.79, recorded in glyphosate formulated (0.05 mg/kg) soil. In the glyphosate formulated soil, number of cocoons generally reduced with increase in concentration of the herbicide. The number of cocoons however increased at the concentrations of 0.2 and 0.5 mg/kg of glyphosate formulation. In 2, 4 - Dichlorophenoxyacetic acid treatment, the number of cocoons decreased with increase in concentration of the herbicide except at the concentration of 0.1 mg/kg where the number of cocoons recorded was higher than that of the 0.07 mg/kg concentration (Fig. 2).

For the atrazine formulations, the quantity of cocoons produced reduced along the concentrations of 0.05, 0.07 and 0.1 mg/kg. However, at 0.2 mg/kg, the number increased to 3± 0.80 and then reduced to 1± 0.80 at 0.5 mg/kg formulation (Fig. 2). Fewer cocoons were produced at high concentrations of the herbicide formulations. This provided some evidence of a dose-related suppression of cocoon production at higher concentrations.

The observation of coiling of the worms receiving atrazine and 2, 4-D treatments was implicated in the low reproduction successes recorded in these treatments. In the coiled state, earthworms find partners less easily and also, copulation cannot take place. The herbicides also possibly caused abnormalities in reproductive cells of the worms. Gupta and Saxena found sperm head abnormalities even at the lowest test concentration of 0.125 mg/kg when they studied the effects of carbaryl, an N-methyl carbamate insecticide, on the reproductive profiles of the earthworm, *Metaphire posthuma* [25]. Similarly, Gestel & Dis concluded that, cocoon production and hatching of *E. fetida* were influenced by pentachlorophenol in soil [26].

It was also possible that, inert ingredients in the herbicide formulations contributed to the toxicity effect of the herbicides on the earthworms. Inert ingredients have been found to increase the penetration and toxicity of herbicides. For example, Everett and Dickerson observed that a glyphosate formulation was 100 times more toxic than just the compound glyphosate when they studied its effects on ciliated protozoans [27]. Chemicals such as naphthalene and xylene are used as inert ingredients in herbicide formulations and these have their own toxicities aside the possible interaction effects between active ingredients and these inert chemicals.

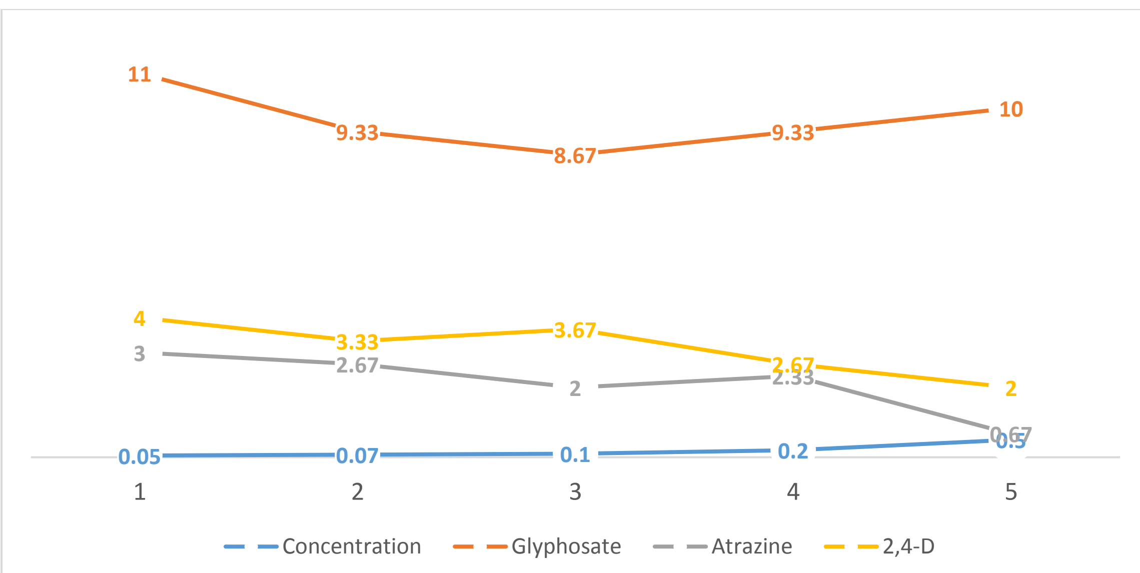

Fig.2. Number of cocoons per concentration of herbicide formulation

2.3 Number of Unhatched Cocoons at Day 56

The mean number of unhatched cocoons for the various concentrations and type of herbicide formulation are shown in Fig 3. The highest mean number (9 ± 1.33) of unhatched cocoons was recorded in glyphosate formulated soil while the lowest mean number (1 ± 0.13) was recorded in atrazine formulated soil. On the average, the number of unhatched cocoons per control was (3). The number of unhatched cocoons increased with increase in concentration for glyphosate whiles in 2, 4 - Dichlorophenoxyacetic acid, the number of unhatched cocoons was higher for the concentrations of 0.05 and 0.1 mg/kg but was lower at the concentrations of 0.2 and 0.5 mg/kg respectively (Fig. 3). There was no increase in the quantity of unhatched cocoons in the atrazine formulations, except at the concentration of 0.5 mg/kg as shown in Fig. 3.

The observation that cocoon production was generally high in all the glyphosate treatments at day 28 but proceeded with less hatching success at day 56, was attributed to two main factors. Firstly, chemical effect of herbicides on hormones. This is articulated in Britannica as; the development of cocoons is dependent on hormones and enzymes and thus any external factor be it chemical (herbicides) or physical (experimental conditions) that inhibits the hormonal and enzyme balance of cocoons might as well inhibit their development. The second factor attributed to the less hatching success of the cocoons was the herbicides prolongation of cocoon incubation time over the period of study. Normally, it takes 2-3 weeks for earthworm cocoons to hatch, but in this study, 4 weeks after incubation, the cocoons could not hatch. A similar observation was reported by Reinecke and Venter who indicated that many agricultural chemicals adversely affect hatching success of cocoons and these chemicals prolong incubation periods [28]. It was also possible that; the herbicides under study interfered with the manifestation of the hatching enzyme of cocoons as reported by Marc *et al* [29].

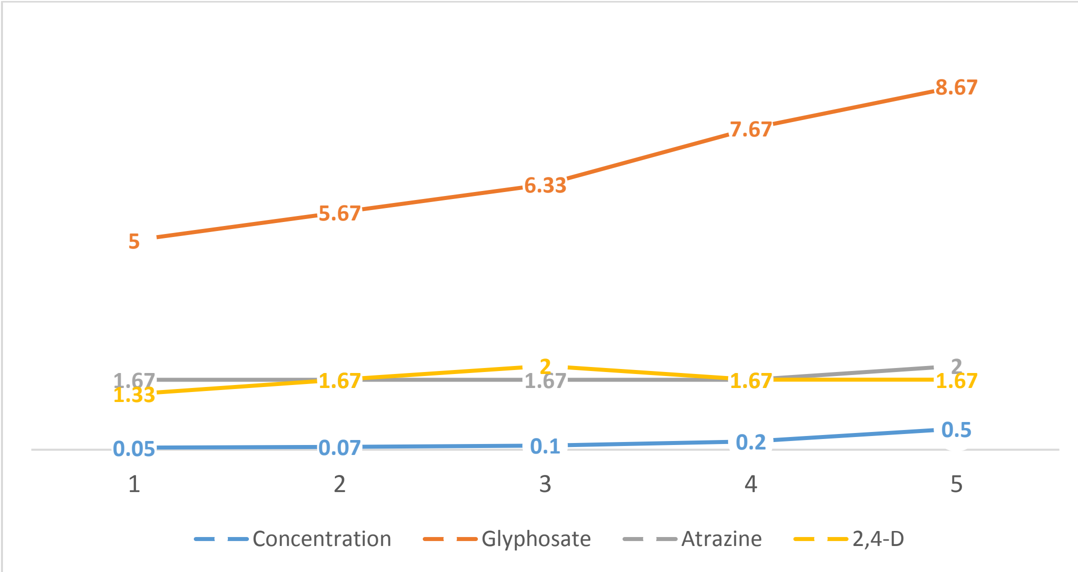

Fig.3. Numbers of unhatched cocoons per concentration of herbicide formulation

3. EXPERIMENTAL

3.1 Construction and set-up of Experimental Containers

Fifty-four wooden boxes of dimension 20 cm x 15 cm x 10 cm were constructed using 8 cm plywood. Loamy sand soil was collected from four sampling stations near Evangelical Presbyterian Collage of Education, Bimbilla, Ghana and sterilized. For each test, 750 g weight of loamy sand soil was placed into each wooden box and an aqueous solution of the test herbicides mixed with the soil. To maintain good organic matter content, each soil sample was mixed with ten grams (10 g) of finely ground cattle dung. The water level in the wooden boxes was adjusted to moderate level using the method of OECD. According to the OECD method, moderate level is achieved when the test soil can be molded into a ball [30].

3.2 Sampling of Worms

Lumbricus terrestris was the test specimen used in this study. The species (*Lumbricus terrestris*) was identified using the taxonomic key developed by Worm Watch [31]. The earthworms were collected along the banks of nearby streams; Jilo, Kpagi turi and Kukoo streams, because the experimental period was in the dry season (harmattan). At each sampling site, the soil was dug and the earthworms were carefully hand sorted on site. The sampling technique used (hand sorting) is considered to be an efficient way to sample epigeic species [32, 33, 34, 35]. The presence or absence of fully developed clitellum was used to identify adult and juvenile worms. Five hundred and forty (540) worms were sampled. The adult earthworms were then put in a plastic container containing soil from the site and watered to keep them moist and alive. They were transported to the Biology laboratory of Evangelical Presbyterian College of Education, Bimbilla where they were kept in wooden boxes filled with the test soil to condition them for at least 24 hours.

3.3 Experimental Procedure

Five concentrations of the herbicides glyphosate, atrazine and 2, 4-dichlorophenoxyacetic acid (2-4-D) in geometric series; 0.05, 0.07, 0.1, 0.2 and 0.5 mg/kg were introduced separately into the test soils as aqueous suspensions. Ten (10) earthworms were put on the surface of the test substrate (soil with herbicides). To prevent the test substrate from drying, but allowing free exchange of gases, the containers were covered with perforated plastic films. Each test container was weighed and recorded at weekly intervals to monitor moisture loss from the test soils and where necessary, watered to replenished lost moisture. The same conditions were maintained between the control group and the experimental groups except that the control was not supplied with herbicides. Adult worms were hand sorted, after 28 days of exposure and mortality checked. To allow for cocoon development, the test soil and worms that survived were put back into the test containers and incubated for another 28 days. At day 57, juveniles were removed from the test soil and counted. The final endpoints studied were, cocoon production and hatchability at day 57. The experiments were triplicated and mean values calculated. Toxicity was assessed using the guidelines of ISO and OECD [36]. According to the principle of the test method, a range of concentrations of the herbicides (glyphosate, atrazine and 2, 4-D formulations) must have been applied to samples of a precisely defined soil (loam sand soil) and the earthworms kept in it [36]. The condition for the validity of the test according to the toxicity test guideline by OECD is that, the death toll in the controls should not be more than 10 per cent (10%) at the end of the test [30]. The experiment was carried out under the same environmental conditions (temperature and moisture levels) in the laboratory.

4. CONCLUSION

In general, all the three herbicides under study had an adverse effect on cocoon production and hatchability. Atrazine had the highest effect, followed by 2, 4-Dichlorophenoxyacetic acid with glyphosate showing the laest effect. The negative effects of the herbicides on cocoon production and hatching success increased with increasing concentrations of the herbicides. Survival of adult worms also decreased with increasing concentrations of the herbicides.

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