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Effects of Two Commonly Used Herbicides on Soil Microbes and Organic Matter of Soil from Maize and Cocoa Plantation

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Abstract:
Herbicide application had been an effective method of weed control over the years, however, the effect of herbicides on the non-target soil microbes that play roles in degrading organic matter and nutrient recycling needs to be considered. The effects of two (2) most commonly used herbicides in Osun State on soil microbes of maize and cocoa plantation were assessed over a period of thirteen continuous days (exposure period). The herbicide treatments were the normal recommended field rate, (0.72 mg active ingredient per gram of soil for 2, 4-D amine and 0.24 mg for glyphosate, double the recommended field rate, (1.44 mg of active ingredient per gram of soil for 2,4-D amine and 0.48 mg for glyphosate). The bacteria and fungi populations were then determined at a three-day interval up to the 13th day after treatment. Result indicated that herbicides treatment had a pronounced toxic effect on microbes population at 4 days after treatment however the toxicity gradually reduced as the exposure time increases. Double recommended field rate has higher toxic effect than the normal field rate. Also, glyphosate has less inhibitory effect on bacterial population than 2,4-D amine. Thus, glyphosate is recommended at the normal recommended field rate for maize and cocoa plantation.

Keywords: *Soil –microbes, glyphosate, 2,4 D amine, maize-plantation and cocoa-plantation*

1. Introduction

The soil serves as a major habitat for most microbial communities such as soil bacteria, fungi and actinomycetes whose activities influences the soil fertility through organic matter degradation and decomposition as well nutrients cycling (De-Lorenzo *et al.,* 2001; Hutsch, 2001; Rosli *et al.,* 2013). It had been reported that, indiscriminate use of these chemicals may inhibit some of the natural processes and decrease the performance of the non-target soil organisms. However, some of these organisms may use these herbicides in the process of degradation as carbon energy source for their metabolic activities (Subhani *et al.,* 2000). Numerous studies have shown that the level of contamination of soil with these chemicals depends on the persistency of the herbicides in the soil's environment, the quantity, frequency of application and the toxicity of the chemicals (Sebiomo *et al*., 2011). Farmers apply the herbicides to the target organisms without paying due attention to the herbicide recommended rate of application. Also, most farmers are ignorant of proper way of disposing the excess herbicides after its application. These pose a challenge to the normal functioning of the microbes in the soil (Rosli *et al*., 2013). Most farmers in Osun State concentrate on cocoa and maize plantations on which they applied these herbicides indiscriminately thus, there is need to assess the effects of two commonly used herbicides on some of the beneficial soil microorganisms and soil organic matter of soil from maize and cocoa plantations.

2. Materials and Methods

Soil samples were collected from top (0-5 cm) from two different locations; cocoa plantation at Igando in Ilesha, maize plantation at Ata Farm in Ile-Ife in Osun State with no prior herbicide treatment. The soil samples were collected from different points within the plantation and mixed to obtain a composite sample for each plantation. The composite soil sample of each plantation was air-dried, sieved with a 2.0 mm mesh and taken to the laboratory for the experiment. The two commonly used herbicides, glyphosate and 2,4-D amine were purchased from a local agrochemical seller at Ile Ife, Osun State, Nigeria.

The soil samples were treated with 2 different concentrations, the normal recommended field rate (NRFR) and the double recommended field rate (DRFR). Soil samples without treatment were maintained as the control. The experiment was set up in triplicates. The rate of treatment of the manufacturer of the herbicides recommended rate of 0.24 mg of the active ingredient per a gram of soil for Forceup (glyphosate) and 0.72mg per gram of soil and for 2,4-D amine. The treatment was calculated using the formula of Zain *et al.* (2013)

$$Y\ (mg/g) = \frac{RFR\ (g\ a.i\ /\ ha)}{Am.\ AiF\ (g\ a.i\ /\ L) \times 450\ L/ha} \times \frac{1000\ mg}{1g}$$

Where; Y - milligrams of chemical per gram of soil
RFR- recommended field rate
Am. AiF - amount of active ingredient in formulation

The enumeration of the bacteria population and fungal population were done at 3 days interval till 13 days after treatment (DAT). Bacteria enumeration was done using Pour Plate Counter. The plate count agar was prepared by suspending 20 g Nutrient agar in one litre of distilled water. The agar was poured into a flask and sterilized in an autoclave at 121^0 C for 15 minutes. One gram of each treated soil sample was weighed and serially diluted. 1ml aliquot was taking from an inch below the surface with sterilized 1ml pipette and placed in an empty sterile plate. 15 ml of the melted plate count agar which was cooled to 45^0C was poured into the diluted sample. This was done swirled to ensure that the mixture is thoroughly mixed and cooled to solidify on a flat laboratory bench before incubation was done. The labelled specimens were inverted to prevent it from being soaked through condensation. Incubation was done at room temperature of 37^0 C for 24 – 48 hours. Total viable colony on each plate was counted using the colony counter and the data recorded. (Sebiomo *et al.,* 2011).

Fungal enumeration was done by using Potato Dextrose Agar (PDA) supplemented with each of tetracycline and streptomycin to inhibit bacterial growth. The PDA was prepared by weighing 39g and dissolved in 1litre of distilled water in a flask. The content was then sterilized in an autoclave at 121^0 C for 15 minutes. 1 ml of the test samples was added to a sterile Petri dish and then a required amount of sterile, molten agar was added to the test sample. The content was allowed to cool at 45^0C and swirled gently to mix well before it was allowed to solidify. Incubation of the fungi was done at room temperature of 25^0C -32^0C for 48 to 72 hours and identified with reference to Bergey's manual of systematic bacteriology. The total number of particular organisms on each plate was identified and scored based on a maximum count on a particular plate (Sebiomo *et al.,* 2011).

The organic matter content was determined by Loss on Ignition analysis using the procedure of Sarah (2011) using the formula:

$$\%\ \text{Organic Matter} = \frac{\text{Pre-ignition weight (g)} - \text{Post-ignition weight (g)}}{\text{Pre-ignition weight (g)}}\ x\ 100\%$$

Data generated from microbial enumeration was subjected to Analysis of variance using Statistical Package for Social Sciences (SPSS). The mean differences were compared using pairwise comparison and Duncan Multiple Range Test (DMRT) at 5% level of probability (Steel and Torrie, 1984). The mean values were presented in figures and tables.

3. Result

The result of the effects of different exposure periods and concentration of glyphosate on bacterial populations of the soil from maize plantation is presented in Figure 1. The highest bacteria count was observed from untreated soil (control) 4 days after treatment (DAT) with 1.80×10^3cfu/ml. There was significant reduction in the bacterial population at 4 DAT. However, there was gradual increase in the bacterial population till 13 DAT. At 13 DAT, there was no significant different ($p = 0.01$) between the soil treated with normal recommended field rate (NRFR) and the untreated soil (Fig. 1).
The result of the different exposure period and concentration of 2,4-D amine on the bacterial population of the soil from the maize plantation is presented in Fig. 2. There was significant reduction in the bacterial population at 4 DAT till 13 DAT at both NFRF and DRFR (Fig. 2).

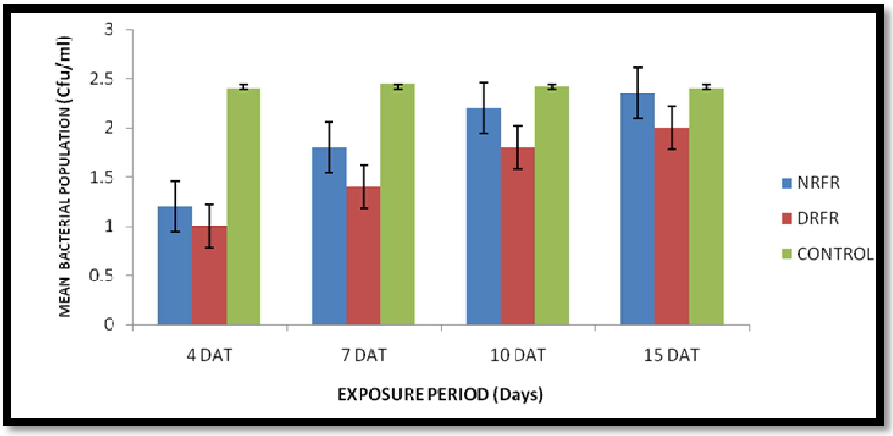

Figure 1: Effect of Glyphosate Exposure on Bacterial Population of Soil from Maize Plantation

NRFR=Normal recommended field rate, DRFR=Double recommended field rate, DAT=Days after treatment, Cfu/ml=colony forming unit per milliliter

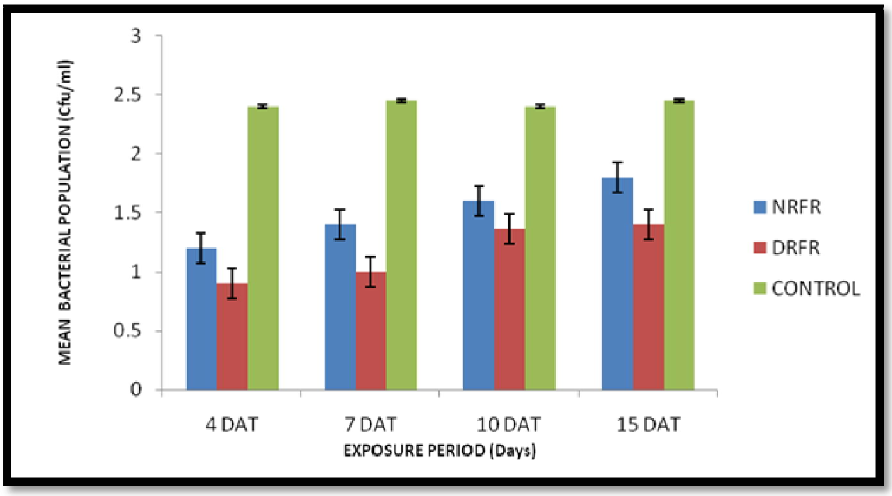

Figure 2: Effect of 2, 4 D Amine Exposure on Bacterial Population of Soil from Maize Plantation

NRFR=Normal recommended field rate, DRFR=Double recommended field rate, DAT=Days after treatment, Cfu/ml=colony forming unit per milliliter. The bacterial population of the treated soil from the cocoa plantation followed the same trend (Fig. 3 and 4).

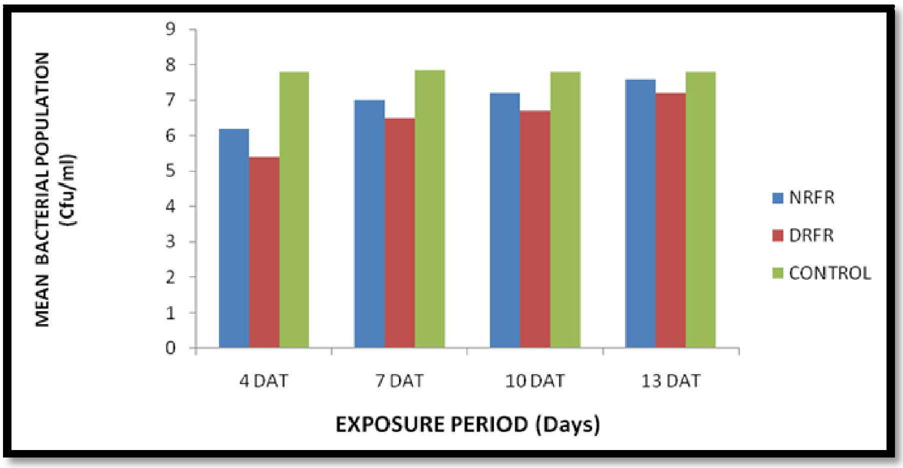

Figure 3: Effect of Glyphosate Exposure on Bacterial Population of Soil from Cocoa Plantation

NRFR=Normal recommended field rate, DRFR=Double recommended field rate, DAT=Days after treatment, Cfu/ml=colony forming unit per milliliter.

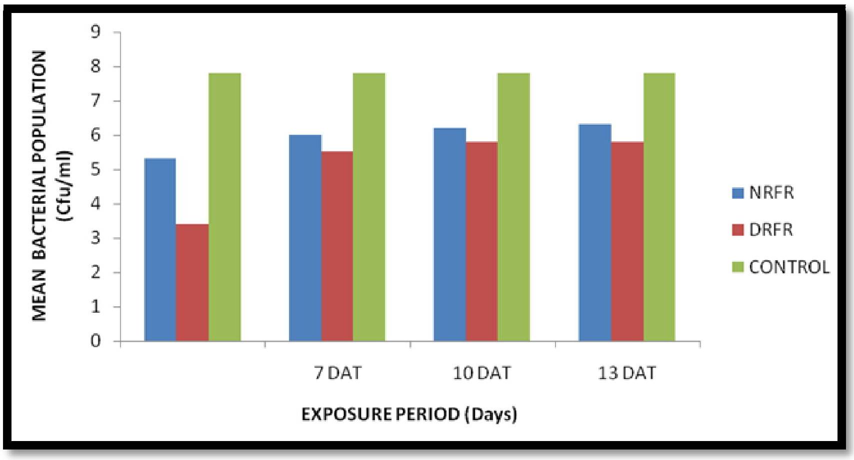

Figure 4: Effect of 2, 4 D Amine Exposure on Bacterial Population of Soil from Cocoa Plantation

NRFR=Normal recommended field rate, DRFR=Double recommended field rate, DAT=Days after treatment, Cfu/ml=colony forming unit per milliliter. The fungal population of the treated soil from maize and cocoa plantation followed the same trend. In both cases, there were significant reduction at 4 DAT followed by gradual increase in fungal population till 13 DAT (Fig. 5-8) such that the fungal population of the soil treated with normal recommended field rate was higher significantly higher than the untreated plots at 13 DAT.

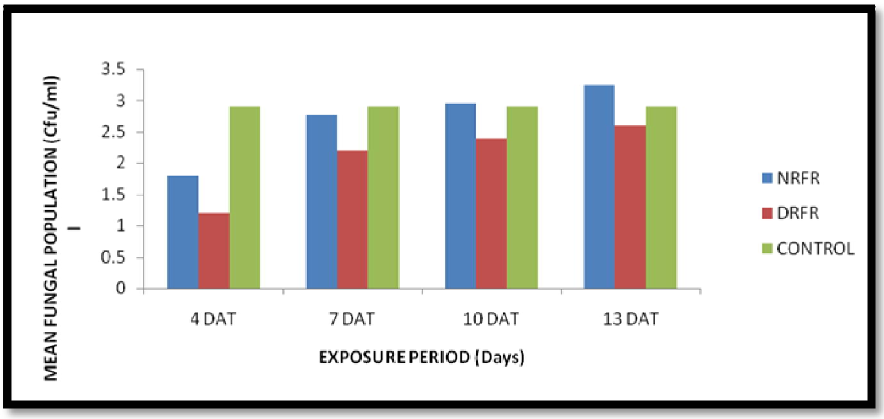

Figure 5: Effect of Glyphosate Exposure on Fungal Population of Soil from Maize Plantation

NRFR=Normal recommended field rate, DRFR=Double recommended field rate, DAT=Days after treatment, Cfu/ml=colony forming unit per milliliter.

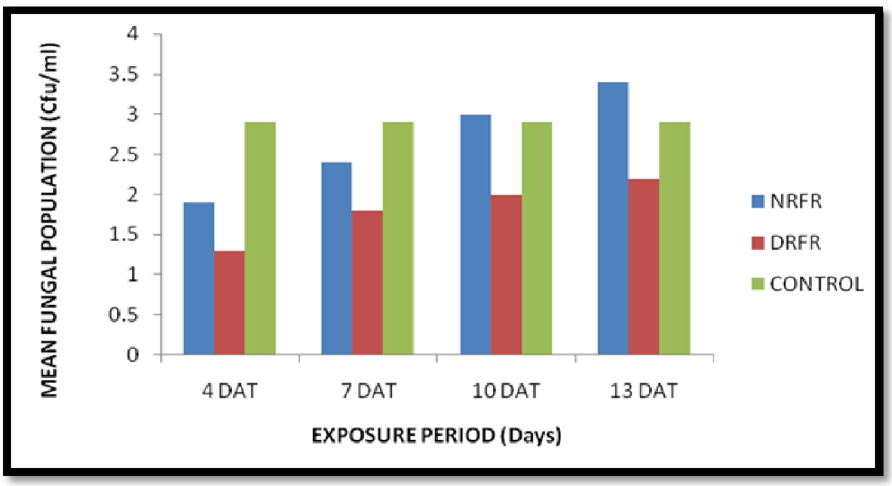

Figure 6: Effect of 2, 4 D-Amine Exposure on Fungal Population of Soil from Maize Plantation

NRFR=Normal recommended field rate, DRFR=Double recommended field rate, DAT=Days after treatment, Cfu/ml=colony forming unit per milliliter.

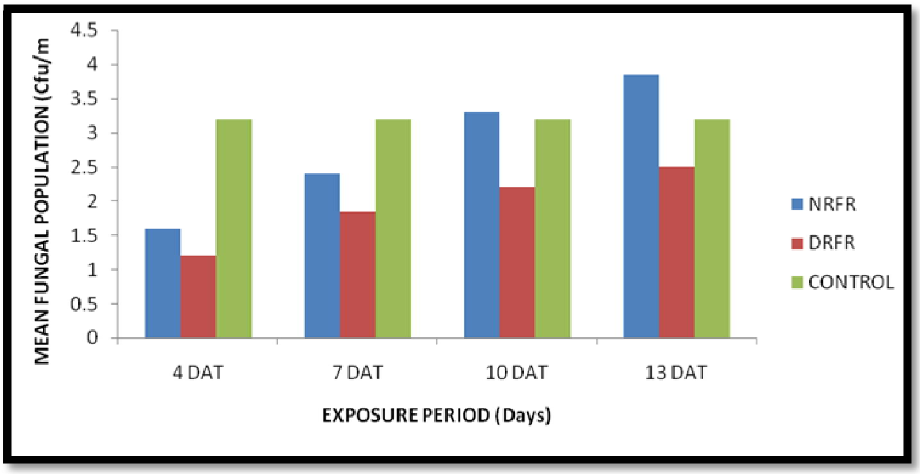

Figure 7: Effect of Glyphosate Exposure on Fungal Population of Soil from Cocoa Plantation

NRFR=Normal recommended field rate, DRFR=Double recommended field rate, DAT=Days after treatment, Cfu/ml=colony forming unit per milliliter

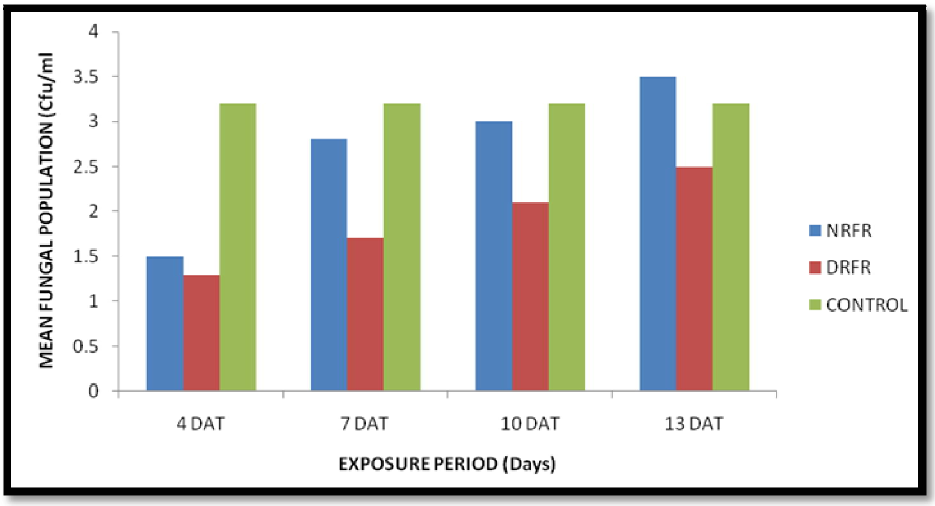

Figure 8: Effect of 2, 4 D Amine Exposure on Fungal Population of Soil from Cocoa Plantation

NRFR=Normal recommended field rate, DRFR=Double recommended field rate, DAT=Days after treatment, Cfu/ml=colony forming unit per milliliter.

The mean values of the percentage organic matter of the different concentrations of herbicides treated soil from maize and cocoa plantations are presented in Table 1 and 2 respectively. In the maize plantation soil, the double recommended field rate of glyphosate recorded the highest mean value of 4.00 ± 0.50. However, this was not significantly different (p = 0.02) from the mean percentage organic matter of the untreated soil and other treated soil (Table 1). However, for the soil from cocoa plantation soil, the control recorded the highest mean value of percentage organic matter 4.17± 0.3, this was not significantly higher (p = 0.01) from the mean value from the treated soil.

Treatments	Mean Percentage Organic Matter
NRFR of glyphosate	3.60 ± 0.20
DRFR of glyphosate	4.00 ± 0.50
NFRF of 2,4 D amine	3.77 ± 0.60
DFRF of 2,4 D amine	3.80 ± 0.70
Control	3.90 ± 0.10

Table 1: Percentage Organic Matter of Soil from Maize Plantation under Different Concentration of Herbicides

NRFR=Normal recommended field rate, DRFR=Double recommended field rate, DAT=Days after treatment.
The result of the effect of the herbicide on percentage soil organic matter of soil from cocoa plantation is presented in Table 1.

Treatments	Mean Percentage Organic Matter
NRFR of glyphosate	4.10 ± 0.30
DRFR of glyphosate	3.80 ± 0.60
NFRF of 2,4 D amine	3.90 ± 0.65
DFRF of 2,4 D amine	3.80 ± 0.60
CONTROL	4.17 ± 0.55

Table 2: Percentage Organic Matter of Soil from Cocoa Plantation Under Different Concentration of Herbicides

NRFR=Normal recommended field rate, DRFR=Double recommended field rate, DAT=Days after treatment.

4. Discussion

Result of this study indicated that glyphosate has significant toxic effect on bacterial population at 4 days after treatment. However, the bacterial population gradually increased as the exposure period increases till 13 days after treatment. This is partially in line the findings of Sebiomo *et al*. (2011) who reported who reported an increase in bacterial population in the first and second week of herbicide treatment. However, 2,4-D amine application resulted in consistence reduction in bacterial population in relation to the untreated soil till 13 days after treatment. This corroborated the findings of Zain *et al*. (2013) who discovered there was a free fall in microbial population as the exposure period increases. Also, the fungal population of the treated soil exhibited significant decrease at 4 DAT, however, this was followed by gradual increase in fungal population till 13 DAT such that the fungal population of the soil treated with normal recommended field rate was higher significantly higher than the untreated plots at 13 DAT. This corroborated the report of Michael and Stephen (2016) who discovered that the fungal population gradually increase after degradation probably because the fungi can degrade the herbicides and convert it to carbon source of energy.

The study revealed that the herbicide treatments did not have significant effect on the % organic matter as the % organic matter of the treated soil revolve around the mean value of the control. This might be due to short time nature of the exposure period for herbicide treatment. It had been reported that the soil organic matter increased after continuous application from the second week to six weeks of treatment (Sebiomo *et al*. (2011).

5. Conclusion

The result of this study indicated that glyphosate is less toxic than 2,4-D amine and that herbicide toxic effect gradually decrease after degradation when the recommended field rate is applied than when the rate is doubled. Glyphosate is therefore recommended for weed control in maize and cocoa plantation with minimal effect on beneficial soil microbes.

6. References

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