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# Soil Microbial Inhibitions on the Impact of Four Herbicides on Plantain Plantation (*Musa paradisiaca*) in FECOLART, Owerri- West, Imo State, Nigeria.


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## Abstract

The increased use of herbicides in agricultural soils causes contamination of the soil with toxic chemicals. These chemicals exert certain effects on non-target organisms, including soil microorganisms which are responsible for the numerous biological processes essential for increase in soil fertility and food production. In line with this, the study assesses the Soil Microbial inhibitions of Four Herbicides using concentration level of 0.33mg/l diluted with 10 litres of water and applied on 1 hectare of land: Glyphosinate; Basta -15; Roundup and Atrazine were applied on Plantain Plantation (*Musa* paradisiaca) in FECOLART. Soil samples were collected at 0-15cm and 15 – 30 cm depths with the herbicides evaluation: 1DAT, 2DAT, 3DAT, 4DAT 10DAT respectively. The results showed that , the herbicides treatments significantly inhibited the development of microbial populations in the soil and the degree of inhibitions closely related to the rates of their applications which varied with the type of herbicide used. Glyphosinate caused the highest percentage of inhibitory effect on bacterial populations at 15-30cm with a scanty growth of Streptococcus progenies when applied at the recommended field rate, but at 0-15cm, yielded a moderate growth of Clostridium when isolated, and when sporadically applied Basta-15 yielded no bacterial growth in the soil. The results also shown that, the fungal populations were mostly affected by Roundup at 3DAT.Atrazine recorded the least inhibitory effect on soil microbial population. The highest fungal inhibition was observed at 3 days after treatment (DAT) followed by a drastic decrease of inhibition by 4 DAT, while 10 DAT recorded zero inhibition. Ultimately, the result showed that inhibition of fungi and bacterial growth increased with increased herbicide rates. The degree of fungal growth inhibition by the herbicides were in order of Glyphosinate >3DAT, Roundup >3DAT, Basta >2DAT and Atrazine> 1DAT. The degree of growth inhibition by the herbicides on bacteria were in order of Basta >1DAT; Glyphosinate > 2DAT; Roundup >3DAT, and Atrazine>2DAT.



**Keywords**: Soil Microorganisms, Inhibition, Herbicides, *Musa- paradisiaca.*

## Introduction

Herbicides are used quite extensively to control weeds in integrated weed management programs in plantation crops (Zain *et al*, 2013) . However, when applied in the field, herbicides not only control targeted weeds, but may also have potential residual impact in soil (Zabaloy *et al.,* 2008), and considerably expose of microbes to the herbicides (Pampulha and Oliveira, 2006).The microbial biomass plays an important role in the soil ecosystem where they fulfill a crucial function in nutrient cycling and decomposition (De-Lorenzo *et. al*., 2001; Chowdhury *et al. (*2008).

Glyphosate has been successfully used in integrated weed management programs in oil palm plantations to control weeds (Wibawa *et. al.*, 2009; Halimah *et. al.*, 2010). However, Franz et. al.(1997) discussed that fungal growth inhibition by glyphosate was due to the blocking of EPSPS enzyme in the shikimic acid pathway that ultimately affected the amino acid synthesis in microorganisms. As farmers continue to realize the usefulness of herbicides, larger quantities are applied to the soil (Sebiomo *et. al*, 2011).According to Handa *et. al.* (1999), Ubuoh *et al*. *(*2012), any adverse impact of chemical on soil characteristics and microorganism may lead to ultimate loss of soil fertility. In general, herbicides affect microbes indirectly, causing physiological changes, increased enzymatic production or when applied in high doses, death of susceptible groups of microorganisms (Cervelli *et. al.*, 1978).

The environmental fate of herbicides is a matter of grave concern given that only a small fraction of the chemicals reach the target organisms (Pimentel et. al , 1995), leading to potential impacts of residual herbicides in soil, human, animal and crop health. But the fate of these compounds in the soil is also important because they may leached or persist in the top soil (Ayansina *et. al.*, 2003).

Herbicide application is a regular practice in modern agricultural production. While in developed countries, weeds and pests reduce yields of agricultural crops by 15 to 20%, reductions can be as high as 50% in undeveloped regions (Dobrovoljskiy and Grishina, 1981).Increase in herbicide dose by farmers tends to amplify the negative effects on microorganisms Konstantinoviã *et al.*, 1998; Miloševiã *et al.,* 2001), incorrect and indiscriminate application of herbicides negatively affects the health of humans, plants and animals. Particularly hazardous are the poorly degradable herbicides whose persistence may lead to long-term accumulation. Thereby reducing crop yield and subsequently food insecurity (Barrett, 2002). Therefore, microbial activity measurements appear as good indicators of the degree of pollution of contaminated soils (Nordgren *et al*., 1988; Aoyama and Nagumo, 1995; Miloševiã and Govedarica, 2002). It has been observed that plantains planted had stunted growth with yellowish leaves following herbicide application. Unforeseen consequences of herbicide use on microbial communities especially soil bacteria and fungi is essential to provide deeper insight into herbicide risk management in soils from *Musa paradisiaca* plantations. The study was undertaken to assess the response of microbial populations to different rates of herbicide applications in soil environment.

## Materials and Methods

The study area is the Federal College of Land Resources Technology permanent site at Oforola in Owerri West Local Government Area of Imo State, situated along Owerri Port-Harcourt express road. It lies on latitude $6^{0}56$” N and longitude $6^{0}3$” E. The study was carried out on Plantain Plantation (*Musa paradisiaca*) plantation and the major characteristics of the study area include the mean annual rainfall of 213.7mm, minimum and maximum temperature of $26^{o}$C and $28^{0}$C of the area respectively. Humidity ranged between 50.5 – 70.5 %. It is in bio-climate rainforest zone with geology that is made up of sedimentary rock and soil type being Sandy-loam (Ubuoh *et. al*, 2012).

**Herbicides Types:** The herbicides used in this study were obtained from FECOLART, Owerri. Herbicide used include Glyphosate, Basta -15, Roundup, paraquat and Atrazine which delays in mineralization (Barriuso and Houot (1996), and paraquat were used. These herbicideswere applied on weeds within the Plantation, and the residual effects were evaluated.

**Application of the Selected Herbicides on Soils:**

The selected herbicides were applied to the growing suckers of plantain at a concentration level of 0.33% each mixed with 10 liters of water and applied to the plot. The plot size is 1 hectare of land and is away from other plots at an interval of 20meters respectively. The herbicide was applied using sprayer used only for herbicide application. Soil auger was used in collecting soil samples from the surrounding plantain rows. The soil samples were taken from each plot size at the depth of 0-15cm and 15-30cm respectively placed in polythene bags and taken to the College Science Laboratory for analyses.

**Enumeration of Microbial Population in Sampled Soil:**

Enumeration of microbial population was done using specific growth media for each microorganism. Three different growth media were supplemented with inhibitors of Potatoes Dextrose Agar (PDA, DIFCO) for enumeration of fungi and Nutrient Agar (NA, OXOID) and bacteria respectively. Soil samples were collected from each microcosm at 1, 2, 3 and 10 days after treatment (DAT) respectively to assess the herbicide inhibition by soil microbial communities present in soils supporting plantain growth. Enumerations of these colonies were further done using drop plate method under sterile conditions (Halimah *et. al.*, 2010).

**Isolation of Bacteria and Fungi in Sampled Soils:**

Soil samples with three bacteria and fungi isolates were subjected to preliminary toxicity screening test to obtain isolates best grown at 2*RD (2 times double recommended dose). Sequences of the tested isolates were affiliated according to tested DNA genes to members of 5 genera namely: Clostridium, Streptococcus, Progenies, Staphylococcus aureus and Mycobacterium. Resistant bacteria and fungi were enriched with 2*RD of Atrazine liquid cultures for 10 days to select the most promising acclimatized bacteria and fungi which may be effective for biodegradation in contaminated plantain plantation soils.

**Results and Discussion**

The percentage inhibition of herbicide effect by soil microbes in the plantain plantation is presented in Table 1 and Table 2.

**Inhibition of Herbicide effect by Soil Fungi in Plantain Plantation:**

Table 1 reveals the soil fungal inhibition by comparing the control with herbicide treated soil in plantain plantation using 0.33 mg/l in concentration with recommended field rates of 1, 2, 3, 4 days and 10 days after treatment (DAT) respectively. It is evident that there was much inhibition of herbicides by the soil fungi leading to quantitative reductions as the results of herbicides applications such as glyphosinate which reveals the percentage of fungal inhibitions of herbicide that ranged between 0.0– 6.5% being the minimum and 0.0- 63% as maximum in microorganisms. Roundup at the same recommended field rates exerted inhibition percentage values that varied between 0.0 – 75.5% as maximum and 0.0-6.2% as minimum. Basta 15 inhibitive values ranged between0.0 – 81.4% as maximum and 0.0 – 8.6% minimum and Atrazine ranged between 0.0 – 71.1% and 0.0 -8.9% respectively. From the results above, the 3DAT recorded the highest percentage of soil fungal inhibition at the highest dose of application of herbicides, while 10 DAT had no trace of inhibition at different recommended field rates (RFR).

**Table 1: Soil Fungal inhibitions impact of Selected Herbicides at Different Levels of application**

| S/N | Herbicide (mg/l) | RFR | 1DAT | 2DAT | 3DAT | 4DAT | 10DAT |
|---|---|---|---|---|---|---|---|
| **1** | **Glyphosinate** | | | | | | |
| | 0.33 | 0.5* | 14.8+2.7 | 23.3+5.1 | 45.2+3.3 | 0.2+4.2 | 0.0+0.0 |
| | 0.33 | 1* | 25.8+1.8 | 33.0+1.4 | 54.3+1.7 | 2.3+0.7 | 0.0+0.0 |
| | 0.33 | 2* | **44.2**+1.4 | **59.1**+6.0 | **63.1+6.5** | **12.4+**1.5 | 0.0+0.0 |
| 2 | Roundup | | | | | | |
| | 0.33 | 0.5* | 37.9+4.9 | 40.6+4.9 | 54.1+6.2 | 9.7+1.2 | 0.0+0.0 |
| | 0.33 | 1* | 44.0+0.9 | 46.1+2.1 | 59.3+4.3 | 10.6+1.3 | 0.0+0.0 |
| | 0.33 | 2* | **57.8+5.0** | **60.8+5.9** | **75.5**+4.0 | **14.4**+2.0 | 0.0+0.0 |
| ***3*** | ***Basta-15*** | | | | | | |
| | 0.33 | 0.5* | 10.3+1.1 | 46.2+3.9 | 44.1+8.3 | 0.6+0.3 | 0.0+0.0 |
| | 0.33 | 1* | 40.2+5.6 | 55.6+5.8 | 53.3+8.6 | 5.1+0.6 | 0.0+0.0 |
| | 0.33 | 2* | **58.3+4.3** | **81.4+8.2** | **59.7**+6.5 | **7+6.5** | 0.0+0.0 |
| ***4*** | ***Atrazine*** | | | | | | |
| | 0.33 | 0.5* | 42.2+5.0 | 36.7+6.4 | 35.7+7.5 | 5.3+8.5 | 0.0+0.0 |
| | 0.33 | 1* | 54.0+7.0 | 53.7+8.9 | 48.2+5.3 | 6.1+1.3 | 0.0+0.0 |
| | ***0.33*** | ***2**** | **71.1+3.2** | **70.3**+3.6 | **68.7**+6.3 | **12.7+**4.3 | 0.0+0.0 |
| ***5*** | ***Control*** | | | | | | |
| | | | 0.0+0.0 | 0.0+0.0 | 0.0+0.0 | 0.0+0.0 | 0.0+0.0 |

***RFR: Recommended Field Rate, DAT: Day after Treatment, MG/L: Milligram per Litre**

The results are consistent with the findings of Zain *et. al*. (2013) who observed increased inhibition of the fungal colony development with increased treatment rates of each herbicide. Also Govedarica *et al*. (1993), Konstantinoviã *et al.* (1998), Miloševiã *et al*. (2001) observed that increase in herbicide dose tends to amplify its negative effect on soil micro-organisms. Ismail *et al.* (1996) showed that microbial population decreased when the concentrations of metsulfuron-methyl increased during the first 3-9 days after application, depending on soil types. Subsequently, the inhibition percentages of the fungal colony development at recommended field rate which are insignificant among the herbicides are from 3 days after treatment (DAT) onwards. Inhibition percentages of fungal colonies were abruptly low for all the treatments at 4 DAT. The fungal colonies therefore showed their ability to recover from the toxic effect by 4 days after treatment and at 10 days after treatment no further inhibition or full colony recovery were observed. This result conforms with the findings of Boschin *et. al.* (2003) and Yu *et al.* (2005). It is also observed the least inhibitory effects to fungal species, which might stem from its low doses and its ability to be degraded by the soil fungi (He *et al.*, 2006).

**Inhibition of Soil Bacteria by Herbicides in Plantain Plantation.**

Bacterial population development in soil was also affected significantly within 4 days after treatment by glyphosinate, Roundup and Basta-15 , Atrazine and the percentage inhibition of the bacterial colony development was found to be relative to the control (Table 2). The herbicides caused higher inhibition of bacterial population development compared to that of fungi. The herbicides inhibition percentages of the bacterial colonies were higher than those observed for the fungal colony development except for the basta-15 at 2 days after treatment (DAT) and 3 days after treatment (DAT). The highest inhibitions of the bacterial population were from 77.9% to 87.9%. These highest inhibitions, however, were observed from the 0.5*, 1* and 2*times (Table 2).The recommended field rate also indicated an increase in the inhibition percentages due to the sporadic treatment rate from 1 to 4 days after treatment. However, the lowest treatment at 0.5* the field recommended rate caused a high inhibition of the colony development compared with the control and those of the recommended field rate.

**Table 2: Soil Bacterial inhibitions of Selected Herbicides at Different Levels of Application.**

| HERBICIDE(mg/L) | RED | 1DAT | 2DAT | 3DAT | 4DAT | 10DAT |
|---|---|---|---|---|---|---|
| **Glyphosinate** | | | | | | |
| 0.33 | 0.5* | 30.1+1.0 | 73.6+2.5 | 62.2+5.2 | 17.8+3.2 | 0.0+0.0 |
| 0.33 | 1* | 45.5+3.0 | 82.0+3.5 | 67.9+4.9 | 22.8+6.5 | 0.0+0.0 |
| 0.33 | 2* | **46.7+3.7** | **87.9+**4.5 | **82.9+**2.6 | **32.9+**1.4 | 0.0+0.0 |
| **Roundup** | | | | | | |
| 0.33 | 0.5* | 52.8+0.9 | 73.3+4.4 | 63.5+2.2 | 13.3+4.9 | 0.0+0.0 |
| 0.33 | 1* | 55.0+5.8 | 74.0+5.7 | 67.9+4.4 | 14.6+2.7 | 0.0+0.0 |
| 0.33 | 2* | **67.0+2.6** | **81.0+**2.4 | **83.7+2.4** | **18.3+6.2** | 0.0+0.0 |
| **Basta – 15** | | | | | | |
| 0.33 | 0.5* | 69.5+6.1 | 39.0+3.4 | 27.1+6.1 | 0.6+0.6 | 0.0+0.0 |
| 0.33 | 1* | 73.0+3.6 | 48.0+3.7 | 30.9+2.3 | 8.0+5.3 | 0.0+0.0 |
| **0.33** | **2*** | **82.1+**2.3 | **66.6+**4.4 | **35.7+**1.1 | **17.0+**2.1 | 0.0+0.0 |
| **Atrazine** | | | | | | |
| 0.33 | 0.5* | 53.8+5.3 | 68.1+3.5 | 58.9+2.3 | 10.4+6.2 | 0.0+0.0 |
| 0.33 | 1* | 67.3+3.9 | 68.7+4.8 | 59.2+1.0 | 17.3+5.0 | 0.0+0.0 |
| 0.33 | 2* | **69.6+5.5** | **77.9+**3.2 | **67.2+7.3** | **21.1+**1.4 | 0.0+0.0 |
| **Control** | nil | 0.0+0.0 | 0.0+0.0 | 0.0+0.0 | 0.0+0.0 | 0.0+0.0 |

**RFR: Recommended Field Rate, DAT: Day after Treatment, MG/L: Milligram per Litre**

At the recommended rate, bacterial population, the highest inhibition of bacterial growth was recorded at 68.7%, 74% and 82% at 2DAT for Roundup, glyphosinate, Basta-15 and Atrazine at 1 DAT. Basta-15 caused the maximum suppression through growth inhibition of the bacterial colony development (73%) at faster rate (1 DAT) than glyphosinate, Roundup and Atrazine with 45.5%, 55% and 67.3% respectively (Table 2). The inhibition percentages of bacterial populations for all treatments reduced significantly by 4 DAT with a range of 8% to 22%. No inhibition was observed at 10 DAT which indicates that the bacterial population recovered from the earlier effects similar to the fungal population. The glyphosinate at the depth of 0-15cm yielded a moderate growth of Clostridium after 1 DAT at $37^{o}C$ depth of 15 -30 cm yielded scanty growth of Streptococcus, probably due the act of leaching the soil (Walker, 1975). Progenies resulting in insufficient growth grasses and loss of soil productivity. Sporadically, the applied Atrazine at 0-15cm yielded scanty growth of Cory bacterium after 1 DAT as the only bacteria in the soil but at further depth of 15 -30cm and at 2 DAT there was no bacteria present in the soil. Basta-15 yielded a moderate growth of Clostridium after 1 DAT at a depth of 0-15cm but a depth of 15-30 cm there was no bacterial growth (Table 2). In a similar way, the Roundup at 0-15cm yielded a growth of Streptococcus Progenies and Staphylococcus aureus after 1 DAT, but at 15.1-30 cm it yielded a poor growth of Mycobacterium indicating that when applying herbicides sporadically, it may results in loss of soil productivity due to residual effects.

**Conclusion:**

From the study, it was observed that increase in the concentration level of herbicides coupled with increase in the rate of application, led to grater loss of soil microorganisms and the inhibition reduced with increased time resulting to the poor growth rate of the plantain seedlings with yellowish leaves with some falling. Indiscriminate applications of Roundup, Atrazine, Basta-15 and glyphosinate caused significant but short term inhibitory effects on growth of fungal and bacterial colonies in soil microorganisms in plantain plantation in FECOLART, Owerri. However, the exposure of the microorganisms upon herbicide application caused short term changes on the growth and development of microbial communities in Plantain Plantation in FECOLART, Owerri. This has resulted to stunted growth and death of plantain seedlings of an improved variety *Musa paradisisiaca*. The herbicides treatment to soil indicated short term inhibitory growth effects on soil microbial population, exhibiting rapid decreasing trends after 3 DAT which had reduced effect by 10 DAT, indicating full recovery of the microbial populations from the initial herbicidal effects. The recovery after initial depression may be due to biodegradation and activities of the microbial population in conjunction with leaching due to sandy clay soil. Disappearance of the herbicides applied to the soil due to leaching that took place.

Therefore, since the microbes in the soils are essential for enhancement of productivity, much care is required when applying herbicides, and the use of environmental friendly herbicides be encouraged especially bio-pesticides to ensure food sustainability for the teeming population.

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