Effects of Herbicide Application on Soil Bacterial Load under Ginger Production in Kaduna, Nigeria

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K E Y W O R D S

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A B S T R A C T

Herbicides are commonly used to control weeds in crop productions, in addition to their impact on weeds, these herbicides are also affecting soil microorganisms which are responsible for numerous biological processes essential for crop production. This study was undertaken to determine the some selected physicochemical property of the soil, assess the effects of herbicide application on bacterial load and to identify the bacteria that are presents in the soil samples for the sampling sites. A soil sample that had history of herbicides application was collected from National Root Crops Research Institute Ginger Station, Kajuru, Kaduna State. The physicochemical analysis of the soil property was carried out based on the standard procedures and the colony counts were obtained using serial dilution technique. Bacteria were isolated and identified based on colonial and biochemical characteristics. Results obtained showed that each sampling point had an average bacterial counts of; Point A (1.4×10^6 cfu/g), Point B (1.5×10^6 cfu/g), Point C (1.4×10^6 cfu/g) and Point D which is the control (2.7×10^6 cfu/g). The bacterial isolates obtained in the study included Proteus sp., Staphylococcus sp., Micrococcus sp., Staphylococcus sp., Bacillus sp. and Enterobacter sp. Conclusively, this study shows that use of herbicides applications at field recommended rate may not have an adverse effects on the bacterial load.

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INTRODUCTION

Fertile soil is inhabited by the root systems of higher plants, rodents, insects and worms and tremendous numbers of microorganisms. Usually, the density of organisms is less in cultivated soil than uncultivated/virgin land and population decreases with soil acidity (Stanley, *et al.*, 2013). In modern agricultural production, herbicide application is a regular practice. The problems caused by the increased application of herbicides and resulted in decreases of number of soil microorganisms Stanley, *et al.* (2013). Herbicides are chemical substances or preparations designed to kill plants, especially weeds, or to inhibit their growth. Herbicides for example Atrazine, Paraquat, Glyphosate etc become incorporated in soil directly, during plant treatment and indirectly, via water or residues of plant and animal origin (Stanley, *et al.*, 2013).

The increased use of herbicides in agricultural soils causes the contamination of the soil with toxic chemicals. When herbicides are applied, the possibilities exist that these chemicals may exert certain effects on non-target organisms, including soil bacteria (Parkinson, 1990). The microbial biomass plays an important role in the soil ecosystem where they fulfill a crucial role in nutrient cycling and decomposition (De-Lorenzo *et al*., 2001).

Atrazine is an example of triazine herbicides with the trade name, Multrazine. The triazines were shown to inhibit photosystem II (Trebst, 2008) while paraquat is an example of quaternary ammonium herbicides with the trade name, Gramoxone. Paraquat is known to act on the Phosystem I within the photosynthetic membrane of plants. On microorganisms, they have inhibitory effects, repressing effects, reduces enzyme activity and mycelia growth. Having known the actions of these herbicides on plants the aim and objectives of

the present study were to determine the physicochemical property of the soil, assess the effects of herbicide application on bacterial load as an indicators of soil fertility and to identify the bacteria that are presents in the soil for the sampling sites.

MATERIALS AND METHODS

Sampling Site

A soil samples were collected from four different sampling points (ABCD) from a ginger research farm of the National Root Crops Research Institute, Maro, Kajuru Local Government, Kaduna State. The farm is located at Iburu along Kachia road 20 Km away from Kajuru. Kajuru Local Government Area is located on longitude 9º 59'N and 10º 55'N and latitude 7º 34'E and 8º 13'E with an area of 2464km^2 (Toro, 2001). The ecology of the study area is underlain by gneisses, migmatites and metasediments of the Precambrian age which have been intruded by a series of granitic rocks of late Precambrian to lower Palaeozoic age. The entire land structure consists of an undulating Plateau with major rivers in the State including River Kaduna in addition to several streams. The whole state is covered by the red-brown to red-yellow ferruginous tropical soils which are heavily weathered and markedly laterized. They are mostly formed on granite and gneiss parent materials, and on aeolian and many sedimentary deposits. The study area is covered by the tropical grassland vegetation with the density of trees and other plants decreasing as one move northwards (Iliya *et al*., 2015). The climate of the study area is the tropical dry-and-wet type. The wet season lasts from April through mid-October with a peak in August, while the dry season extends from mid- October of one calendar-year to April of the next year. The annual average rainfall in the state is about 1323mm (Iliya *et al*., 2015).

Sample Collection:

Soil sampling was started from a distance of 3m away from the farm land border into the farmland where soil samples were collected every distance of 50m inside clean polythene bags from four different sampling points (ABCD). A modified sterile soil core sampler was used at a depth of 20cm and to ensure a fair representation of soil sample, the sample was collected in triplicate to form a composite of about 1kg of the soil. The field was solely ginger farm and the management was done both chemical (Herbicides) and manual (Hand picking). The Herbicides used were Paraquat, Glyphosate and Atrazine. Herbicides were applied 4 to 5 weeks after planting at the rate of 4L/Hectre each before shoots to emerge. The soil samples were divided into two for physicochemical and microbiological analysis and were taken to the Microbiology research laboratory of Bayero University, Kano for further analysis. In line with standard procedures as described by Dem, (2007), the samples were sieved through a pore size of 0.5mm brass sieve and was done inside safety cabinet chamber and stored at room temperature for further analysis.

Determination of the selected soil physicochemical property

Temperature

The temperature of the soil was taken using mercury-in-bulb thermometer at each point of collection during sampling. This was achieved by dipped the thermometer 5-10cm depth and waited for 2-3 minutes and the reading was recorded.

Soil pH

A soil: water ratio of 1:2 was used. A 10g of soil was suspended in to 20ml of distilled water and a digital pH meter model EQ-610 was immersed in to the suspension and waited for seconds and the result was recorded as employed by Onyeike and Osuji (2003).

Moisture content

The laboratory determination of the moisture content of soil samples was carried out by placing 10g of soil sample in a weighing glass beaker initially weighed, followed by oven dried at 70°C for 24h. Glass beaker contained the dried soil were then be weighed again to get the final weight of the soil. The moisture content was calculated as percentage using the formula as described by ASTM, (2010).

Water Holding Capacity (WHC)

Water Holding Capacity (WHC) of the soil was determined by placing 3g of soil samples on a piece of Whatman filter paper which had been initially weighed, followed by oven drying at 70°C for 24h. Oven-dried soil on the weighed Whatman filter paper was dipped and saturated. The soil was then placed in humid enclosure to drain off the water and weighed again, and finally calculated using the formula below (ASTM, 2010).

$$\text{Water Holding Capacity (WHC): } = \frac{\text{mass of water contained in saturated soil}}{\text{Mass of saturated soil}} \times 100$$

Organic matter

The soil organic matter was determined by Walkley-Black Wet Oxidation Method adopted by Eno, *et al.* (2009).

Soil texture

The texture of the soil was determined by the hydrometer method for the mechanical analysis of particle size distribution employed by Gee and Bauder (1986) was used.

Enumeration of the Total Aerobic Bacterial Plate Count in Herbicide Cultivated Soil Samples

A Nutrient Agar (NA, Oxoid) medium supplemented with 0.1g/L cyclohexamide specific media was used and were prepared according to the manufacturer's instruction.

A twenty five gram (25g) of herbicides treated and control soil samples from uncultivated site were weighted aseptically in to a conical flask containing 225ml of sterile distilled water. A homogenizer was used for proper mixing. Serial dilutions of up to 10^{-5} fold were prepared by transferring 1ml from stock solution in a test tube containing 9ml of sterile distilled water. A 0.1ml of 10^{-5} dilution of each suspension were plated aseptically using spread plate method on the prepared nutrient agar. The plates were prepared in triplicates, covered and dried. After 1h, the plates were inverted and sealed with parafilm to avoid contamination and incubated at 37°C for a period of 24 hours. After incubation the colonies were counted and expressed as colony forming unit per/g. The isolates obtained were sub cultured and the pure bacterial colonies were slanted and stored in the refrigerator at 4^0C (Odetunde *et al.,* 2014).

Isolation, Characterization and Identification of Bacteria from Herbicide Cultivated Soil Samples

A pure isolates obtained were subjected for the following identifications techniques.

Gram Staining: This technique was used to determine the nature of the bacterium. Colonies grown on nutrient agar was gram stained as per the procedure explained by Todar *et al*., (2005). **Motility Test:** Bacterial motility was done by hanging loop method. Few drops of liquid culture were place onto the cover slip in sterile condition. Depression slide was taken and the concave portion over the drop was pressed on the slide onto the cover slip. The slide was inverted quickly to keep from disrupting the drop. Then the motility was examined under microscope at 40× magnification Todar *et al*., (2005).

Biochemical Tests: Biochemical test such as indole test, methyl red test, citrate utilization test, voges proskauer test, catalase test, Oxidase and Urease test was carried out for the identifications of the isolates as adopted by Musliu and Salawudeen, (2012).

RESULTS

Selected Physicochemical Properties of the Soil Samples for the four sampling sites

Table 1 shows the result for the selected physicochemical properties of the soil sample at different sampling site of the ginger research farm. The temperature of the sampling point A and C had the highest average value of 40.0°C each and point B and control point D had 39.0°C respectively. Point A had the highest pH of 6.59 and point D with least of 5.99. In case of moisture contents point B had the highest value of 0.280% with the least water holding capacity of 60.67%. Point D had the least moisture contents 0.225 but with 65.43% water holding capacity while A had the highest water holding capacity of 67.57% with 0.229 moisture contents. However, the textural class for all the four point were sandy loam soil table.

Table 1: Physicochemical properties of soil sample for the four sampling sites

		Sampling	**Site**		
Parameter	A	B	C	D (Control)	Standard limits
Temperature	40.00	39.00	40.00	39.00	<40°C*
pH (H_2O)	6.59	6.45	6.53	5.99	6.0-9.0*
Moisture contents (%)	0.229	0.280	0.231	0.225	
Water Holding capacity	67.57	60.67	64.10	65.43	
Organic Matter (%)	1.878	1.88	1.769	1.996	0.5% – 3.0%*
Sand (%)	62	59[a]	61	60	
Silt (%)	23	24	19	24	
Clay (%)	15	17	20	16	
Textural class	Sandy loam	Sandyloam	Sandy loam	Sandy loam	

Statistically not significant p>0.05 (P=0.14) , FEPA (1991).

Average Bacterial Colony Forming Unit per Gram (cfu/g) of Soil for the four sampling site

Table 2 shows the result of mean bacterial colony forming unit per gram (cfu/g) of soil samples analyzed. The results from the table indicates that point D had the highest counts with 2.7×10^6 followed by point B with 1.6×10^6 and the lowest counts were recorded in point A and point C with similar average of 1.4×10^6 respectively.

Table 2: Average Bacterial Colony Forming Unit/Gram (cfu/g) of Soil samples for the four Sampling sites

Sampling site	CFU/G	Fertility level
A	1.4×10^6	$1O^6$ - 10^8cfu/g FAO, 2016
B	1.5×10^6	
C	1.4×10^6	
D	2.7×10^6	

Statistically not significant p>0.05 (P=0.081)

Morphological and Biochemical Properties of the Different Bacterial Isolates

Table 3 shows the morphological and biochemical properties of the different bacterial isolates. This shows that six bacterial isolates were identified and characterized based on the differences on their morphological and biochemical properties and the bacterial isolates were identified are *Proteus mirabilis, Staphylococcus sp., Micrococcus sp., Staphylococcus sp., Bacillus sp.* and *Enterobacter sp.*

Table 3: Morphological and Biochemical Properties of Different Bacterial Isolates

Lab. Code	Morphology /Arrengement.	Endo Spore	Motility	Grams test	Catalase	Oxidase.	Citrate.	Voges Proscour	Methyl Red	Urease.	Indole.	Expected Bacteria
1	Rod/ Single	-	+	-	+	-	+	-	+	+	-	*Prpteus. sp.*
7a	Coccus/irre. Clusters	-	-	+	+	-	+	+		+	-	*Staphylococcus sp.*
5	Coccus/clusters	-	-	+	+	+	+	+		+	-	*Micrococcus sp.*
8	Coccus/irre. Clusters	-	-	+	+	-	-	+		+	-	*Staphylococcus sp.*
7	Rod/pairs,chain	+	+	+	+	-	+	-		+	-	*Bacillus sp.*
16	Rod/ Single	-	+	-	+	-	+	+	+	+	-	*Enterobacter sp.*

DISCUSSION

The present research shows that physicochemical properties of the soil, aerobic bacterial counts and the predominant bacteria presents

The selected physicochemical properties of the soil sample of different site was determine in order to come up with the differences or similarities that exist in the site. From the results obtained the temperature of the site A and C with the average value of 40.0 respectively, site B and D had 39.0 and there is no statistically significant between the sampling sites ($P > 0.05$) and this may be due to the fact that they have similar ecological factors and the temperature range for the sites are within the standard limit of <40°C according to FEPA (1991). Site A had the highest pH of 6.59 and site D with least of 5.99. In case of moisture contents B had the highest value of 0.280 with the least water holding capacity of 60.67. Site D had the least moisture contents 0.225 but with 65.43 water holding capacity while A had the highest water holding capacity of 67.57 with 0.229 moisture contents and both the pH and moisture contents are within the standard limit of 6.0 – 9.0 and 0.5% – 3.0% respectively FEPA (1991). However, the textural class for all the four sampling points were sandy loam soil.

The enumeration of soil bacterial counts was carry out and the average counts were 2.7×10^6, 1.6×10^6cfu/g, 1.4×10^6cfu/g and 1.4×10^6cfu/g for point D (control), point A, B and C respectively even though there was no statistically significant differences between the points (p=0.081) but the little differences that exist between point D and the remaining three sites was due to the fact that the three points ABC had the history of herbicides applications while point D (control) is a virgin land. These findings are in consistent with the works by Ubuoh, *et al*. (2012) that applications of pesticides result in decline of the bacterial population which further stressed the soil the bacterial *species* present in the soil which will invariable reduce the action of bacteria in the soil that might lead to decline in soil fertility in the farmland. And persist in the soil for long usually affect soil resulting to chemical degradation of the soil (Kamrin, 1997; Gupta, 2001). However, the results of this study shows that 10^6 bacterial colony forming unit per gram of soil which are within the

range of 10^6 -10^8cfu/g culturable bacteria present per gram of soil would be considerd a healthy number and number less than 10^6cfu/g indicates poorer soil health (FAO, 2016) which may be due to a lack of nutrients as food in low organic matter soil, abiotic stress imposed by extreme soil pH values (pH< 5 or > 8) or toxicity imposed by organic and in organic anthropogenic contaminants and in lines with works by Stanley, *et al*. (2013) that enumerates and identified numerous microorganisms in the soil which includes Proteus species and Actinomycetes which were sensitive to herbicides application and therefore, may serve as a reliable indicator of the biological value of soil.

Six different bacterial isolates were isolated, identified and characterized based morphological and biochemical properties. The bacterial isolates were characterized are both gram positive and gram negative bacteria with the predominant of gram positive. The gram positive bacteria are, *Staphylococcus sp.*, *Micrococcus sp.*, *Staphylococcus sp*., *Bacillus sp.* and gram negative bacteria are *Proteus sp.* and *Enterobacter sp*. The presents study is in agreement with Stanley, *et al*. (2013) works on the effect of two herbicides, atrazine and paraquat on soil bacterial population with the predominant of gram positive *Bacillus species* and *Micrococcus species* and with less observed of gram negative species of *Proteus* and *Pseudomonas* bacterial. However, the predominant species of *Bacillus* and *micrococcus* respectively were isolated by Ubuoh, *et al*., (2012) on the research conducted on the effects of pesticide application on soil microbial spectrum: case study fecolart demonstration farm, Owerri-west, Imo State, Nigeria. But the present research is in contrast with the Benslama and Boulahrouf, (2013) works on isolation and characterization of glyphosate degrading bacteria from different soils of Algeria were isolated gram negative species of *Pseudomonas, Enterobacter, Serratia, Rahnella* and *Escherichia.* Conclusively, this study shows that use of herbicides applications at field recommended rate may not have an adverse effects on the bacterial load.

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