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Research Article

Antibiotic Susceptibility of Bacteria Isolated from Soil Contaminated with Glyphosphate

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ABSTRACT

Glyphosate has been a widely used herbicide for close to half a century to control weeds through a biochemical process by acting as an enzymatic inhibitor in the shikimate pathway of aromatic amino acid synthesis. The aim of this work is to assess the impact of glyphosate usage in farms on the bacteria ecosystem and its influence on the development of antibiotic resistance in the bacteria exposed to it.

Four soil samples were collected at four different locations. Three were collected at a farm in Owo in Ondo state, where the herbicide is frequently used to control weeds (samples A, B, and C), while sample D was collected at a nearby farm with no use of the herbicide in the past. The organisms were cultured using the serial pour plate method, bacteria count was enumerated and the pure culture was obtained. The bacteria were identified using biochemical methods. Antibiotic sensitivity testing using common antibiotics was used against the bacteria isolated. The result showed a count of $6.6 \pm 0.02 \times 10^5$ cfu/gm for sample A, $5.9 \pm 0.01 \times 10^5$ cfu/gm for sample B, $4.9 \pm 0.01 \times 10^5$ cfu/gm for sample C, and $7.0 \pm 0.02 \times 10^5$ cfu/gm for sample D (control). The identified organisms in contaminated soil are *Bacillus cereus*, *Micrococcus luteus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus aureus*, and *Flavobacterium lutescens*, while the organisms found in control soil are *Clostridium botulinum* and *Bacillus subtilis*. Most of the isolated organisms were susceptible to most antibiotics except *Staphylococcus aureus* and amoxicillin, which was the most common antibiotic resisted by most bacteria. Glyphosate has selective pressure on the state of biomass and bacteria in an area exposed to it, and it could affect antibiotic sensitivity to some degree. However, this may need further evaluation.

Keywords: Antibiotic; Bacteria; Count; Glyphosate; Ecosystem; Weed control; Herbicide

INTRODUCTION

Glyphosate is a non-selective, systemic herbicide, discovered in 1971, an active chemical present in many common herbicides such as roundup and many more. Herbicide generally which is a group under pesticide family of chemical, help in the control of annual and perennial weeds. Weed control is one of the major challenges and labor-intensive activity in agriculture and with advent of herbicide this has been laid to partial rest [1]. Though the United States' EPA (Environmental Protection Agency) website, opined that glyphosate is harmless to humans, animals and the environment. However, other researchers were of contrary opinion based on their findings [2-4].

After application of glyphosate to the farmland, it is absorbed by plants and translocated within the plants to the site of action. In the absence of deactivation mechanism and continuous accumulation to toxic levels at the site of action, glyphosate act by inhibiting Aromatic Amino Acids (AAA) synthesis in plants and microorganisms that have shikimate pathway of amino acid biosynthesis. This is done via inhibition of enzyme 5-Enolpyruvylshikimate-3-Phosphate Synthase (EPSPS), a catalyst in the penultimate reaction of the shikimate [5]. Thus, glyphosate tampers with stabled ecosystem by possibly killing off bacteria needed for maintenance of soil fertility. However, not all organisms have shikimate pathway or have some class EPSPS not susceptible to Glyphosate, such organism can escape the deleterious effect of the chemical [6,7].

There are unbreakable interconnections that exist in the ecosystem, which makes the impact of one chemical substance have a wide effect. Glyphosate, acting as a selective pressure that has a known specific mechanism of action, can shape the type of bacteria present in an environment [8]. The herbicide is used on

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farms where human and livestock are exposed. Thus, if there is a selection of bacteria resistant to common antibiotics, this could pose a big problem for society. Research has shown the potential of glyphosate to cause antibiotic resistance in different settings [9]. Beyond the identification of a shift in population, there is a need to critically assess the impact of glyphosate on the resistance of bacteria exposed to it to common limited antibiotics.

MATERIALS AND METHODS

Study area

This study was carried out on different farmlands in Owo, Ondo state.

Sample collection

Portion of the Soil samples of 200 g was collected from three different glyphosate contaminated sites, from the top soil layer profile of 0 cm-20 cm; in a sterile plastic bag and stored in ambient temperature. A sample devoid of glyphosate was collected from the region which will serve as control

Isolation and enumeration of bacteria from the contaminated soil

Isolation and enumeration of total viable bacteria on the test soils and controls was carried out using conventional methods [10].

Phenotypic and biochemical identification of isolates

Phenotypic and biochemical identification of bacterial isolates was done by carrying out Gram staining reactions, catalase test, coagulase test, oxidase test, spore test, motility test, and growth on differential media, citrate utilization, Methyl Red, Voges Proskauer reactions, urease production, nitrate reduction, and sugar fermentation tests [11].

Antibiotics sensitivity test

The tests were carried out in nutrient agar plate and antibiotics (optan wig) were aseptically placed on the media after innoculating the agar plate with the organism in an anticlockwise manner. The test organism was screened against Ciprofloxacin 10 µg, Nofloxacin 10 µg, Gentamycin 10 µg, Augumentin 30 µg, Ampicillin 30 µg, Chloramphenicol 10 µg, Tarvid 10 µg, Reflacine 10 µg, Streptomycin 30 µg, Ceporex 10 µg, Nalidixic Acid 30 µg, Septrin 30 µg, Amoxil 20 µg, Rifampicin 20 µg, Erythromycin 30 µg, Ampliclox 20 µg and Levofloxacin 20 µg. The Kirby-bauer disc diffusion method was employed, Multi discs containing the antibiotics named above were aseptically placed on the nutrient agar plate inoculated with the test organism. The discs were allowed for 1 hour to diffuse through the plate and were then incubated at 37°C for 24 hours. After incubation, Inhibition Zone Diameters (IZDs) around each antibiotic were measured. Inhibition Zone Diameters were measured in mm and were recorded by calculating the mean of IZDs in duplicate plates. The results were interpreted according to CLSI standards (Table 1).

Table 1: Sample grouping.

Sample code	Locations
A	Cocoa Farm(contaminated)
B	Cassava Farm(contaminated)
C	Plantain Farm(contaminated)
D (Control)	Outside school

RESULTS AND DISCUSSION

Statistical analysis

Results were expressed as the means ± standard error of mean and excel sheet for descriptive analysis (Tables 2-6).

Table 2: Colony count of soil samples collected from different farm land in Emure Ile.

Samples	TBC × 10^5 (cfu/g)
Soil A	6.6 ± 0.02
Soil B	5.9 ± 0.01
Soil C	4.9 ± 0.03
Soil D (Control)	7.0 ± 0.01

Note: TBC: Total Bacterial Count.

Table 3: Morphological and biochemical characteristics of bacterial isolated from the soil sample.

Samples	Isolates	margin	Colour	Elevation	Texture	Shape	Gram Strain	Catalase	Coagulse	Glucose	lactose	Most Probable organism Isolated
A	A1	Undulate	Off white	Flat	Dry	Rod	+	+	-	+	-	*Bacillus cereus*
	A2	Entire	Yellow	Raised	Mucoid	Cocci	+	+	-	+	-	*Micrococcus luteus*
B	B1	Undulate	White	Flat	Dry	Rod	+	+	+	+	-	*Bacillus subtilis*
	B2	Entire	Yellow	Raised	Moist	Cocci	+	+	+	+	+	*Staphylococcus aureus*
	B3	Undulate	Greyish white	Convex	Dry	Filamentous	-	+	-	-	-	*Escherichia coli*
C	C1	Entire	Milky	Raised	Moist	Cocci	+	+	+	+	-	*Staphylococcus aureus*
	C2	Filamentous	Milky	Flat	Moist	Rod	-	+	-	+	+	*Flavobacterium lutescens*
D	D1	Filamentous	Milky	Flat	Dry	Filamentous	+	-	-	+	-	*Clostridium botulinum*
	D2	Undulate	White	Flat	Dry	Rod	+	+	+	-	-	*Bacillus subtilis*

Note: +: positive; -: negative; A, B, C: Diesel contaminated soil; D: Uncontaminated soil.

Table 4: Antibiotic susceptibility test (+VE disc).

Isolate	CPX	E	LEV	CN	APX	RD	AMX	S	NB	CH
Bacillus cereus	S	S	S	S	S	S	R	S	R	R
Bacillus subtilis	S	S	S	S	S	S	R	S	R	S
Micrococcus luteus	S	S	S	S	S	S	S	S	S	S
Staphylococcus aureus	S	S	S	S	R	R	R	S	R	S
Flavobacterium lutescens	S	S	S	S	S	S	S	S	S	S

Table 5: Antibiotic susceptibility test (-VE disc).

Isolate	OFX	NA	PEF	CN	AU	CPX	SXT	S	PN	CEP
Escherichia coli	S	S	S	S	S	S	S	S	S	S

Note: R: Resistance to the antibiotics; S: Susceptible to antibiotics.

Table 6: The antibiotics present in the disc and abbreviations.

S.No	Antibiotic	Abbreviation	Concentration
1	Tarivid	OFX	10 mcg
2	Ciproflox	CPX	10 mcg
3	Erythromycin	E	30 mcg
4	Levofloxacin	LEV	20 mcg
5	Gentamycin	CN	10 mcg
6	Amplicox	APX	20 mcg
7	Rifampicin	RD	20 mcg
8	Amoxil	AML	20 mcg
9	Streptomycin	S	30 mcg
10	Norfloxacin	NB	10 mcg
11	Chloramphenicol	CH	30 mcg
12	Ceporex	CEP	10 mcg
13	Nalidixic acid,:	NA	30 mcg
14	Reflecine	PEF	10 mcg
15	Augmentin	AU	30 mcg
16	Septrin	SXT	30 mcg
17	Amplicin	PN	30 mcg

The bacterial population and diversity in a soil could be an indicator of its fertility and its assessment becomes paramount. Despite glyphosate's wide use for weed control in agriculture, questions remain about the herbicide's effect on soil microbial communities. Like other toxicant, glyphosate may have effect on unicellular and multicellular organism that thus makes studies on it necessary [12]. The findings of studies on the impact of glyphosate on the population and variety are debatable because some argue that most bacteria can utilize it as a source of carbon for metabolism, such as *Pseudomonas* spp., which would promote bacterial development [13]. Research conducted in 2006 by Ratcliff indicated a rise in the number of culturable bacteria in high concentrations of glyphosate. It was concluded that when used at the approved field rate, glyphosate in commercial formulations has a benign effect on community structure [14].

According to the findings of another study [15], the use of glyphosate may modify the population and activity of soil microbes. This finding, however, did not support the assumption that administration increased bacterial biomass. This is consistent with the observation that glyphosate-treated soil has a smaller bacterial population than untreated soil, and that the application of the herbicide causes a partial disruption of the soil's bacterial connection network [16]. The relative abundance of organisms in the roots did not differ between the microbial communities in the roots of plants that got a foliar application of glyphosate and those that did not [17].

Bacillus cereus, *Micrococcus luteus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus aureus*, and *Flavobacterium lutescens* were the probable organisms found on pesticide-treated soil, while *Clostridium botulinum* and *Bacillus subtilis* are likely organisms found on control soil. The application of the herbicide glyphosate causes a shift in the bacterial balance in the plant-endophyte, favoring some microbial groups that can use the herbicide as a source of energy and nutrients while being hazardous to other groups. *Acinetobacter calcoaceticus*, *A. junii*, *Burkholderia* sp., *B. gladioli*, *Enterobacter sakazaki*, *Klebsiella pneumoniae*, *Pseudomonas oryzihabitans*, *P. straminea*, *Ralstonia pickettii*, and *Sphingomonas* sp. were among the cultivable endophytic bacterial community

recovered from soybean leaves, stems, and roots [18]. The research done by Lalevi in 2019 was able to retrieve two strains of bacteria (PP-23 and NT-11) following application of glyphosate on maize and raspberry plantations, suggesting that the bacteria that can grow and use glyphosate may be exploited for bioremedative roles. They are therefore attractive candidates for bioremediation of glyphosate-contaminated soil as they were able to thrive on mineral medium supplemented with glyphosate at concentrations of 1 and 2 percent (v/v) [19].

By using the enrichment culture technique, bacterial strains that can use glyphosate as their only carbon source were isolated from contaminated soil. These strains were then recognized using a partial 16S rRNA gene sequence analysis. The best glyphosate-degrading *Pseudomonas* spp. strains were GA07, GA09, and GC04, which were employed in glyphosate bioremediation laboratory tests [20]. There is a need to examine glyphosate as a selection pressure for glyphosate-resistance in bacteria because this could cause changes in the composition of the microbiome and an increase in antibiotic resistance to clinically important antimicrobial agents. Research on a connection between glyphosate and antibiotic resistance is still lacking [21]. The majority of the organisms are, nevertheless, susceptible to the existing antibiotics. *Staphylococcus aureus*, among the organisms, is the most resistant to the majority of antibiotics, and amoxicillin is the most resistant antibiotic. The use of glyphosate may have sparked the emergence of resistance in other bacteria, posing difficulties for producers of live livestock and those treating affected humans. It has been shown that soil bacteria exposed to herbicides like glyphosate are more likely to develop genetic resistance to antibiotics [22-25]. The findings show that natural freshwater bacteria cross-select for antibiotic resistance [26].

There is proof that glyphosate, an herbicide and antibiotic, may be a factor in the development of antibiotic resistance in countries where it is widely used because it alters the microbial ecology. In the last 40 years, glyphosate use has increased globally, which is associated with the emergence of bacterial resistance [24]. Additionally, glyphosate prevents the development of bacteria that have an EPSP synthase [22]. Certain bacteria develop glyphosate resistance by gaining mutations in the EPSP synthase gene, making the encoded enzyme less sensitive to the herbicide. The molecular mechanisms underpinning glyphosate entail raising EPSP synthase synthesis, detoxifying or destroying glyphosate, and reducing or increasing the uptake or export of the herbicide [25,27].

CONCLUSION

Glyphosate is an environmental selective pressure that could affect the population and diversity of bacteria. Some bacteria become resistant either by nature or through acquiescence of genes needed to avert the effect of it and use it as a sole source of carbon. This change in population and strain reflects changes in antibiotic sensitivity, which confers a risk of development of bacteria resistance to available antibiotics. This can pose a great challenge to the management of clinical conditions caused by such organisms, both to humans and livestock.

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CONFLICT OF INTEREST

There is no conflict of interest in the research.

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