Potentiality Assessment of *Acidovorax sp*. and *Aeromonas sp.* for Degradation of Glyphosate and Paraquat Herbicides

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A B S T R A C T

In agriculture, weed control through chemical herbicides, creates spray drift hazards and adversely affects the environment. The search for an alternate method for the degradations of herbicides through the use of bacteria which is eco-friendly for the bioremediations of herbicides contaminated soil. This study aimed at assessing the biodegradation of glyphosate and paraquat herbicides by bacteria isolated from soil. Soil samples were collected from Research Farm of National Root Crops Research Institute, Ginger Station, Kajuru, Kaduna State, Nigeria. Bacteria were isolated and identified based on colonial and biochemical characteristics. The assessment for the potential of the bacterial isolates to degrade glyphosate and paraquat herbicides was carried out using microcosms study. The bacterial isolates were phenotypically identified as Proteus sp., Acidovorax sp., Micrococcus sp., Staphylococcus aureus, Bacillus sp. and Aeromonas sp. Among the isolates, Acidovorax sp. and Aeromonas sp. had the highest potential to utilize glyphosate and paraquat as sole source of carbon. In the presence of glyphosate Acidovorax sp. and Aeromonas sp. had cells count of 1.30×108cfu/g and 1.90×108cfu/g respectively. However, in the presents of paraquat as a source of carbon Acidovorax sp. and Aeromonas sp. had the counts of 1.90×107cfu/g and 2.60×106cfu/g respectively. The quantifications of glyphosate and paraquat herbicides residues in amended soil using GC/MS shows that Acidovorax sp. have degraded 77.1% while Aeromonas sp. 88.1% of glyphosate. However, Acidovorax sp. degraded 51.5% while Aeromonas sp. 59.8% of paraquat. This study showed that Acidovorax sp. and Aeromonas sp. had the potential to degrade glyphosate and paraquat herbicides.

INTRODUCTION

Herbicides are chemical substances or preparations designed to kill or inhibit the growth of plants, especially weeds. Herbicides are commonly used to control weeds in crop production. In addition, herbicides influence soil microorganisms responsible for numerous biological processes and crop production (Saleh, *et al*., 2020). However, excess use of herbicides in agricultural soils could contaminate the soil with toxic chemicals. When herbicides are applied, it is possible that certain chemicals may exert significant effects on non-target organisms such as soil bacteria (De-Lorenzo *et al*., 2001). Herbicides such as glyphosate and paraquat become incorporated in to soil directly during weed control and indirectly via water or residues of plant and animal origin. After applications, herbicides may evaporate (volatilize) and be washed away through surface run-offs or leached into deep soil strata (Kortekamp, 2011). Cost effective treatment and removal of such environmental pollutants become an essential part of environmental remediation. Some commonly employed methods toward the removal of glyphosate and paraquat herbicides residues from the soil include, chemical oxidation, photo oxidation, sorption, volatilization, leaching and biodegradation (Bogen *et al*., 2008).

Biodegradation is considered to be a cheaper, environmentally eco- friendly process involved in the remediation of glyphosate and paraquat contaminated soil (Yuan *et al*., 2002). The use of bacteria in the clean-up of the environment is the most suitable, non –toxic, economical and easy to carry out (Prakash and Irfan, 2011). Biodegradation is the process by which organic substances are broken down into smaller compounds by living microorganisms (Marinescu *et al*., 2009). Biological decontamination methods are preferable to conventional approaches because in general, microorganisms degrade numerous environmental pollutants without producing toxic intermediates (Olawale *et al*., 2011). Many bacteria that are able to degrade glyphosate and paraquat herbicides have been isolated from soil around the World (Desaint *et al*., 2000).

MATERIALS AND METHODS

Sampling Site and Collection

Soil samples were collected from Ginger Research Farm at the National Root Crops Research Institute, Maro, Kajuru Local Government Area of Kaduna State Northwestern Nigeria. The farm is located at Iburu along Kachia road 20 Km away from Kajuru town. 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 (GIS, 2021). The soil sampling was started 3m away from the entrance of the farmland. The soil samples were collected every 50m inside clean polythene bags. The soil samples were collected from four different sites using soil corer at a depth of 20cm. To ensure a fair representation of soil sample, the soil samples were collected in triplicate. The soil samples were transported 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 air-dried and sieved through a pore size of 0.5mm brass sieve and stored at ambient temperature until further analysis (Dem, 2007).

The herbicides were purchased from a local agricultural dealer store in Kaduna. The herbicides are Paraquat and Glyphosate (Paraeforce and Force up respectively) and were identified and authenticated by comparing it with glyphosate and paraquat standard at the Department of Quality Assurance of the National Root Crops Research Institute, Umudike; Abia State, Nigeria. Paraquat contains 276g paraquat dichloride (200g paraquat ion) per litre. The Force up contains 360g glyphosate per litre in the form of 480 Grams per litre isopropylamine salt of soluble liquid (SL).

Isolation and Identification of Isolated Bacteria from Soil Samples

The isolates obtained from the soil by repeating sub culture of the bacterial colonies were identified using morphological, biochemical test and Molecular identifications (Todar *et al*., 2005).

Assessment of the Potential of the Isolated Bacteria to Degrade Glyphosate and Paraquat Herbicides

These were assessed in the laboratory using soil microcosm experiment (Pal and Das Gupta, 1994). Bacterial isolate obtained from pure culture were screened by inoculating the different bacterial isolates on a prepared solid mineral salt medium (MSM) enriched with 0.1% each of glyphosate and paraquat herbicides and incubated for 24 to 120 hours. The colonies were counted and isolates that recorded highest number of cells were considered for the assessment. Soil samples were collected and sterilized using autoclave at 121°C for 15 minutes. Soil samples (100g) was weighed into 500ml tapered bottles. A test soil in each tapered bottle was spiked with glyphosate and paraquat herbicides at a concentration of 6 mg kg-1 each and a control without herbicides. Five milliliters (5ml) suspension of a screened bacterial isolates equivalent to 0.5 Macfarland standard turbidity was added in to the respective bottles. A sterile distilled water was added to adjust to 60% water holding Potential (WHC) in each bottle. The experiment was monitored for a period of five weeks at a controlled temperature of 37 °C. Soil moisture contents was measure and maintain to constant weight by adding an appropriate amount of distilled water weekly (Pal and Das Gupta, 1994). At the expiration of five weeks of incubation, the soil (test soil) was taken for GCMS analysis for quantification of glyphosate and paraquat herbicides residues at Department Of Chemistry, Yobe State University, Damaturu.

Extraction of Glyphosate and Paraquat Herbicides Residues from Herbicides Amended Soil

One gram (1g) of glyphosate herbicide contaminated soil sample was extracted with 10mL of acetonitrile. The mixture was mixed at high speed using vortex mixer for 1min. Exactly 0.1 g of NaCl and 0.2g of activated anhydrous MgSO4 were added to the mixture, and mixing continued for an additional 60 seconds. The mixture was centrifuged for 5 minutes at 5000 rpm. The supernatant was transferred to a 15 mL tube containing 2g MgSO4. After shaking for 1minute and centrifuged for 5min at 5000 rpm, 4mL of the supernatant was transferred to a 5mL vial and evaporated to dryness. The residue was reconstituted by acetonitrile to obtain 1mL solution, and after shaking for 3minutes, 2µL of the solution was analyzed using gas chromatograph to determine the residues of glyphosate herbicide (Karyn and Ronald, 2012). Similar treatment was done for the paraquat amended soil sample.

GC/MS Analysis of Glyphosate and Paraquat Herbicides Residues from Contaminated Soil and the Ginger Rhizomes

Two microliters (2µL) of each of the solution (extracts) were injected separately into gas chromatograph to determine the herbicides residues. The GC/MS was equipped with helium as the carrier gas at a constant flow of 1 mL/minute. The oven initial temperature

setting was 80°C for 3 minutes then ramped at the rate of 15°C/minute to 290°C and held for 5 minutes. Injection port was adjusted at 250°C and split less injection mode was used. After acquisition of the total ion chromate gram for the mixed stock standard solutions in scan mode, peaks were identified by their retention time and mass spectra. The most abundant ion that showed no evidence of chromatographic interference and had the highest signal-to-noise ratio was selected for quantification purposes (Karyn and Ronald, 2012).

RESULTS

The potential of the isolated bacteria to degrade glyphosate and paraquat and use it as carbon and energy source for the increases in bacterial populations were presented in Table 1. The two bacteria (*Acidovorax* sp. and *Aeromonas* sp.) were found to have the highest potential ability to utilized glyphosate and Paraquat herbicides as carbon source. *Acidovorax* sp. and *Aeromonas* sp. shows cells count of 1.30×10^{8}cfu/g and 1.90×10^{8}cfu/g respectively in the presents of glyphosate while *Proteus* sp. (2.30×10^{6}cfu/g), *Micrococcus* sp. (1.40×10^{6}cfu/g), *Bacillus* sp. (1.20×10^{6}cfu/g) and *Staphylococcus* sp. (1.11×10^{6}cfu/g). However, in the presents of paraquat as a source of carbon *Acidovorax* sp. and *Aeromonas* sp. has the counts of 1.90×10^{7}cfu/g and 2.60×10^{6}cfu/g respectively as shown in table 1.

Table 1. Potentiality of the Isolated Bacteria to Utilize Glyphosate and Paraquat Herbicides for Growth

Bacteria	Glyphosate Herbicide	Paraquat Herbicide
Proteus sp.	2.30×10^{6}cfu/g	1.10×10^{5}cfu/g
Acidovorax sp.	1.30×10^{8}cfu/g	1.90×10^{7}cfu/g
Micrococcus sp.	1.40×10^{6}cfu/g	1.80×10^{5}cfu/g
Bacillus sp.	1.20×10^{6}cfu/g	1.70×10^{5}cfu/g
Staphylococcus sp.	1.11×10^{6}cfu/g	2.90×10^{5}cfu/g
Aeromonas sp.	1.90×10^{8}cfu/g	2.60×10^{6}cfu/g

Glyphosate residues in soil and their percentage degradations by the bacterial isolates is presented in Table 2. The results reveals that, the highest percentage degradations was recorded in treatment B (Soil + Glyphosate + *Aeromonas* sp.) with percentage value of 88.1% while treatment A (Soil + Glyphosate + *Acidovorax* sp.) and C (Soil + Glyphosate + *Acidovorax* sp. + *Aeromonas* sp.) were 77.1% and 70.6% respectively. The D (Control) soil had percentage value of 11.9% only (Table 2.).

Table 2. Percentage Degradation of Glyphosate Herbicide

Treatments	Initial concentration (6mg/kg)	Final concentration (6mg/kg)	Percentage degradation (%)
A	0.109	0.025	77.1
B	0.109	0.013	88.1
C	0.109	0.032	70.6
D (Control)	0.109	0.096	11.9

Key:A= Soil + Glyphosate + *Acidovorax* sp., B= Soil + Glyphosate + *Aeromonas* sp., C= Soil + Glyphosate + *Acidovorax* sp. and *Aeromonas* sp. And D= Soil + Glyphosate (Control)

Paraquat resdues in soil and their percentage degradations by bacteria (*Acidovorax* sp. and *Aeromonas* sp.) were assessed. The highest percentage degradations was recorded in treatment C (Soil + Paraquat + *Acidovorax* sp. + *Aeromonas* sp.) with values of 61.9% and the control treatment D (Soil + Paraquat) had the least percentage degradation (8.2%) (Table 3.).

Table 3. Percentage Degradation of Paraquat Herbicide

Treatments:	Initial concentration (6mg/kg)	Final concentration (6mg/kg)	Percentage degradation (%)
A	0.097	0.047	51.5
B	0.097	0.039	59.8
C	0.097	0.037	61.9
D (Control)	0.097	0.089	8.2

Key: A= Soil + Paraquat + *Acidovorax* sp., B= Soil + Paraquat + *Aeromonas* sp., C= Soil + Paraquat + *Acidovorax* sp. and *Aeromonas* sp. And D= Soil + Paraquat (Control)

DISCUSSION

Biodegradation is the process by which organic substances are broken down into smaller compounds by living microorganisms (Marinescu *et al*., 2009). The use of bacteria in the degradation and detoxification of many toxic xenobiotics, especially glyphosate and paraquat herbicides, is an efficient tool for the decontamination of polluted sites in the environment (Mohammed, 2009). Glyphosate and paraquat herbicides used in this research show an effects on growth and development of *Acidovorax* sp. and *Aeromonas* sp. The highest increase in bacterial counts was observed in the presence of glyphosate when compared with the paraquat. Glyphosate was reported to be less toxics than paraquat due to the presence of phosphate group in their structural formular while paraquat contains ammonium compound. According to Carlisle and Trevors (1988), glyphosate and paraquat can either stimulate or inhibit soil microorganisms depending on the soil type, herbicide concentration and environmental conditions. In relation to degradation of herbicides, it was observed that, the highest degradations was recorded in glyphosate using *Aeromonas* sp. in comparison with paraquat. This might be due to the fact that glyphosate was observed to be less toxic than paraquat to the bacterial cells and this findings were supported by other studies (Turkington, *et al*., 2001; Wyss and Muller-Scharer, 2001; Anderson and Kolmer, 2005). However, few studies contradicts this result (Wardle and Parkinson, 1990; Busse *et al*., 2001; Weaver *et al*., 2007).

This finding is in line with Isenring (2006) that paraquat inhibit several microorganisms in soil. They reported that paraquat could inhibit a great number of cellulolytic microflora and that might cause injurious effects to symbiotic, anaerobic and nitrogen fixing microorganisms. Paraquat is also known to be bounded strongly and coherently to soil components, including clay minerals and organic matter and therefore these limits the access of microorganisms to paraquat in soil water (Bromilow 2003; Isenring, 2006). Thus, adsorption of paraquat to soil rapidly decreases the bioavailability of the herbicide in the soil environment and demonstrated the capability of adsorption process to deactivate hundreds or even thousands of paraquat application over many soil types (Roberts, *et al*., 2002). The sandy loam characteristics of the experimental soils might have reduced the binding of paraquat to soil components and thus increasing the availability of paraquat in soil water, and hence affecting the soil microorganisms significantly. Conclusively, thus, the bacteria (*Aeromonas* sp. and *Acidovorax* sp.) can be exploited for biodegradation of glyphosate and paraquat and should be further studies for their ability to degrade other classes of herbicides.

REFERENCE

Anderson, J. A. and Kolmer, J. A. (2005): Rust control in glyphosate tolerant wheat following application of the herbicide glyphosate. *Plant Diseases* 89:1136-1142.

Bogen K. T., Beson,J. M., Yost G. S., Morris J. B., Dahl A. R. and Clewell H. J. (2008): Naphthalene Metabolism in Relation to Target Tissue Anatomy, Physiology, Cytotoxicity and Tumorigenic Mechanism of Action. *Regulatory Toxicology and Pharmacology*, *51*:27-36

Bromilow R. H. (2003): Paraquat and sustainable agriculture, *Pesticide Management Science* 60: 340-349.

Busse M., Ratcliff A. M., Shestak C. J. and Powers R. F. (2001): Glyphosate Toxicity and the Effects of Long Term Vegetation Control on Soil Microbial Communites. *Soil Biology and Biochemistry33*: 1777-1789.

Carlisle S. M. and Trevors J. T. (1988): Glyphosate in the environment. W*ater Air and Soil Pollution* 39: 409-420.

De Lorenzo, M. E., Scott, G. I. and Ross, P. E. (2001): Toxicity of pesticides to aquatic microorganisms: a review. *Environmental Toxicology Chemistry*, 20: 84-98.

Dem, S. B. (2007). Pesticide Residues in Soil and Water from Four Cotton Growing Areas of Mali, West Africa. *Agricultural, Food and Environmental Sciences.*1:1. 1-12.

Desaint, S., Hartman A., Parekh, N. R. and Fournier J. C. (2000): Genetic Diversity of Carbofuran- Degrading Soil Bacterial. *FEMS Microbiology Ecology*, 34:173-180.

Isenring, R. (2006). Effect of paraqut application In R. Madeley (Ed.). Paraquat – Unacceptable health risks for users, pp. 6-70, UK: PAN UK.

Karyn, U. and Ronald, E. M. (2012): Analysis of pesticide residues using GCMS. Agilent, Inc. Wilmington. DE 19808 USA manual.

Kortekamp A. (2011) Herbicides and environment. Publish In Technical education hand book pp59

Marinescu M., Dumitru M. and Lacatusu A. (2009): Biodegradation of Petroleum Hydrocarbonsin an Artificial Polluted Soil. *Research Journal of Agricultural Science* 41:2. Materials, p2980

Mohammed M. S. (2009): Degradation of Methomyl by the Novel Bacterial Strain Strain *Stenotrophomonas Maltophilia* M1. *Biotechnology*. 12:1-6.

Olawale, A., Akintobi K. and Olubiyi A. (2011): Biodegradation of Glyphosate Pesticide by Bacteria isolated from Agricultural Soil, *Report and Opinion*, 3:1:124-128.

Pal S. k. and Das Gupta S. K. (1994): Pest Control (Skill Development Series no. 15) Training and Fellowship proGram, International Crop Research Institute for SemiArid Tropics (ICRISAT), Patancheru 502324, Andhra Pradesh, India. pp 15-19.

Prakash B. and Irfan M. (2011): Pseudomonas Aeroginosa is Present in Crude Oil Contaminated Samples of Barmer Region (India) *Journal of Bioremediation and Biodegradation*, 2:5

Roberts T. R., Dyson J. S. and Lane M. C. G. (2002): Deactivation of the biological activity of paraquat in the soil environment: a review of long-term environmental fate. *Journal of Agricutural Food Chemistry* 50:3623-3631.

Saleh, A., Kawo, A. H., Yahaya, S., Riskuwa, L.M., Ali, M. and Ibrahim, U. B. (2020). Effects Of Glyphosate And Paraquat Herbicides Application On Soil Bacterial Population, Proceedings of 7th National Society for Conservation Biology Biodiversity Conference 92-97

Todar, K., Ubukata, M. and Hamada, M. (2005): Microbiology of Human Perspective. Mc Graw Hill publisher, London. Pp 203-208

Turkington, T. K., Orr, D. D. and Xi K. (2001): The influence of Roundup® on *in vitro* growth and sporulation of *Rhynchosporium secalis* and *Pyrenophora teres. Canadian Journal of Plant Pathology* 23:307-311.

Wardle D. A. and Parkinson D. (1990): Influence of the herbicide glyphosate on soil microbial community structure. *Plant Soil* 122:29-37.

Weaver M. A., Krutz L. J., Zablotowicz R. M. and Reddy K. N. (2007): Effects of glyphosate on soil microbial communities and its mineralization in a Mississippi soil. *Journal of Pest Managemrnt Science* 63:388-393.

Wyss G. S. and Muller-Scharer H. (2001): Effects of selected herbicides on the germination and infection process of *Puccinia lagenophora*, a biocontrol pathogen of *Senecio vulgaris*. *Biological Control*, 20:160-166.

Yuan S. Y., Liu C., Liao C. S. and Chang B. V. (2002): Occurrence and Microbial Degradation of Phthalate Esters in Taiwan River Sediment. *Chemosphere*, 49:1295-1299