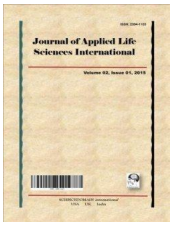

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Monitoring the Bio-Utilization of Herbicide (Paraquat Dichloride) by Some Bacterial Species Isolated from Soil in Calabar, Cross-River State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author TOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CAE and EUE managed the analyses of the study. Author OKA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Herbicides though capable of killing weeds, also affect soil microorganisms depending on the dosage applied. They can also be used as sources of food for energy and growth by microorganisms. Some herbicides persist in the soil for a long period of time. These groups can be degraded by soil microorganisms. Thus this research was aimed at examining and isolating soil bacteria capable of utilizing the herbicide (paraquat dichloride), applied at different concentrations on the plot and also to assess its effects on the bacterial isolates. Using nutrient agar medium incorporated with nystatin to inhibit the growth of fungi, bacteria such as *Bacillus sp., Micrococcus sp., Klebsiella sp., Pseudomonas sp., Listeria sp., Proteus sp., Streptococcus sp.,* and *Staphylococcus sp were* isolated. Screening test with mineral salt medium showed that all the isolates were able to utilize paraquat as substrate for growth and energy. The study also showed that *Micrococcus sp., Bacillus* and *Proteus* species especially were able to degrade the herbicide. *Bacillus* persisted till the eight weeks and was the most frequently isolated, followed by

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Micrococcus and *Proteus sp*. An increase was observed in microbial count within the first four weeks (5 *10 ^{8} to 5.34 *10^{8} and 5.3 *10 ^{8} to 6. 64 *10 ^{8} for plots 1 and 3 respectively) but by the sixth week the count decreased (4.70 * 10^{8}, 6.38 *10^{8} for plots 1 and 3 respectively) as a result of substrate utilization simultaneously with degradation.

Keywords: Herbicides; paraquat dichloride; soil; bacteria; Nigeria.

1. INTRODUCTION

Herbicides are chemicals which are used for killing undesirable vegetation commonly known as weeds and affecting plant growth. They are also used as sources of food for energy and growth for microorganisms. Bio-utilization of herbicides is a process by which microorganisms utilize herbicides as a source of food for energy and growth. For microorganisms to be able to use a herbicide, that herbicide must have a molecular configuration that makes it suitable for use by soil microbes [1]. Paraquat or ***N, N′-*** dimethyl-4, 4′ bipyridinium dichloride is the organic compound with the chemical formula [(C6H7N)2]Cl2. It is classified as a viologen, a family of redox-active heterocycles of similar structure. This salt is one of the most widely used herbicides. It is quick-acting and non-selective; killing green plant tissue on contact. It is also toxic to human beings and animals. It is linked to development of Parkinson's disease [2,3]. Paraquat is a non-selective, post emergence herbicide, with fast contact action and thus affects both monocots and dicots. Uptake of diquat and paraquat (of the same class) is very rapid since rain falling shortly after application does not affect its action [4]. Their action is dependent on light and thus acts rapidly on bright tropical sunlight. Diquat and paraquat act by blocking the oxidation-reduction phenomenon and are inactivated upon contact with the soil and thus have no residual effect.

2. MATERIALS AND METHODS

2.1 Sample Preparation and Herbicide Application

On a bright morning, herbicide was mixed to a concentration of 0/04, 0.06 and 0.08 litres and sprayed on the three subplots. The concentration of the active ingredient of paraquat dichloride was 0.003 kg/ha.

2.2 Sample Collection

Four random samples were taken from each of the subplots at a depth of 0 to 15 cm respectively, were bulked into a composite sample and transported to the laboratory for bacteriological analysis within 24 hrs of collection.

2.3 Isolation and Enumeration

The nutrient agar medium incorporated with nystatin to inhibit the growth of fungiwas used for isolation and enumeration.

2.4 Screening Test for the Utilization of Paraquat Dichloride

This was achieved by the use of modified Mineral Salt medium of Zarji and Suppeston (1972). A mineral salt medium was prepared and were dispensed into sterile test tubes. Then, 0.1 mL paraquat dichloride was added to the tubes respectively and 1 mL of 24 hrs broth culture of each of the bacterial isolate added and incubated at 28°C for three to five days. Controls were set up for each of the isolate, one lacks the source of the organic carbon (paraquat) and the other not inoculated. After incubation the tubes were observed for turbidity which was rated as high (+++), moderate (++), low (+) and no turbidity. A spectrophotometer was also used to read absorbance at 540 nm.

3. RESULTS

The Fig. 1 showed heterotrophic bacterial count at concentrations of 0.04 l/ha, 0.06 l/ha and 0.08 l/ha for plots 1, 2 and 3 respectively. From the results it was observed that bacterial counts increases with increased concentration of herbicide, from 0.04 l/ha to 0.08 l/ha. There was also marked increase in bacterial count during the second week after application; this was assumed to be the time of highest activity by the microorganisms. Also as the herbicide disappeared slowly, the heterotrophic count decreased.

The standard deviation is 0.092 which is an insignificant value.

3.1 Biochemical Characterization of Isolates

Table 1 showed the result of biochemical characterization of the isolates, the following organisms were isolated; *Pseudomonas species, Streptococcus sp.*, *Staphylococcus sp.*, *Escherichia coli*, *Micrococcus sp.*, *Listeria sp.*, *Bacillus sp.*, *Proteus sp.,* and *Klebsiella sp.*

3.2 Persistence of Bacterial Isolates after Application of Paraquat Dichloride

From Table 2, it was observed that Micrococcus and *Bacillus* sp., persisted until the eight weeks though *Micrococcus sp.,* did not reappear during the sixth week of analysis. *Proteus sp* persisted until the sixth week while *Streptococcus sp.,* did not persist at all.

3.3 Screening Test for the Utilization of Paraquat Dichloride by Bacterial Isolates

The result from the figure below showed that *Bacillus sp* was able to utilize paraquat more than all the other isolates while *Pseudomonas sp* was the least.

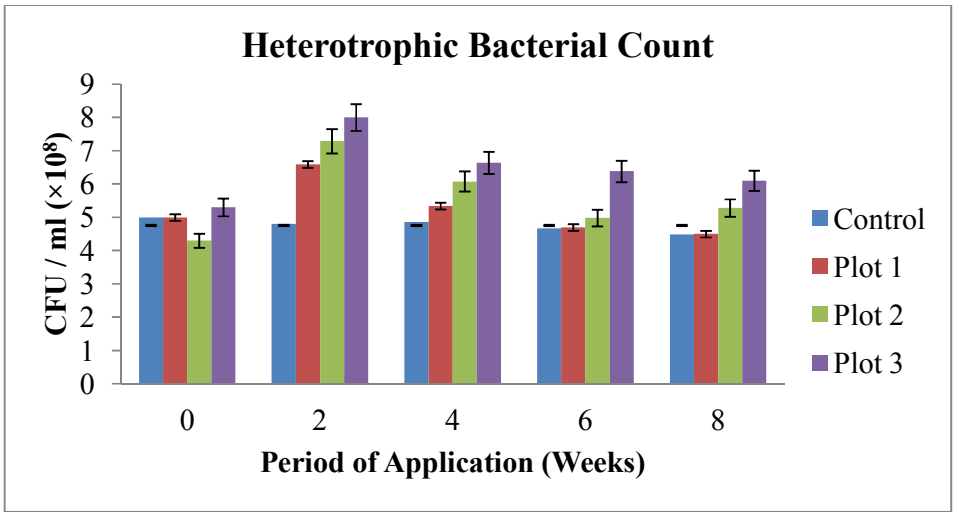

Fig. 1. Heterotrophic bacterial count at different concentrations of application

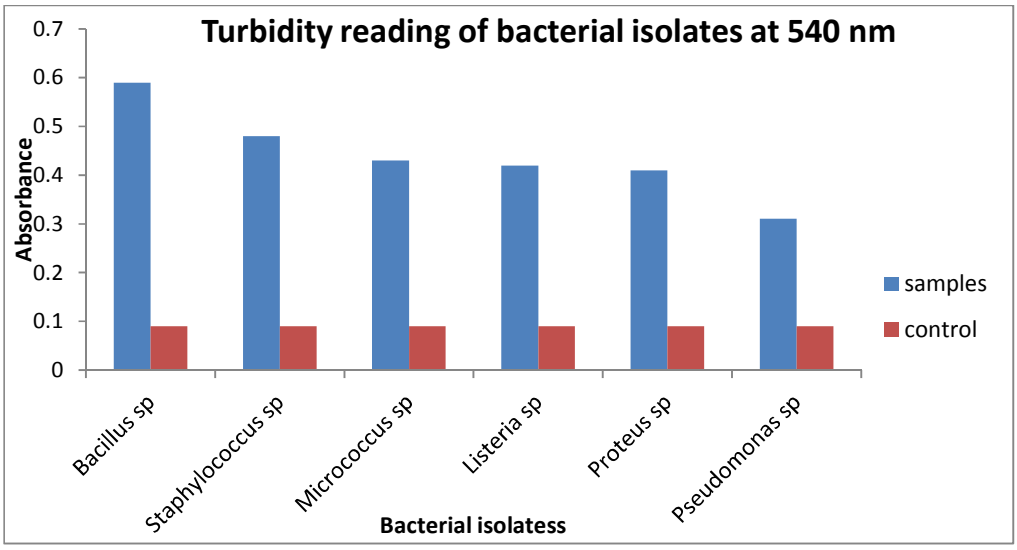

Fig. 2. Absorbance reading of bacterial isolates using a spectrophotometer at 540 nm

Table 1. Biochemical characterization of isolates

Gram's reaction	Oxidase	Coagulase	Catalase	Motility	Indole	Urease	Starch hydrolysis	Growth on TSI			Growth on CLED	Microorganism
								Slope	Butt	Gas		
G+ Cocci	+	-	+	-	+	-	+					*Micrococcus sp*
G+ Rods (short rods)	-	-	-	-	+	-	+	Y	Y	+		*Klebsiella sp*
G+ Cocci (in clusters)	+	-	+	-	-	-	-					*Staphylococcus sp*
G- Rods (short rods)	-	-	+	+	+	-	-	Y	Y	+	Yellowish colony	*Escherichia sp*
Rods	-	-	-	+	-	+	+	R	Y	+		*Bacillus sp*
G+ Rods (cocobacilli)	-	-	+	+	-	-	-					*Listeria sp*
Cocci (in chains)	+	-	-	-	-	-	-					*Streptococcus sp*
G- Rods (long rods)	-	-	+	+	-	+	+	R	Y	-	Did not swarm	*Proteus sp*
G- Rods (short rods)	-	-	+	+	+	-	-	R	R	-	Greenish colony	*Pseudomonas sp*

KEY: *G +ve = Gram positive, G -ve = Gram negative, R = Red, Y = Yellow, - = Negative, + = Positive*

Table 2. Persistence of bacterial isolates after application of paraquat dichloride

Bacterial isolates	Persistence			
	2	4	6	8
Bacillus sp.	+	+	+	+
Staphylococcus sp.	-	-	-	+
Micrococcus sp.	+	+	-	-
Proteus sp.	+	+	+	-
Streptococcus sp.	-	-	-	-
Escherichia coli	-	+	-	+
Listeria sp.	-	-	+	-
Pseudomonas sp.	-	-	-	+
Klebsiella sp.	+	-	-	-

KEY: + = isolated, - = not isolated

4. DISCUSSION

From the research study, it was observed that bacteria of the genera *Pseudomonas sp.*, *Klebsiella sp.*, *Micrococcus sp., Bacillus sp., Escherichia coli* etcetera can utilize and biodegrade the herbicide paraquat dichloride. This report agrees with some of the several reports on the degradation of herbicides by soil organisms as been documented by some researchers; [5] reported profluralin degradation by *Cellulomonas* and *Arthrobacter simplex;* [6] demonstrated the degradation of 2- sec- butyl- 4, 6- dinitrophenol by a strain of *Clostridium bifermentans* known as KmR-1 which was able to use Dinoseb as sole carbon but degradation occurred via cometabolism in the presence of fermentable carbon source. [7] came up with the first report of a pure bacterial isolate with the ability to cleave the s- triazine rings structure of atrazine, they demonstrated the degradation of atrazine by a bacterium isolate which is capable of utilizing atrazine under aerobic conditions as sole source of carbon and nitrogen,, during this process, it was found that a *Pseudomonas sp.,* carried out the deisopropylation step. [8] reported degradation of atrazine by *Pseudomonas sp,* [9] also demonstrated the biodegradation of atrazine by *Pseudomonas sp* while [10] observed that *Klebsiella planticola* degraded simazine in about 70% in 20 days, and that the bigger the dose of simazine in culture (on membrane lipid fatty acid), the higher the level of utilization is. [11] reported the biodegradation of triflurain by *Klebsiella oxyecota, Pseudomonas aeruginosa* and *Bacillus sp*. The study also showed that, the concentration of the herbicide has direct relationship with the microbial populations; Table 1 and as such using concentrations above standard will be detrimental to these organisms. Also it was observed that *Micrococcus* and *Bacillus sp*., persisted until the eight weeks though *Micrococcus sp.,* did not reappear during the sixth week of analysis. *Proteus sp.,* persisted until the sixth week while *Streptococcus sp.,* did not persist at all.

5. CONCLUSION

Herbicides are chemicals which are used for killing undesirable vegetation commonly known as weeds and affecting plant growth. They are also used as sources of food for energy and growth for microorganisms. Bio-utilization of herbicides is a process by which microorganisms utilize herbicides as a source of food for energy and growth. The genera of bacteria that can utilize and biodegrade paraquat dichloride include; *Micrococcus sp.*, *Pseudomonas sp., Klebsiella sp., Bacillus sp.*, and *Proteus sp*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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