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BIODEGRADATION OF PARAQUAT

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ABSTRACT

Studies on the biodegradation of the herbicide paraquat (NN-dimethyl-4-4-bi pyridinium dichloride) was carried out. Four concentrations (0.5, 0.10, 0.15 and 0.20%) of paraquat were screened against four fungal isolates (*Rhizopus* sp, *Aspergillus* sp, *Mucor* sp, *Penicillium* sp) and one bacterial isolate (Micrococcus sp) in culture. Effectiveness of the isolates was measured through assessing the cell mass yield, growth tolerance and the rate of carbon (iv) oxide evolution. Rhizopus species ranked the best ($p<0.05$) in cell mass yield recording values of 85 and 7mg after 10 and 15 days of incubation respectively. *Penicillium* sp was the least effective and accumulated 6mg cell mass after 15 days of incubation. *Micrococcus* sp recorded a higher cell mass yield compared to *Aspergillus* sp and *Penicillium* sp within the test period. Carbon (iv) oxide evolution was assessed in *Micrococcus sp* and *Rhizopus* sp of the two isolates, *Rhizopus* sp produced lower volume (8.80mg) of CO_2 and the production progressed till the end of the experiment. *Micrococcus sp* produced a higher volume of CO_2 in the first five days of incubation which declined and thereafter terminated on the day 10. *Rhizopus* sp was the best paraquat degrader followed by *Micrococcus* sp.

Keywords: Paraquat, biodegradation, microorganisms

1. INTRODUCTION

Herbicides are inorganic and biological agents used in weed control (Oritz-Hernadez and Sanchez-Salinas 2010). Weeds consist of animals and perennials plants that grow in association with crop plants. The use of herbicides does not disturb the crop plants. Herbicides include phenoxy acetic acids, chlorinated benzoic acids, phenotic nitrides, dinitrophenols, phenylcarbonates, carbamates, hydrocarbon oils and more recently quaternary ammonium compounds such as paraquat and disquat. Paraquat (Gramaxone) was developed as a chemical substitute for the plough (Zonghi *et al.*, 2001; Horne *et al.*, 2002, Chang *et al.*, 2005). The use of herbicides has immensely contributed to the reduction in labour requirement and also helps in prevention of soil erosion by eliminating repeated cultivation. Herbicides have made it possible for farmers to have increased food production and also made farming more attractive and economical.

Inspite of these advantages, the presence of herbicides in the soil is becoming hazardous as they are leached down to the water table hence carried by surface runoffs to water bodies. Soil microflora and fauna are killed by this chemical (Oluwole and Cheke, 2009). Microorganisms which are widely distributed in nature have the ability to utilize herbicides as a sole source of carbon and energy thus transforming the herbicides into harmless compounds such as water and carbon iv oxide. Microbial degradation is the safest means of checking pollution in the environment (Ijah and Essien, 1993, Kang *et al.*, 2006, Natala and Ochofe, 2009, Asogwa and Donge, 2009, 2009). Several researchers have studied the biodegradation of herbicides by soil microorganisms (Amakiri, 1982, Ijah and Essien, 1993, Elena *et al.*, 2010). However there is little or no information about biodegradation of paraquat which is commonly used in Cross River State. This paper is presented to bridge this gap to proffer solutions to bioaccumulation of paraquat in the environment as well as to create awareness on the dangers associated with the use of paraquat in weed control by farmers.

2. MATERIALS AND METHODS

Soil samples were collected from the botanic garden of University of Calabar in polythene bags and transported to the laboratory, the herbicide
paraquat (Gramaxone) was bought at 34 Hewet Street, Calabar CRS and stored in the laboratory.

2.1 Isolation of Paraquat degraders

Soil enrichment medium method of Amakiri (1982) was used. About 150ml of liquid medium was poured into a 250ml conical flask. A 0.15ml paraquat was introduced into the liquid culture medium using pipette. The mixture was thoroughly shaken. Then 10g of soil sample was added. The flask was plugged with non-absorbent cotton wool and incubated at room temperature (28 ± 2^0C) in an incubator shaker at 200rpm for 8 days. Following this 0.1ml of the enriched broth was plated out on nutrient agar and malt extract agar and incubated at 28 ± 2^0C for 5 days.

The bacterial isolates obtained were sub-cultured to obtain pure colonies. The isolates were characterized and identified using gram stain, motility, oxidase, catalase, coagulase, citrate, starch hydrolysis, methyl red, voges-proskauer and sugar fermentation tests. The fungal isolates were identified using hyphae staining technique.

The ability of microbes to utilize paraquat as their sole carbon source was determined using the method of Amakiri (1982). The broth was dispensed in 10ml test tubes. A 0.01ml of Paraquat was aseptically transferred into each test tube. A 0.1ml of 24 hours old microorganisms grown on nutrient and czepek Dox broth were introduced into the test-tubes using pipettes. The test tubes were incubated at $28+2^0$C for 5 days without shaking. A positive control was set up. Broth turbidity was an indication of microbial growth.

3.1 Microbial tolerance of different paraquat concentrations

Tolerance of different concentrations of paraquat by microbial isolates was investigated. A 20ml nutrient broth was dispensed into sixteen 50ml capacity conical flasks. Different concentrations of paraquat viz 0.05%, 0.10%, 0.15% and 0.20% were separately added to the conical flasks. The cell mass of the inocula was determined on the first day before incubation at 27^0C. Difference in cell mass of the inoculums was determined after five days interval. A positive control flask was set up. At the end of the incubation period the tolerance of paraquat by microbial isolates was determined.

Degradation of Paraquat by microbes

The rate of degradation of paraquat by microbial isolates was determined using the method of Okpokwasil and Nwosu (1990). One hundred milliliters of liquid medium containing 1ml of paraquat was pipetted into three capped flasks. The first flask was inoculated with *Micrococcus* sp, the second with *Rhizopus* and the third flask which served as the control was not inoculated with any microorganism. A 0.5mg of Barium peroxide dissolved in 4.5ml of distilled water was dispensed into each of the flasks to absorb the carbon IV oxide resulting from the breakdown of the herbicide. All the flasks were incubated at room temperature for ten days.

At intervals of 5 days vials containing Barium carbonate and Barium hydroxide were washed with 40ml distilled water into 250ml conical flask and the residual BOH titrated with IN HCl using phenolphalein as indicator. The indicator imparts a pink colour to the solution. A colourless change indicates end point of titration.

3. RESULTS

Five paraquat degraders were isolated from the soil sample analysed. The microbial isolates were characterized and identified as *Micrococcus* sp, *Aspergillus niger*, *Penicillium* sp and *Mucor* sp (Tables 1 and 2).

Screen test for the utilization of paraquat by microbes revealed that the microbes degraded paraquat at different rates. The bacterial isolate *Micrococcus* sp utilized paraquat at a moderate rate (++). Among the fungal isolates, *Rhizopus sp* utilized paraquat at a high rate (++++),

while *Penicillium* sp, *Aspergillus niger* and *Mucor* sp utilized the paraquat minimally (+) each. The result is shown in Table 3.

Growth tolerance of the five organisms at the different concentrations of paraquat varied greatly. The cell mass yield of the microbes decreased with increased concentration of the paraquat except for *Penicillium* sp where increased yield was observed at increased herbicide concentrations. This is shown on Table 4.

The degradation of paraquat in liquid medium by the different isolates was measured by the level of carbon iv oxide (CO_2) evolved as seen in Fig. 1. *Micrococcus* sp produced a greater amount (57.20mg) of CO_2 in the first five days of incubation compared to 4.40mg evolved by *Rhizopus* sp within the same period. However on the 10th day of incubation, the level of CO_2 evolution by *Micrococcus* sp declined to 52.80mg which was still higher than the 8.80mg CO_2 recorded by *Rhizopus* sp during the period. Carbon (iv) oxide (CO_2) evolution by *Micrococcus* sp did not progress beyond the 10th day of incubation but *Rhizopus* sp was still active even after 15 days of incubation. However the total CO_2 evolved by *Micrococcus sp* was higher when compared with that evolved by *Rhizopus* sp.

4. DISCUSSION

Micrococcus sp, *Rhizopus* sp, *Aspergillus* sp and *Mucor* sp are among microbial isolates identified as effective herbicide degraders. (Asogwa and Dongo 2009, Elena *et al.*, 2010, Agary *et al.*, 2013). In this study, these three organisms effectively degraded paraquat with *Rhizopus* sp being the most effective. Among the paraquat degraders fungi constitute 80% while bacteria constitute 20%. A survey of the ability of the isolates to utilize paraquat as the sole source of carbon and energy revealed that the isolates utilized paraquat at different rates. This indicates the varying ability of microorganisms to degrade herbicide. The difference in the paraquat degrading capability of the isolates may be a function of the difference in adhesion, cell membrane properties and/or the maintenance of their regimes in the test medium (Naqvi *et al.*, 2011 and Nsikak *et al.*, 2011). It was observed that *Rhizopus* sp yielded the highest cell mass when compared with other isolates screened. This was observed at 5 and 10 days of incubation and at all levels of concentration. *Micrococcus sp* was more tolerant than *A. niger* and *Penicillium* sp. At 0.10% concentration of the herbicide there was no difference between *A. niger* and *Penicillium* sp at 5 and 10 days. At 0.15% concentration the growth tolerance was statistically significant at $P<0.05$ for *A. niger* and *Penicillium* sp. At 0.2% *A. niger* recorded no growth. The growth yield was however the same (30mg) for *Penicillium* species at both days (5 and 10) of culture.

The efficient enzyme system possessed by *Rhizopus* sp which facilitates breakdown and accumulation of carbon may be responsible for higher cell mass accumulation. Increase in paraquat concentration promoted cell mass yield in *Penicillium* sp. This suggests that the herbicide may play a role in activating the enzyme system of *Penicillium* sp. Cell mass yield of other isolates progressively decreased as the concentration of herbicide paraquat was increased. The total growth ceasation exhibited by *Aspergillus* sp at 0.20% concentration of the herbicide could be due to interference of the herbicide with the mechanism of action of the organism's enzyme system at higher herbicide concentration.

5. CONCLUSION

Microbial degradation of paraquat can be effected by some fungi and bacteria organisms. The genera of organisms effectively involved in paraquat degradation include fungi: *Rhizopus, Penicillium, Aspergillus* and *Mucor species* and the bacteria, *Micrococcus sp*. The best degraders were *Micrococcus* and *Rhizopus species*. Paraquat should only be applied for the control of weeds when absolutely necessary. In such circumstances it should be applied at low concentrations.

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Table 1: Characterization and identification of Paraquat- degrading organisms

Code	Gram recreation and morphology	Motility	Oxi	Cat	Coag	Cit	Starch Hydrolysis	MR	VP	Sugar Fermentation				Probable Organism
										Glucose	Lactose	Maltose	Manitol	
HD 01	Gram Positive Cocci	Nil	Neg	Pos	Neg	Pos	Pos	Pos	Neg	A	AG	A	AG	*Micrococcus* sp

Oxi - Oxidase, Cat = catalase, Coag – Coagulase, Cit = Citrate, MR = Methyl Red, VP - Voges Proskauer, A, Acid, AG = Acid
and Gas, Pos = Positive, Neg = Negative.

Table 2: Physical charactersitcis of Paraquat degrading fungi

Isolate Code	Colony Morphology	Cell Morphology	Probable Organism
HD 02	Very large colonies with greenish velvety central region	Mass of hyohae metulae absent, conidiophores sterigmata conidia arrangement	*Aspergillus* sp
HD 03	Very large colonies with greenish velvety central region	Mass of hyphae metulae seen, conidiophores sterigmata conidia arrangement	*Penicillium* sp
HD 04	Abundant colony mycelium which appear to fill the plate	Non-septate has atolons and rhizoids, sporangiophores arising at the node where rhizoids are formed	*Rhizopus* sp
HD 05	White fluffy colonies	Spores are spherical and hyaline, collumella round, sporangiophore unbranched and hyphae are aseptate	*Mucor* sp

Table 3: Screen Test For Utilization Of Paraquat By Microbes Isolated From Soil

Isolate code	Probable organism	Growth in Paraquat 5 days
HD 01	*Micrococcus* sp	++
HD 02	*Aspergillus niger*	+
HD 03	*Penicillium* sp	+
HD 04	*Rhizopus* sp	+++
HD 05	*Mucor* sp	+

+++ - Maximum growth; ++ - Moderate growth; + - Minimum growth

Table 4: Growth tolerance of Microbial isolates to different concentrations of Paraquat in culture

Isolates	Paraquat Concentration (%)/Incubation Period (Days)											
	0				5				10			
	0.05	0.10	0.15	0.20	0.05	0.10	0.15	0.20	0.05	0.10	0.15	0.20
Micrococcus sp	2	2	2	2	20	6	5	4	30	7	6	5
Aspergillus niger	1.5	1.5	1.5	1.5	12	12	8	0	13	12	8	0
Penicillium sp	1.2	1.2	1.2	1.2	5	12	14	0	6	13	14	30
Rhizopus sp	2.0	2.0	2.0	2.0	85	72	61	63	87	75	63	53
Mucor sp	1.3	1.3	1.3	1.3	27	27	21	12	29	29	21	12
LSD	1.90											

Values are means of 3 replicates.

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