BACTERIAL GROWTH ABILITIES IN CARBOFURAN AND PARAQUAT

ABSTRACT

Introduction: The norm of pesticides use is very crucial in protecting the agriculturalists' venture in seeds, fertilizer and labour as they provide a sure protection from damage by pests. The use of pesticides is thus unavoidable and the associated environmental contamination owing to these toxicants and their deposits will remain a concern.
Aim: The research aimed at isolating Carbofuran and Paraquat degrading bacterial species and assessing their growth capacities at different concentrations of these pesticides.
Study design: Microcosms were set-up in test tubes in replicates (320 test tubes) and sacrificial sampling technique was adopted during growth test.
Place and duration of study: The study was carried out at the Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun, Delta State/Three months.
Methodology: Standard method was used to isolate Carbofuran and Paraquat degrading bacteria. Isolates were screened for their abilities to use Carbofuran and Paraquat as only carbon source. Bacterial isolates were identified and subjected to the growth test at 0.5, 1.0, 1.5 and 2.0% of Carbofuran (w/v) and Paraquat (v/v), respectively. Growth abilities were scored by turbidity and colour variations.
Results: The growth abilities at different pesticide's concentrations were significantly different at *P=.05*.
Conclusion: This research revealed that the bacterial isolates were able to grow at various concentrations using these chemicals but best at 0.5% with higher growth rates in microcosms containing Paraquat. If these test microorganisms can grow in the presence of these toxicants, there is the likelihood that they maybe be able to reduce both Carbofuran and Paraquat hazards of contaminated areas. Thus, these bacteria can be used for the clean-up of these pesticides pollution in farms to ameliorate the problems of pesticide pollution in environment.

Keywords: Gram positive, Gram negative, Concentrations, Carbofuran, Paraquat, Growth

1. INTRODUCTION

Sustainable agricultural practices refer to good stewardship of environment, social and economic reliable agricultural practices that guarantee the security and safety of the future of agriculture. From an environmental point of view, this includes planting schemes that do not deplete soil fertility, wise management of water and soil, minimizing pollution and biodiversity promotion. A sustainable planting scheme has to be reliable and high yielding if the expanding world population will be fed adequately without bringing more virgin land into cultivation [1,2,3].

About 60,000 to 95,000 chemicals are used commercially. Information from third world network affirms the discharge of toxic compounds of about 450,000,000kg into the atmosphere and hydrosphere, worldwide. These toxic compounds are of major concern due to the ecological issues they cause which in turn results in natural imbalances in the ecosystem. These have drawn the attention of environmental scientists across the world to developing various strategies of surmounting these concerns. Although, these scientists speak on the extinction of natural resources at global platforms, less attention is given to their words and several of these toxic chemicals are still in use not minding the toxic effects they leave in the environment [4].

Globally, pesticides represent a class of chemicals which prevents or control pests, diseases, weeds and other plant pathogens in an effort to increase plant productivity and maintain high product quality. They include insecticides, herbicides, fungicides and other varieties of pesticides [5,6].

The toxic effect of Carbofuran (an insecticide of N-methyl carbamates family also applied as acaricide and nematicide) is due to its activity as a cholinesterase inhibitor and is considered a neurotoxic pesticide. Carbofuran even at extremely low doses is a prevailing endocrine disruptor and foundation to transitory modifications in the amounts/concentrations of lots of hormones in animals and humans. These changes may subsequently lead to serious reproductive problems after repeated exposures [7,8]. The World Health Organization (WHO), has reported that over half a million people are diseased in each year by the pesticide with five thousand casualties [9]. Carbofuran extensive use in fields has resulted in contamination of soil and ground water system thus, polluting the environment.

Paraquat (a herbicide and a bypiridinium compound) is labelled a chemical flame gun due to its action on carbon dioxide (CO_2) fixation by inhibiting the variable chlorophyll fluorescene in decreasing oxygen evolution. Its activity is irreversible. It poses a number of health problems to humans that use and handle them. Water polluted with Paraquat is a risk factor for liver, lung, kidney and heart illnesses [10,11]. It has been proven that pesticides significantly add to the danger of Parkinson's disease [12,13,14]. Due to severe impacts on man and the ecosystem, these persistent toxins have been prohibited [15].

Carbofuran and Paraquat have been implicated in ground and surface water contamination in addition to persistence in the surroundings for long term [16,17,18]. There are reports that soil microbes are able to degrade both pesticides (19,20]. The breakdown of pesticides by organisms depends on some physical and chemical environmental factors which include but are not limited to; temperature, moisture and soil pH. Breakdown is also dependent on constituents of the pesticides (which may include; its hydrophilicity, degree of solubilization), microbial population and diversity and biochemical reactions. The breakdown of pesticides by these physical and chemical environmental factors is a resultant effect of physico-chemical alterations or changes of the pesticides by processes which includes; photolysis, hydrolysis, oxidation and reduction [21]. Also, there may be the challenge of bioavailability of these pesticides due to partitioning which results in attachment or adherence of the pesticide compounds to soil and soil colloids still in its original chemical form or structure [22].

Nevertheless, the main way of detoxifying pesticides is through biological means powered by enzymes (enzymatic reactions/changes) present in plants and microorganisms [23,24,25,14,26]. Their growth capabilities in these pesticides contaminated systems will foster the breakdown and removal of these toxicants from such environments.

2. MATERIAL AND METHODS

2.1 Sample collection

Soil samples were collected from three different locations within the Federal University of Petroleum Resources, Effurun, Delta State. A minimum of ten samples were collected from 0-15cm beneath the

surface of soil under aseptic conditions in sterile containers and taken to the laboratory in icepacks for further studies.
Carbofuran (3%) and Paraquat (200g/l) were purchased from the market for the study.

2.2 Culture and isolation of bacteria

Serial dilution agar plating method was adopted for the isolation of microorganisms according to Chikere et al [27]. Serial dilution was done using one (1) gram of soil sample suspended in 9 ml of sterile physiological saline. Aliquots (0.1ml) of the dilutions were plated out using appropriate media for the isolation of microorganisms.

2.3 Screening for pesticide degrading potentials in solid and liquid media

The method of Hamada et al [28] was used for screening. The ability of the bacterial isolates to utilize Carbofuran and Paraquat was screened by culturing them at 37°C for 72 hours on minimal agar containing 150 parts per million (ppm) of the pesticides as the sole source of carbon and nitrogen. Bacteria capable of utilizing pesticides were selected and their degradative abilities were further analyzed in minimal liquid media supplemented with 150 ppm pesticides as the sole source of carbon and nitrogen. These cultures were shaken at 37°C and 220 rpm. Bacterial growth was monitored at 600 nm using a spectrophotometer.

2.4 Bacterial growth at different pesticides concentration

Isolates with biodegradative abilities were exposed to various concentrations of the pesticides according to the protocol of Nisha et al. [29]. The pesticides were the sole carbon source during this study. Pesticides were added to test tubes containing minimal media at 0.5, 1.0, 1.5 and 2.0 % (w/v) for Carbofuran and (v/v) for Paraquat. Test tubes were inoculated with test organisms and incubated at 28 ± 2^{0}C in a rotary shaker at 120rpm. Their growth abilities were assessed by optical density (OD) at 600nm for eight days.

2.5 Statistical Analyses

Two way-ANOVA was used to test the effects of the different agro pesticides use with time (days) on the bacterial growth abilities.

3. RESULTS AND DISCUSSION

3.1 Bacterial identification

Bacterial isolates identified from Carbofuran and Paraquat are represented on Table 1 and Table 2, respectively.

Table 1: Bacterial isolates from Carbofuran during the study

TEST	B1	B2	B3	B4
Glucose	+	+	+	+
Glycine	+	+	+	+
H_2S	-	-	-	-
Urease	-	-	-	-
VP	+	+	-	+
Indole	-	-	-	-
Gelatine	+	+	+	+
Citrate	+	+	-	+
ONPG	+	+	+	+
Fructose	+	+	+	+
Inositol	+	N	N	+
L-Arabinose	+	-	+	+
Sorbitol	+	+	N	+
D-Xylose	+	+	+	+

Maltose	+	+	+	+
Esculin	+	+	+	+
ADH	-	-	-	-
LDC	-	-	-	-
%ID	94'5	99.9	61.8	99.5
Identified organisms	*Bacillus subtilis*	*Paenibacillus polymyxa*	*Bacillus circulans*	*Bacillus megaterium*

Key: H_2S- hydrogen sulphide, VP- Vogue proskaeur, ONPG – O-nitrophenyl-β-galactopyranoside, ADH- arginine dehydrolase, LDC- lysine decarboxylase, N – Not applicable

Table 2: Bacterial isolates from Paraquat during the study

TEST	B5	B6	B7	B8
Glucose	+	+	+	+
Glycine	+	N	N	N
H_2S	-	-	-	-
Urease	-	+	-	-
VP	+	-	-	+
Indole	-	+	-	-
Gelatine	+	+	-	-
Citrate	+	-	+	+
ONPG	-	-	-	+
Inositol	+	-	-	+
L-Arabinose	+	N	N	N
Sorbitol	+	-	-	-
Mannose	N	-	-	+
Esculin	+	N	N	N
ADH	-	-	-	+
LDC	-	-	-	-
%ID	89.5	99.6	99.9	98.4
Identified organisms	*Bacillus amyloliquefaciens*	*Chryseobacterium indologenes*	*Acinetobacter baumannii*	*Enterobacter sakazaki*

Key: H_2S- hydrogen sulphide, VP- Vogue proskaeur, ONPG – O-nitrophenyl-β-galactopyranoside, ADH- arginine dehydrolase, LDC- lysine decarboxylase, N – Not applicable

The soil is a reservoir and a ready source of microorganisms. Several researchers [30,31,32,33,34] have isolated bacterial species including *Bacillus*, *Paenibacillus, Chryseobacterium*, *Acinetobacter, Enterobacter* and others from farmyard soils.

3.2 Isolates with degradative abilities

The bacterial isolates were screened for the ability to grow in each of the pesticides under study using a spectrophotometer. The abilities of the bacterial isolates during the screening are shown in Table 3. Four isolates were chosen for the study and was carried out at four different concentrations (0.5, 1.0, 1.5 and 2.0%), their growth were scored by turbidity and colour changes in the set-up.

Table 3: Screening for degradative capacities

S/N	Isolate	Growth
Carbofuran		

1	*Bacillus subtilis*	++
2	*Paenibacillus polymyxa*	++
3	*Bacillus circulans 2*	++
4	*Bacillus megaterium*	++
Paraquat		
5	*Bacillus amyloliquefaciens*	++
6	*Chryseobacterium indologenes*	++
7	*Acinetobacter baumannii*	++
8	*Enterobacter sakazaki*	++

3.3 Bacterial growth at different pesticides concentration

The ability of four of the selected bacterial isolates after screening to grow in different concentrations of Paraquat and Carbofuran are as shown in Fig.1 to Fig.4 and Fig.5 to Fig.8, respectively. The isolates were able to grow using these chemicals at different concentrations but best at 0.5% as presented in Fig.1 (Paraquat) and Fig.5 (Carbofuran).

At 0.5% Paraquat, *Chryseobacterium indologenes* and *Acinetobacter baumanii* grew best with a steady rise in the former (Fig.1). *Chryseobacterium indologenes* increased throughout the study (0.207 to 0.330). *Bacillus amyloliquefaciens* increased from day 0 (0.207) - day 6 (0.332) and later decreased to 0.292 (day 8). *Enterobacter sakazaki* increased till day 2 (0.253) and decreased by day 4 (0.242), thereafter, it increased till the end of the study. *Acinetobacter baumanii* increased till day 4 (0.306), reduced at day 6 (0.303) and later increased to 0.316 (day 8). The figures below are graphical illustrations of the variation in growth rate of the organisms at different concentration of Paraquat and Carbofuran. A two way ANOVA showed that the variation in the bacterial growth rate with respect to the different pesticides and days was significant at P=0.05.

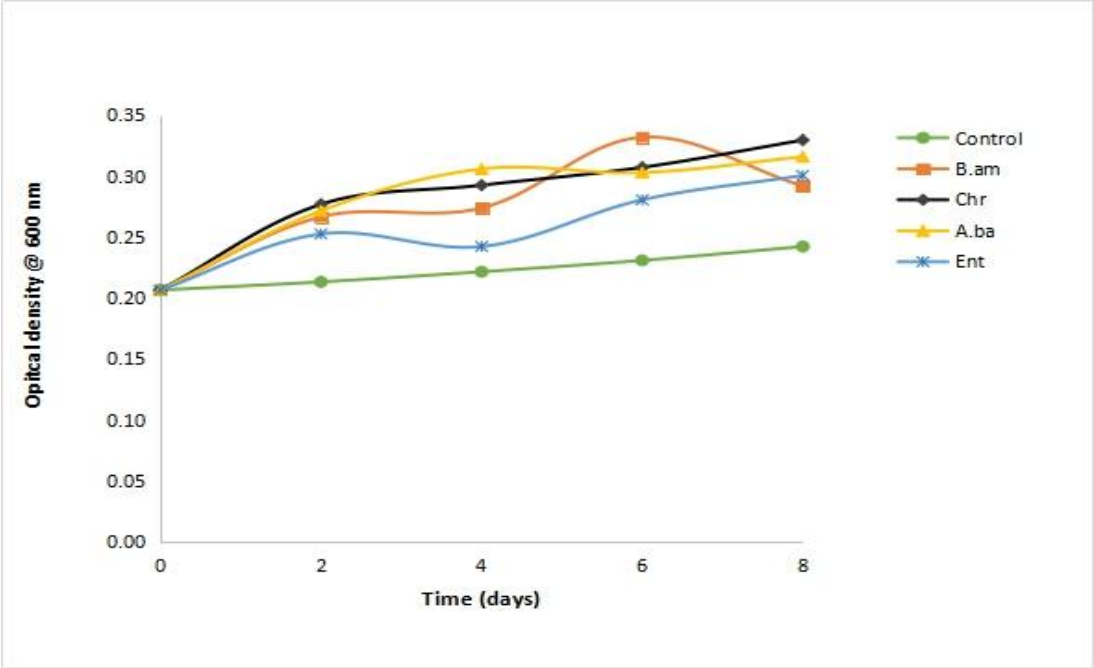

Fig.1. Growth of selected bacterial isolates at 0.5% Paraquat

Key:

B.am - *Bacillus amyloliquefaciens*
Chr - *Chryseobacterium indologenes*
A.ba - *Acinetobacter baumannii*
Ent - *Enterobacter sakazaki*

Figure 2 showed growth rate at 1.0% Paraquat, *Bacillus amyloliquefaciens* had the highest growth at day 2 (0.194) while *Acinetobacter baumanii* recorded the highest growth at day 6 (0.252) and 8 (0.252). All isolates increased in growth till day 8 except *Acinetobacter baumanii* that reduced at day 4 (0.189) but later increased at day 8 (0.252). There were significant differences in bacterial growth abilities with respect to the different pesticides and days at P=0.05.

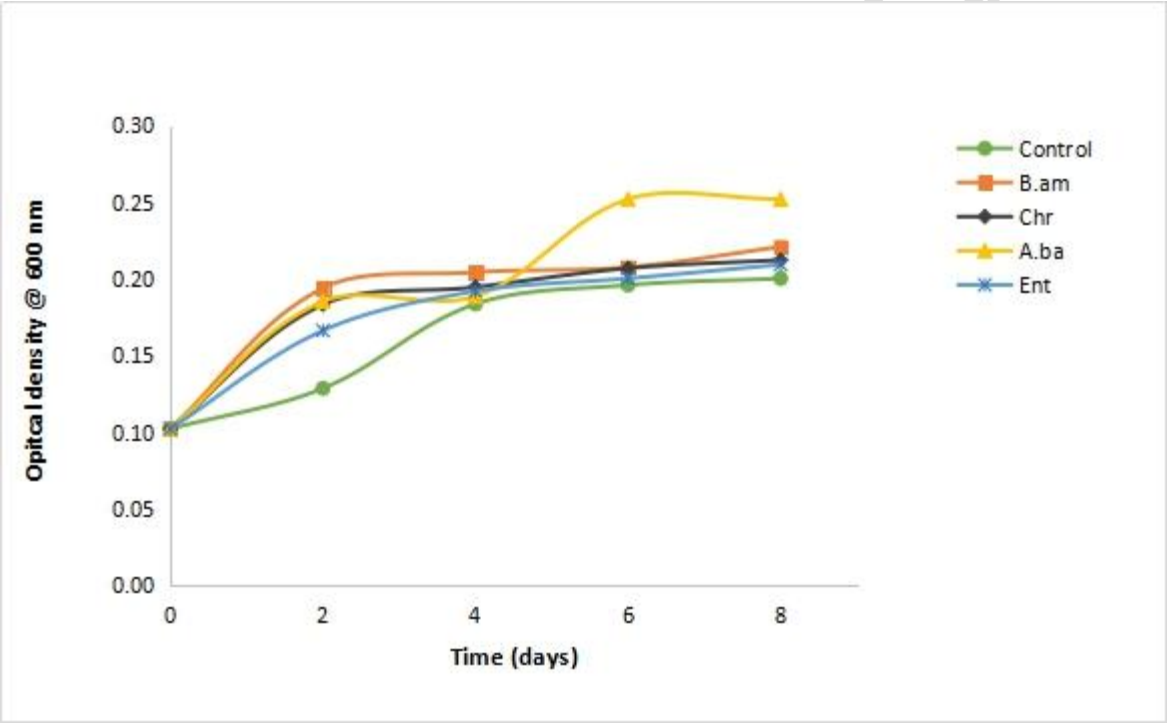

Fig.2. Growth of selected bacterial isolates at 1.0% Paraquat
Key:
B.am - *Bacillus amyloliquefaciens*
Chr - *Chryseobacterium indologenes*
A.ba - *Acinetobacter baumannii*
Ent - *Enterobacter sakazaki*

Bacillus amyloliquefaciens had a steady rise in growth till day 4 (0.347), a decline at day 6 (0.344) then, an increased growth at day 8 (0.429), a gradual but steady rise in growth was observed for *Acinetobacter baumanii* (0.316 to 0.383) and *Chryseobacterium indologenes* peaked at day 6 (0.371) and dropped at day 8 (0.358) in set-ups containing 1.5% Paraquat as presented in Fig.3. A two way ANOVA showed that the variation in the bacterial growth rates with respect to the different pesticides and days was significant at P=0.05.

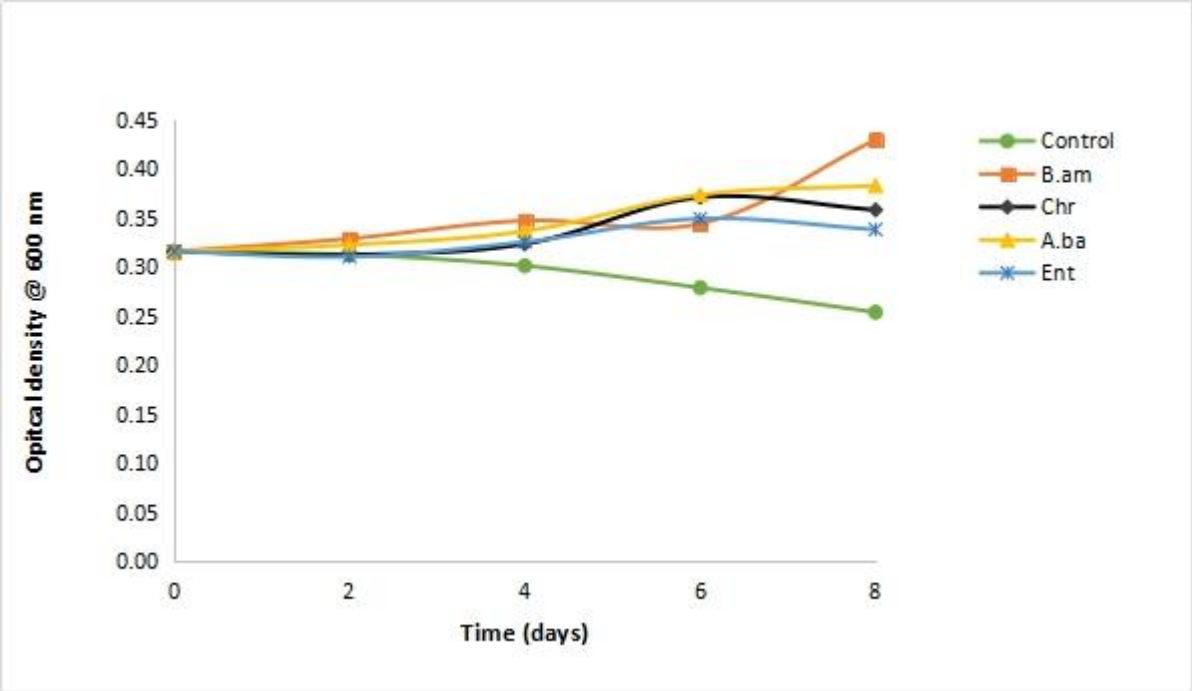

Fig.3. Growth of selected bacterial isolates at 1.5% Paraquat
Key:
B.am - *Bacillus amyloliquefaciens*
Chr - *Chryseobacterium indologenes*
A.ba - *Acinetobacter baumannii*
Ent - *Enterobacter sakazaki*

At 2.0% Paraquat, *Bacillus amyloliquefaciens* and *Enterobacter sakazaki* recorded the highest and least growth rate, respectively (Fig.4). All isolates' growth decreased at day 2 and increased at day 4. *Bacillus amyloliquefaciens* increased in growth from 0.475 (day 6) to 0.504 (day 8). Similar trend was seen in *Acinetobacter baumannii* from 0.454 (day 6) to 0.483 (day 8). *Enterobacter sakazaki* increased till day 6 (0.463) but reduced at day 8 (0.453) while, the reverse trend was observed in *Chryseobacterium indologenes* where growth reduced to 0.459 (day 6) and later increased at day 8 (0.470). A two-way ANOVA showed that the variation in bacterial growth rates for different pesticides and days was significant at P=0.05.

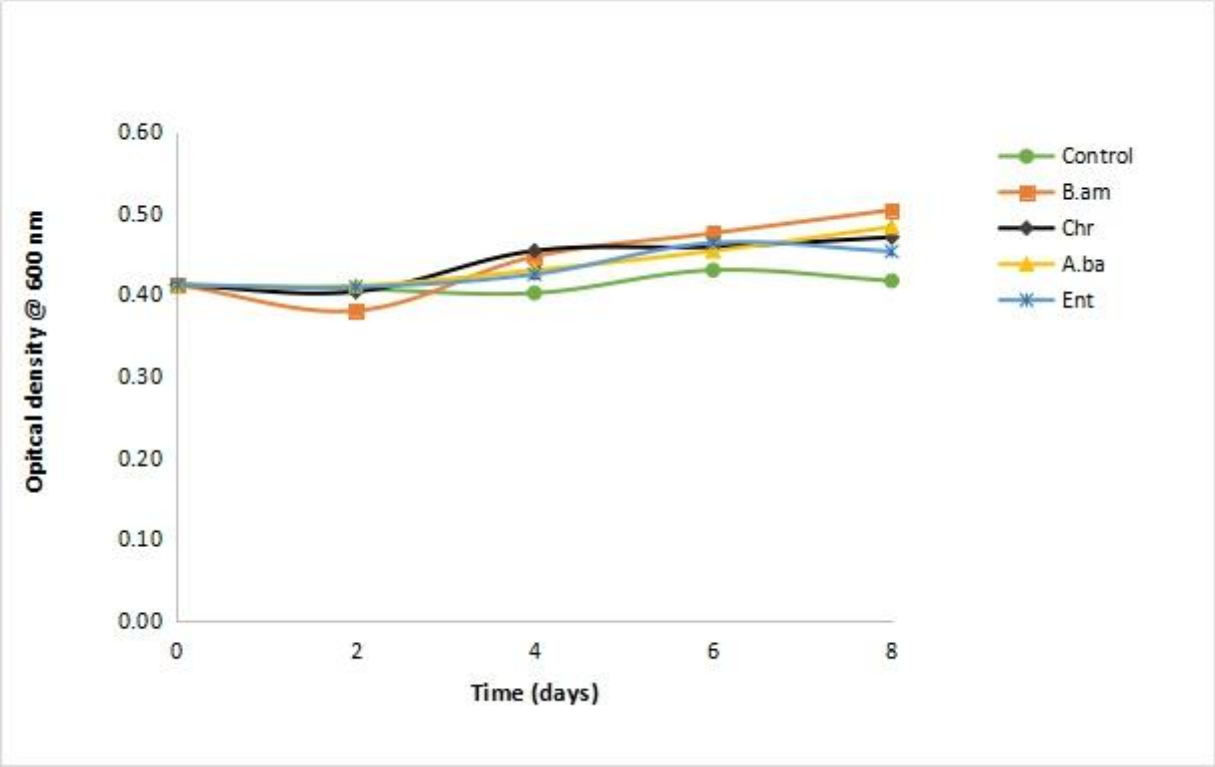

Fig.4. Growth of selected bacterial isolates at 2.0% Paraquat
Key:
B.am - *Bacillus amyloliquefaciens*
Chr - *Chryseobacterium indologenes*
A.ba - *Acinetobacter baumannii*
Ent - *Enterobacter sakazaki*

Figure 5 shows the growth rate at 0.5% Carbofuran, All isolates had good growth rates but *Bacillus subtilis* had the highest rate. *Bacillus circulans* increased at day 2 (2.406) and decreased. *Bacillus megaterium* increased from day 0 (1.62) to day 6 (2.288) but decreased at the end of study (2.135). *Bacillus subtilis* increased till day 2 (2.457), decreased till day 6 (2.189) and later increased at day 8 (2.422). *Paenibacillus polymyxa* had the highest growth at day 6 (2.297). A two way ANOVA showed that the variation in the bacterial growth rates with respect to the different pesticides and days was significant at P=0.05.

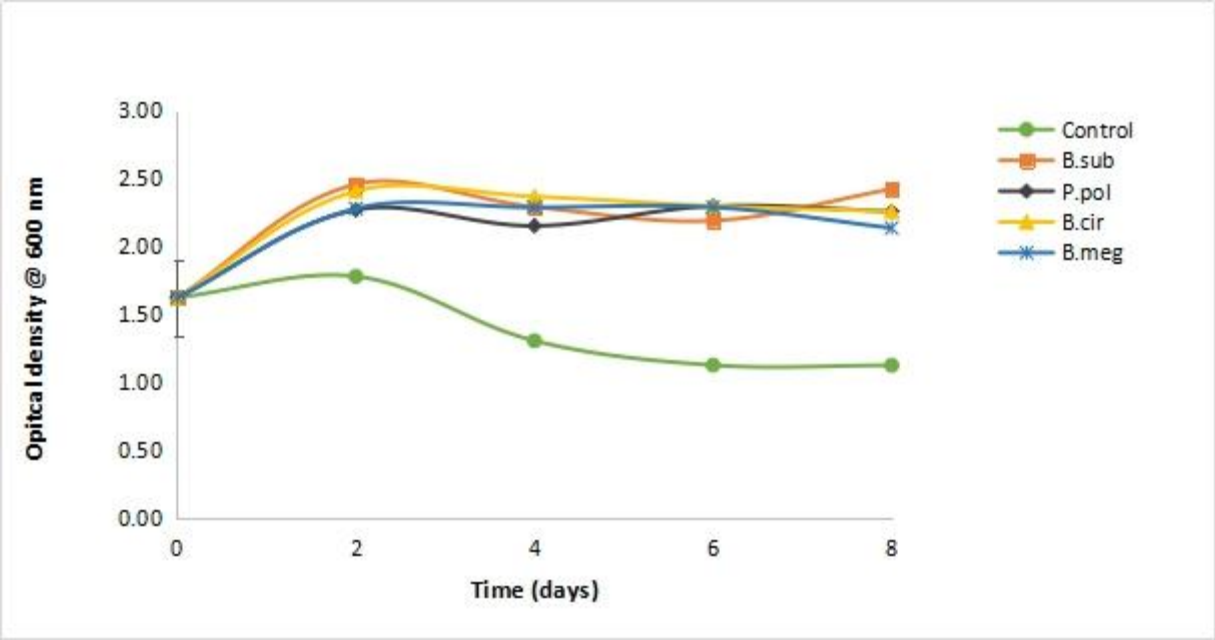

Fig.5. Growth of selected bacterial isolates at 0.5% Carbofuran
Key:
B.sub – Bacillus *subtilis*
P.pol – *Paenibacillus polymyxa*
B.cir – *Bacillus circulans*
B.meg –*Bacillus megaterium*

At 1.0% Carbofuran, there were variations in the different isolates' growth rates but *Bacillus circulans* had the best growth rate as seen in Fig.6. All isolates increased in growth from day 0 to day 2, reduced at day 4. *Bacillus megaterium* increased from day 6 (1.992) till the end of study. *Bacillus circulans* and *Paenibacillus polymyxa* increased at day 6 (2.297, 2.198) and decreased at day 8 (1.920, 1.874), respectively. *Bacillus subtilis* had a steady growth during the study. A two-way ANOVA showed that there was statistical significance in growth abilities with respect to the different pesticides and days at P=0.05 value

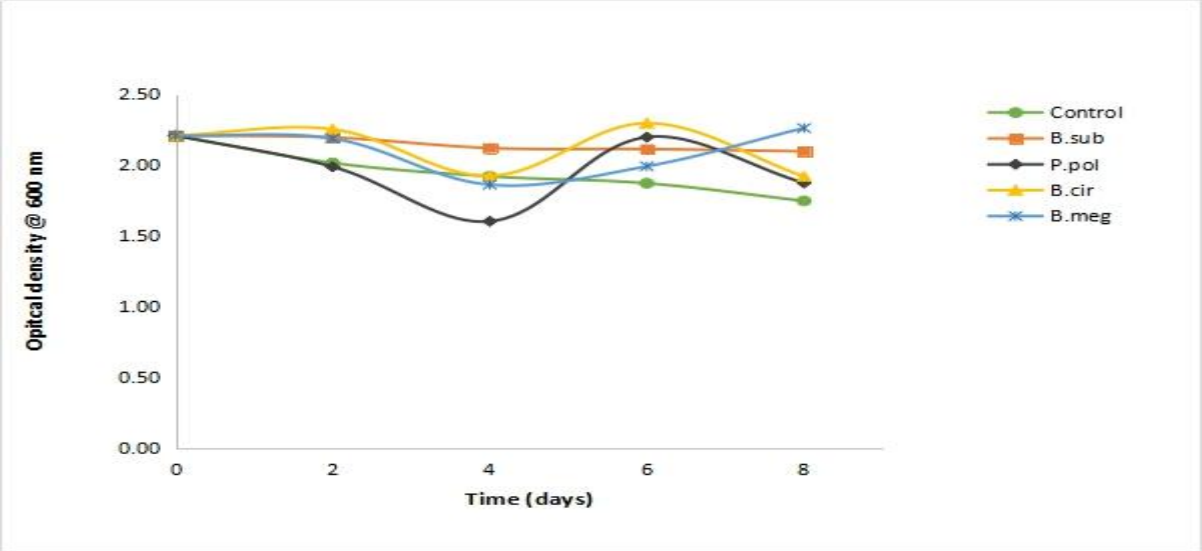

Fig.6. Growth of selected bacterial isolates at 1.0% Carbofuran
Key:
B.sub – *Bacillus subtilis*
P.pol – *Paenibacillus polymyxa*
B.cir – *Bacillus circulans*
B.meg – *Bacillus megaterium*

Paenibacillus polymyxa and *Bacillus subtilis* recorded the highest and least growth rates at 1.5% Carbofuran as shown in Fig.7. There were variations in the growth of *Bacillus megaterium* during the study. *Paenibacillus polymyxa* growth decreased from 2.926 (day 0) to 2.586 (day 4) but increased till end of study. *Bacillus circulans* reduced at day 2 (2.646) increased at day 4 (2.761) and later decreased till end of study. The growth of *Bacillus subtilis* decreased steadily throughout the experiment. At P=0.05 there was a significant difference in bacterial growth abilities with days using the two-way ANOVA

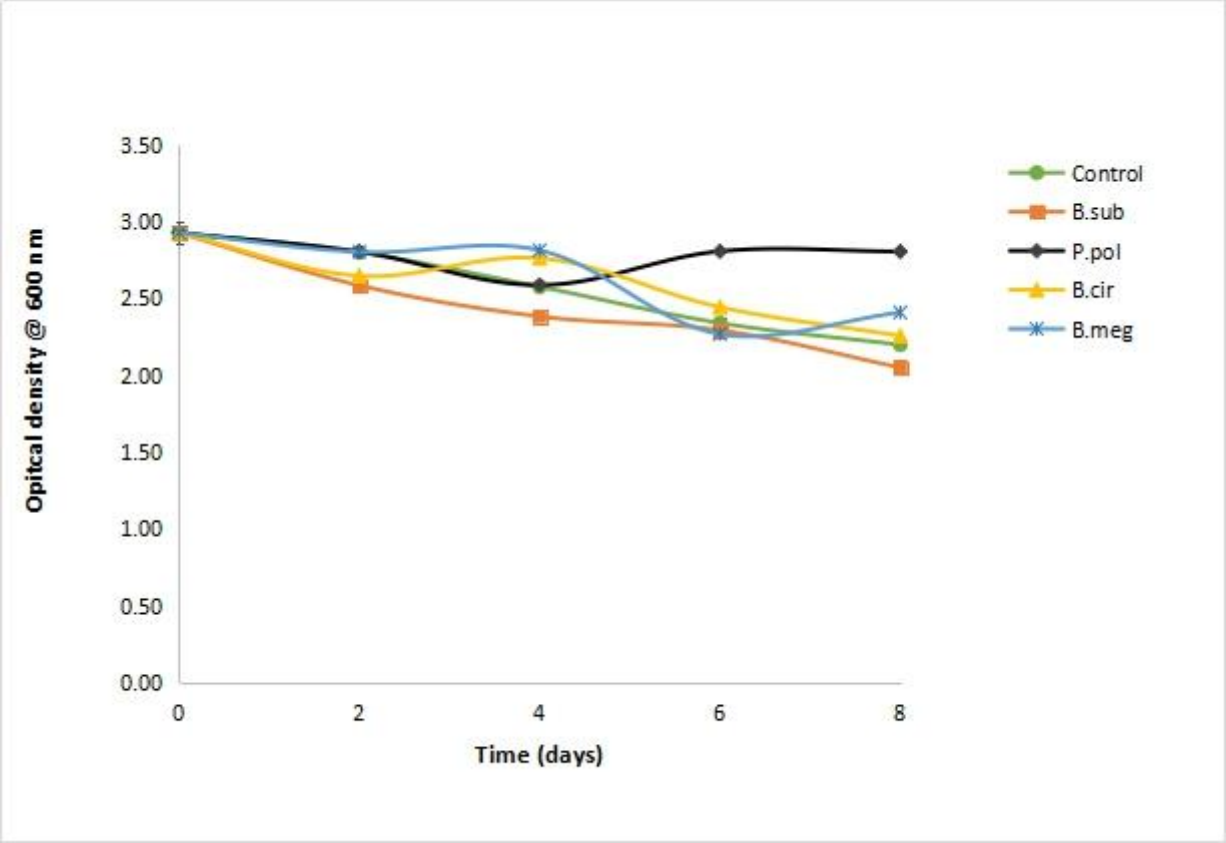

Fig.7. Growth of selected bacterial isolates at 1.5% Carbofuran
Key:
B.sub – *Bacillus subtilis*
P.pol – *Paenibacillus polymyxa*
B.cir – *Bacillus circulans*
B.meg – *Bacillus megaterium*

Bacillus megaterium recorded the highest growth rate followed by *Bacillus subtilis* while *Bacillus circulans* had the least rate at day 8 in microcosms containing 2.0% Carbofuran (Fig.8). There were growth variations for *Bacillus subtilis* throughout the trial. *Bacillus circulans* reduced gradually all through the experiment. Furthermore, there was a continuous decrease in the growth of *Paenibacillus polymyxa* during the study. A two-way ANOVA showed that there was statistical significance among bacterial growth abilities with days at P=0.05.

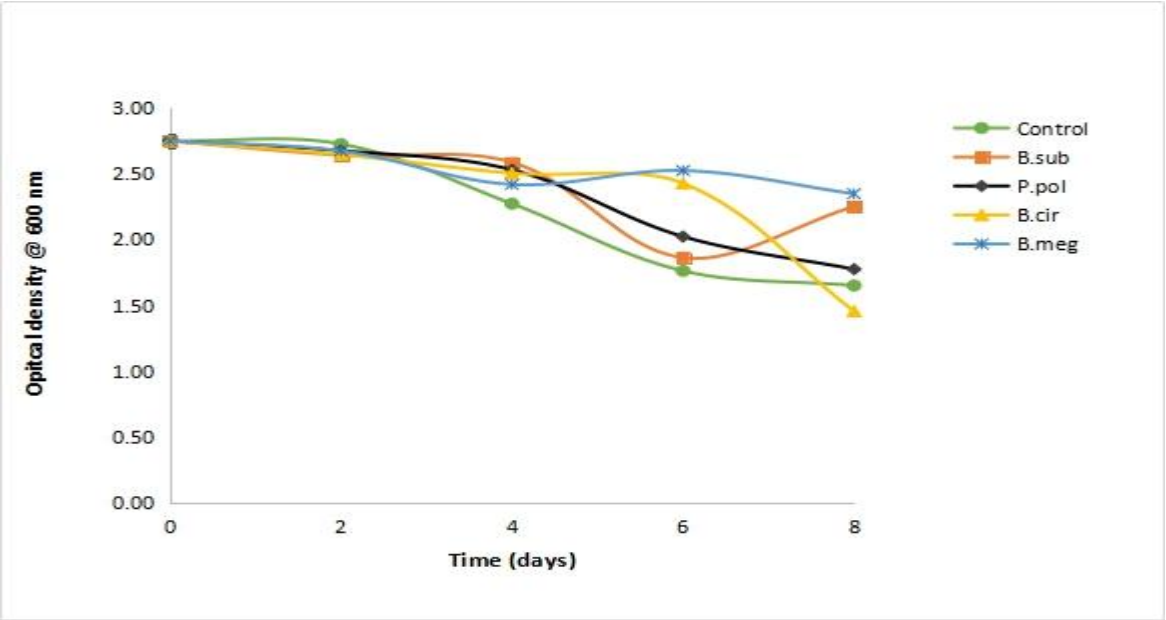

Fig.8. Growth of selected bacterial isolates at 2.0% Carbofuran

Key:
B.sub – *Bacillus subtilis*
P.pol – *Paenibacillus polymyxa*
B.cir – *Bacillus circulans*
B.meg – *Bacillus megaterium*

The test organisms used were isolated from Carbofuran and Paraquat enriched medium containing soil. Selected bacteria were identified as *Bacillus subtilis, B. circulans, B. megaterium* and *Paenibacillus polymyxa*. These organisms were used to test bacterial growth capabilities in Carbofuran as illustrated in figures 5 - 8 above. *Chryseobacterium indologenes, Acinetobacter baumannii, Enterobacter sakazaki* and *Bacillus amyloliquefaciens* were used for Paraquat (Figures 1-4). According to Nisha et al. [29], bacterial species such as *Bacillus, Pseudomonas, Flavobacterium, Arthrobacter* and *Sphingomonas* which are capable of growing in the presence of Carbofuran have been isolated from the soil samples. Obuotor et al. [32] reported the ability of *Pseudomonas, Providentia, Proteus, Citrobacter, Klebsiella* and *Enterobacter* species from fermented corn steep to grow and biodegrade Paraquat dichloride-contaminated soil.

Higher growth rates were obtained in 0.5% 0f Carbofuran and Paraquat for all organisms with faster growth in set-ups containing Paraquat. The increase in turbidity and optical density (OD) were directly proportional to the growth of organisms. These organisms were able to grow in medium containing Carbofuran and Paraquat as a result of their ability to metabolize them as source of carbon and energy to grow. The variation in growth of organisms could be traceable to the number of carbon and nitrogen atoms in each pesticide. Carbofuran contains 12 carbon and 1 nitrogen atoms while Paraquat has 12 carbon and 2 nitrogen atoms. When comparing the growth ability of the test organisms *Bacillus subtilis* had the highest activity in Carbofuran and *Acinetobacter baumannii* in Paraquat.

Omolo et al. [33] reported the growth of *Pseudomonas, Flavobacterium* and *Alcaligenes* species in the presence of Methomyl and Carbofuran in soils of horticultural farms in Rift Valley and Central Kenya. Ammar [34] stated that species of *Micrococcus*, *Streptomyces* and *Bacillus* were able to grow in the presence of three pesticides (diuron, carbaryl and glyphosate) and were able to degrade them within 11days which were contrary to slow degradation earlier reported from studies. If these test microorganisms can grow in the presence of these toxicants, there is the likelihood that they maybe be able to reduce both Carbofuran and Paraquat hazards of contaminated areas. Thus, these bacteria can be used for the clean-up of these pesticides pollution in farms to ameliorate the problems of pesticide pollution in environment.

Furthermore, at higher concentrations of Carbofuran (1.5% and 2.0%) bacterial growth were impaired but later adjusted to the environment. This implies that Carbofuran was toxic at higher concentrations which could be lethal to the organisms thus, suppressing their growth and numbers. Again, there are reports from other researchers on the toxicities of pesticides at higher concentration to microorganisms. Therefore, this study advocates the need for strict adherence to the amounts and use of these chemicals.

4. CONCLUSION

Diverse mixtures (organic and inorganic) are extensively dispersed in the environs as an upshot of their prevalent routine as pesticides, solvents, fire retardants, pharmaceuticals, and lubricants. Environmental hazards and human health problems due to persistence and noxious nature have been recorded from the continuous application of these chemicals. Mechanized farming due to increased need for agricultural products to feed the teaming population has greatly accelerated the large scale manufacturing and usage of pesticides.

The applications of pesticides overtime leads to periodic negative effect on such agricultural fields. It is absolutely pertinent to source for microorganisms capable of removing manmade and recalcitrant substances from the ecosystems that are polluted with these noxious compounds. Microbes have developed catabolic paths to breakdown foreign chemicals due to the presence of catabolic plasmids coding for enzymes responsible for the breakdown and removal of different natural and artificial compounds. This work particularizes the likelihood of the various bacterial agents in decontamination of agricultural soils as they are capable of growing at different pesticides' concentrations making them candidates for application in bio-restoration trials in pesticide polluted environments.

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