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Identification of Carbofuran and Paraquat Degrading Microorganisms from Soil

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Authors' contributions

This work was carried out in collaboration among all authors. Author GCO designed the study and wrote the protocol. Author TLA performed the laboratory analyses, statistical analysis and wrote the first draft of the manuscript. Author POO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Increased rates of pesticide misapplication and follow-on concerns on public health have become subjects of countless distress. The occurrence of pesticides in soils could result in modifications in soil physical, chemical as well as biological properties hence the need for ways to reduce such impacts.
Research Gap: Insufficient literatures on extensive identification of pesticides' degraders from non-impacted soils. Existing literatures are restricted to a particular microbial group (bacteria or fungi).
Aim: The study aimed at isolating, characterizing and testing bacteria, moulds, yeasts and actinomyces from soil for the biodegradation of pesticides.
Place and Duration of Study: The study was carried out at the Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun and Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria/ four months.
Methodology: Carbofuran and Paraquat degrading microorganisms were isolated from a non-pesticides impacted soil using mineral salt medium (MSM). The MSM composed in grams per liter:

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K_2HPO_4, 4.8; KH_2PO_4, 1.2; NH_4NO_3, 1.0; $MgSO_4$ $7H_2O$, 0.2; $Ca(NO_3)_2$ $.4H_20$, 0.4, and $Fe(SO_4)_3$, 0.001 supplemented with 2 mM Carbofuran or Paraquat as the only carbon source. The ability of the microbial isolates to utilize Carbofuran and Paraquat was screened on MSM containing 150 part per million of the pesticides as the only carbon source. The isolates were identified using the analytical profile index (API), microscopic and macroscopic characteristics.

Results: Bacterial species identified were *Bacillus, Pseudomonas, Kocuria, Enterobacter, Chryseobacterium, Corynebacterium, Acinetobacter, Paenibacillus, Lerclercia and Proteus.* Actinomyces were *Actinomyces isrealii*, *Actinomyces naeslundi*, *Actinomyces viscosus* 1, *Actinomyces meyeri* and *Actinomyces viscosus* 2. Yeast isolates were *Candida stellatoidea, Candida krusei* and *Saccharomyces cerevisiae while* moulds were *Talaromyces, Cladosporium carionii* and *Curvularia species*.

Conclusion: These findings indicated that Carbofuran and Paraquat degrading organisms are readily extant in soils and can be used to facilitate the removal of these pesticides from such polluted environments.

Keywords: Carbofuran; Paraquat; pesticide degraders; actinomyces; xenobiotics; soil.

1. INTRODUCTION

Carbofuran (2, 3 – dihydro - 2, 2 – dimethylbenzofuran – 7 – yl methylcarbamate, $C_{12}H_{15}NO_3$, CAS registry no. 1563-66-2) is one of the broad-spectrum, highly toxic carbamate (highly toxic pesticide "Ib") and a nuerotoxin. Carbamates are inhibitors of acetylcholinesterase. The toxic action is a result of its ability to inhibit cholinesterase in the central and peripheral nerve systems where it functions in transmitting nerve impulses [1,2]. It is a wide-range N-methyl carbamate, applied as an insecticide, miticide and acaricide.

Carbofuran is the most commonly used carbamate insecticide in insect control on lawns, home gardens, citrus, fruit, forage and field (rice, corn) crops and potatoes. Agricultural crops with the highest annual use of Carbofuran include rice, corn and potatoes [3,4]. Carbofuran is banned in many countries including Canada and European nations. Environmental Protection Agency (EPA) initiated the ban on all granular formulation in certain ecologically sensitive areas. The ban was initiated to protect birds and not related to human health concerns. Bird kills have occurred when ingested Carbofuran granules that resemble grain seeds or when predatory or scavenging birds ingest small birds or mammals that have ingested Carbofuran granules [5,6,7].

Additionally, there is no ban on the liquid formulation but is grouped as a restricted use pesticides (RUP) due to their acute oral and inhalation toxic impact to man. Carbofuran is a systemic insecticide in plants and contact in insects. It is highly toxic by inhalation and ingestion and moderately toxic by dermal absorption. It disrupts an insect's nervous system and could be lethal if touched or eaten. The impacts of Carbofuran on human and environmental health depend on its concentration, the length and frequency of contact. Effects are also dependent on the healthiness of an individual and/or certain environmental factors. It possesses one of the greatest acute toxicities to humans [8,9].

At low concentrations, it causes alteration of the concentration of hormones. Risks from exposure are especially high for persons with asthma, diabetes, cardiovascular disease, mechanical obstruction of the gastrointestinal or urogastal tracts. The symptoms on human associated with acute Carbofuran exposure may include vomiting, blurred vision, excessive salivation, imbalance, breathing difficulty, muscle weakness, nausea, abdominal cramps, sweating, increased blood pressure, restlessness, lack of controlled urine or faeces release (incontinence), drowsiness, lethargy, inability to concentrate and others. Death may result from respiratory system failure associated with Carbofuran exposure. In contact, burns the skin and irritate eyes. Chronic exposures can damage the nervous and reproductive systems [10,11]. The EPA has proposed 40 part per billion (ppb) as the maximum contaminant level goal.

Paraquat (N, N′ - dimethyl - 4, 4′ - bipyridinium dichloride, $C_{12}H_{14}Cl_2N_2$, CAS registry no.4685-14-7) is classified in category 111 as "slightly toxic". Paraquat, a bipyridinium compound is an example of quaternary ammonium herbicides with the trade name, Gramoxone. Paraquat is known to act on the photosystem I within the

photosynthetic membrane [12]. It inhibits carbon dioxide fixation through inhibiting the variable chlorophyll fluorescene by decreasing oxygen evolution and its activity is irreversible. European Union (EU) proposed a limit of less than 0.1 µg for pesticides and herbicides. Water polluted with Paraquat is a risk factor for liver, lung, kidney and heart illnesses. It is currently banned in 32 countries including European nations based on human concerns [13], but still registered and used in over 90 countries [14]. Several researches targeted at its toxicology in mammals including humans [15,16] at occupational and non-occupational exposures have been conducted [17].

Paraquat is applied post emergence to leaf tissues. It is non selective to plants, quick and lethal to man and animals. It is more acutely lethal to people compared to other herbicide in widespread commercial use and is connected to Parkinson's disease [18,19]. Paraquat is termed a chemical flame gun, in so much as it kills the above-ground parts of plants whilst not damaging the roots. Such effects will usually kill annual weeds, but deeper-rooted and rhizomatous weeds will resprout; agricultural systems have been established to utilize these potentials [20]. Paraquat is a non- selective herbicide used to control most yearly weeds and several broad leaf weeds in field corn, potatoes, rice, cotton, soybeans, tobacco, peanuts and sunflowers. Paraquat is used for no till burn down and in aerial distribution of marijuana, almonds, cotton, grapes, alfalfa and cocoa plantings [21]. It is administered in pre-emergence and early post-emergence weed control.

Although, these pesticides play important roles in protecting agricultural crops from insect pests and weeds, and in controlling disease-transmitting vectors, they cause serious environmental pollution problems [22,23].

Slight losses of Paraquat can result from photodecomposition and volatilization [24]. Paraquat soil half-life is 36 days – 2.6 years [25,26,27]. Carbofuran has a half-life of 30-150 days [28] and degradation could be via chemical hydrolysis, photodegradation and microbial action [7]. The adsorption/desorption to/from soils results to the pollution of ground and surface water.

However, due to these pesticides stability and long half-life, residues in farm products and the natural environs are potential hazards to human health and other biotic life [29,30]. According to [31,32] pesticides may harm non-target organisms possibly threatening human health through the food chain. Similarly, it causes harm to immune-related genes and membrane proteins of non-target organisms [33,34,35]. For the past decades, overuse of these chemicals have severely polluted surface water, soil, food and biota. For that reason, it is imperative to raise fear about its environmental impacts and to advance an operational/effective and possible approach for eliminating their residues [36]. Studies on microbial isolation, identification and degradation are expedient in the development of schemes for the decontamination of pesticides by microorganisms [37].

The restoration of pesticide-contaminated sites is recognized as a cost-effective and reliable method. Microbes are key players during the degradation of these compounds thus, reducing their toxicity. Many bacteria capable of degrading different xenobiotics including pesticides have been isolated and identified [38]. These studies mainly focused on the role of some bacterial genera, such as *Bacillus, Pseudomonas, Flavobacter, Arthrobacter, Diaphorobacter, Klebsiella, Ochrobactrum*, *Agrobacterium* and others [33,39,40,41,42] in degradation of pesticides. However, till to date few data are available in the literature on the isolation of different microbial groups (bacteria, fungi and actinomycetes) involved in pesticides biodegradation. Hence, the present work aimed to isolate and characterize pesticides degrading microbial isolates from farmyard soils.

2. MATERIALS AND METHODS

2.1 Site Description

The study area was located in the Federal University of Petroleum Resources, Effurun, Delta State, Nigeria (7° 23' N; 3° 51'E and 26.7 m above mean sea level). The Niger Delta experiences tropical climate with distinct wet and dry seasons having a bimodal rainfall pattern with rainfall peaks mostly in June to September and average temperature of 25.2°C (78.8°F) - 28°C (82.4°F). The soils were mostly sandy loam at the top, to brown loamy sand sub soil and well drained. Four different representative locations having similar ecological conditions were chosen for this study. The locations had no history of pesticides applications.

2.2 Sample Collection

The surface soil samples were collected from 0 to 15 cm and mixed to form a composite sample at each location. Soil samples were sorted to remove stones, plant and root debris. All soil samples from the four locations were pooled together and transferred to the laboratory in a cooler with ice packs for analysis.

Pesticides (Carbofuran and Paraquat) used in the study were purchased from local retailers in Warri, Delta State.

2.3 Physicochemical Analysis

Fresh soil sample was characterized to assess the physicochemical properties. Soil pH, soil texture, electrical conductivity, moisture content, nitrogen, total organic carbon and phosphates were analyzed following the standard methods by American Public Health Association (APHA) [43].

Calcium, magnesium, potassium, sodium, mercury, arsenic, cadmium and lead were detected by flame analysis method using the atomic adsorption spectrophotometer Model AA500 (PG instruments) following digestion of samples according to the protocol described by APHA [43]. Microbial counts were determined using standard plate counts.

Serial dilution was done using one (1) gram of soil sample suspended in 9 ml of sterile physiological saline. Aliquots (0.1 ml) of the dilutions were plated out using appropriate media for the enumeration of microorganisms. Rose-Bengal chloramphenicol agar was used for the enumeration of fungi [44] and plate count agar (PCA) was used for the enumeration of total heterotrophic bacteria [45]. Actinomycetes were enumerated using starch-casein agar [46] and Pikovskaya's medium for phosphate solubilizing microbes [47]. Ashby agar was used to enumerate nitrogen fixers [44] and individual colonies were recorded as colony forming units (CFU).

2.4 Enrichment of Pesticide Degrading Microorganisms

The method described by Omolo et al. [48] was adopted for the enrichment of Carbofuran-degrading and Paraquat-degrading microorganisms. One gram each of soil sample was suspended in 10 ml of phosphate buffered mineral salt medium (MSM). The MSM composed in grams per liter: K_2HPO_4, 4.8; KH_2PO_4, 1.2; NH_4NO_3, 1.0; $MgSO_4$ $7H_2O$, 0.2; $Ca(NO_3)_2$ $.4H_20$, 0.4, and $Fe(SO_4)_3$, 0.001. The media was supplemented with 2 mM Carbofuran or Paraquat as the sole carbon source. Cultures were grown in 100 ml culture flasks under aseptic conditions at 30°C with shaking in a rotary shaker at 100 rpm for 18 days. Cultures were then streaked onto agar plates containing mineral salt medium supplemented with 2 mM of the pesticides. Single colonies obtained were re-suspended in basal medium (MSM) containing 2 mM of pesticides for 14 days to confirm the ability of the isolates to utilize pesticides. All the solutions, cultures and media were prepared and maintained using aerobic techniques which included covering media with cotton wool and shaking to allow air circulation in the cultures.

2.5 Laboratory Isolation of Pesticide Degrading Microorganisms using Cultural Methods

Serial dilution agar plating method was carried out for the isolation of microorganisms. Luria-Bertani medium containing 0.1 g/liter cycloheximide (Sigma Aldrich, Steinheim, Germany) to suppress fungal growth was used to determine bacterial population [48] while Rose - Bengal agar and starch casein agar were used for fungal and actinomycetes population, respectively. Streptomycin 40 µl/ml and griseofulvin 50 µl/ml were used to prevent bacterial and fungal contaminants [46] in starch casein agar while Rose Bengal contained chloramphenicol [44]. Selected pesticides degrading organisms were characterized by physiological, morphological and biochemical characters [3,4,49] using the analytical profile index (API).

3. RESULTS AND DISCUSSION

3.1 Soil Characteristics

The soil physical, chemical and microbiological properties of the pooled soil are presented on Table 1. The pH of the soil was 6.7. The value of nitrates, phosphates (mg/kg), exchangeable cations (sodium (Na), magnesium (Mg), potassium (K), and calcium (Ca)) present in the soil were 36.28 meq/100 g, 22.15 meq/100 g,

26.32 meq/100 g, 85.98 meq/100 g soil, respectively. The total heterotrophic bacterial, fungal, actinomycetes, nitrifiers and phosphate solibilzers counts in the fresh soil were 6.3 x 10^7 CFU/g, 1.41 x 10^5 CFU/g, 1.99 x 10^4 CFU/g, 1.45 x 10^4 CFU/g and 1.52 x 10^4 CFU/g, respectively. The physicochemical properties of the soil showed that it was a sandy (97.192%) silt (2.65%) soil from the particle size distribution with a close to neutral pH value of 6.70. According to Kyveryga et al. [50] most agricultural soils have a pH in the range of 5.5 to 8.0 but under different agricultural practices soils pH values may increase or reduce [51]. The solubility of soil macronutrients, micronutrients or essential trace elements are influenced by soil pH [52,53]. These macronutrients and micronutrients are highly solubilized in soils between the pH range of 5.5 to 7.0 such that these high pH levels in the soil result in leaching of nutrients and releases aluminium in solubilized forms from its insoluble state [54]. Furthermore, it prevents cell division and growth in the roots; affects the plant's uptake of cations and stimulates organic acid secretion [55]. Heavy metal analysis showed that soil had a higher lead content (19.25) relative to arsenic (0.215), mercury (<0.001) and cadmium (0.725). Heavy metals examination indicated soil had very low levels of the metals hence, large numbers of microorganisms could thrive in the soil. In addition, the soil had appreciable amount of nitrates and phosphates essential for the growth and proper functioning of the microbial communities present in soil.

3.2 Isolation of Pesticides Degraders

Microorganisms are the main degraders of pollutants in the environment. Various organisms were isolated by enrichment to isolate pesticides degraders using Carbofuran and Paraquat, respectively as the sole carbon and energy source from soil. Microorganisms are focal degraders of contaminants in various environment. The taxonomic classification of isolates performed using the API platforms placed the isolates into seventeen bacterial, three yeast and five actinomyces species. Microscopic and macroscopic characteristics of the isolates also supports mould genus assignments.

Table 1. Physicochemical and microbiological characteristics of soil used for the study

Parameters	Value
Physicochemical	
Electrical conductivity (µs/cm)	144
pH	6.70
Total organic carbon (TOC %)	3.316
Total nitrogen (%)	0.3029
Nitrates (mg/kg)	36.28
Phosphates (mg/kg)	26.32
Moisture content (%)	19.41
Calcium (meq/100g)	61.83
Magnesium (meq/100g)	22.15
Sodium (meq/100g)	0.97
Potassium (meq/100g)	1.03
Arsenic (mg/kg)	0.215
Mercury (mg/kg)	<0.001
Lead (mg/kg)	19.25
Cadmium (mg/kg)	0.725
Soil particle size distribution (%)	
Silt	2.65
Sand	97.192
Clay	0.158
Microbiological	
Microbial counts (CFU/g)	
Total heterotrophic bacteria	6.30×10^7
Fungi	1.41×10^5
Actinomycetes	1.99×10^4
Nitrifying bacteria	1.45×10^4
Phosphate solubilizers	1.52×10^4

Thirty five bacterial, fifteen fungal and nine actinomycetes isolates were obtained after pesticides enrichment. Of the bacterial isolates; nineteen were Gram positive and sixteen were Gram negative. The bacterial isolates identified were *Bacillus subtilis, Bacillus amyloliquefaciens, Paenibacillus polymyxa, Bacillus circulans, Bacillus basidium, Bacillus megaterium, Pseudomonas putida, Pseudomonas aeruginosa, Proteus penneri, Lerclercia adecarboxylata* and *Enterobacter* species from Carbofuran (Table 2) while *Bacillus amyloliquefaciens, Bacillus basidium, Bacillus mycoides, Bacillus megaterium, Kocuria varians, Corynebacterium capsicum, Chryseobacterium indologenes, Pseudomonas aeruginosa, Pseudomonas putida, Enterobacter* species*, Acinetobacter baumannii* and *Enterobacter sakazaki* were identified from Paraquat as shown on Table 3. The results of our study were in confirmation with the outcomes of other researches.

Omolo et al. [48] isolated bacterial isolates closely associated to members of *Pseudomonas* from Carbofuran enriched soil using the partial 16S rDNA sequence analysis. Three unalike species of *Bacillus* were isolated by Nisha et al. [4] from Carbofuran enriched soil. Obuotor et al. [37] isolated and identified species of *Pseudomonas* and *Enterobacter* from Paraquat contaminated soil using the API. These reports are in corroboration with our findings. Again, Desaint et al. [56], equally isolated *Pseudomonas* and *Bacillus* species using amplified ribosomal DNA restriction analysis (ARDRA) analysis of 16S rDNA as Carbofuran-degrading bacteria from soil. Report has it that *Burkholderia, Pseudomonas, Cupriavidus, Corynebacterium, Arthrobacter, Micrococcus*, and *Sphingomonas* species have been identified as pesticide degraders using the culture-dependent studies, a number of which use the compound as an exclusive carbon source [57].

Table 4 shows the yeast isolates obtained during the experiment. The yeast species identified were *Candida stellatoidea, Candida krusei* and *Saccharomyces cerevisiae*. Again, Obuotor et al. [37], isolated *Saccharomyces cerevisiae* and *Candida* species from their research which was also true from this present study. Martani [58] reported *Rhodotorula, Cryptococcus* and *Sporobolomyces* as Paraquat degrading yeasts from fibric and saphric peat soils.

Actinomyces species isolated during the study are as presented on Table 5. The actinomycetes identified were *Actinomyces isrealii*, *Actinomyces naeslundi*, *Actinomyces viscosus* 1 (Carbofuran) and *Actinomyces meyeri, Actinomyces naeslundi*, *Actinomyces viscosus* 2 from Paraquat. De Schrivjver & De Mot [59], equally isolated different members of *Actinomyces* during their studies. Fuentes et al. [60], isolated several actinomycetes in the genus *Streptomyces* and a member of *Micromonospora* which was in contrast to our findings. Furthermore, Chougale and Deshmukh [61] reported that seven actinomycetal isolates were isolated and identified as *Streptomyces alanositicus, Streptomyces atratus, Strptoverticillium album, Nocardia farcinica, Norcadia vaccini, Norcadia amarae* and *Micromonospora chalcea* from soils. These actinomycetes when further tested for biodegradation of Carbofuran pesticide by cometabolism and as a sole carbon source were active degraders of the chemical.

Talaromyces, Cladosporium carionii and *Curvularia* were the mould species identified in this research as shown on Table 6. Our results were contrary to other researchers' reports that isolated *Aspergillus, Penicillium, Mucor, Trichotherium* and *Rhizopus* suggesting that the soil type could determine the type of fungi present [62,63]. Also, Martani [58] isolated the moulds; *Penicillium* and *Aspergillus* based on their ability to degrade Paraquat.

It is exciting to note that microbial isolates normally present in soil; a non-pesticide impacted environment, has the capability to utilize Carbofuran and Paraquat as sole source of carbon and energy. Several researchers have reported in their work microbial populations capable of utilising these pesticides as sole source of carbon from soil [37,44,48,64]. There are reports that microbial communities exposed to xenobiotics adapt to this exposure through selective enrichment and genetic changes resulting in an increase in xenobiotic-degradation [65,66]. This pre-exposure of microorganisms make them better suited to degrade the pollutant through higher growth, reproduction and more efficient metabolism thus maximizing the rate of these pesticides removal from the soil.

Table 2. Gram positive bacterial isolates from the study

Code	Glucose	Glycine	H_2S	Urease	VP	Indole	ONPG	Gelatine	Citrate	L-Arabinose	Fructose	Sorbitol	Inositol	Ribose	D-xylose	Esculin	Maltose	ADH	LDC	% ID	Identified Isolate
Carbofuran																					
B1	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	92.5	*Bacillus amyloliquefaciens*
B2	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	94.5	*Bacillus subtilis*
B3	+	+	-	-	+	-	+	+	+	-	+	+	N	+	+	+	+	-	-	99.9	*Paenibacillus polymyxa*
B4	+	+	-	-	-	-	+	+	-	+	+	N	N	+	+	+	+	-	-	61.8	*Bacillus circulans 2*
B5	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	99.5	*Bacillus megaterium*
B6	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	92.5	*Bacillus badius*
Paraquat																					
B7	+	N	N	+	+	N	N	+	N	N	+	N	N	N	-	N	-	-	-	99.0	*Kocuria varians*
B8	+	-	N	-	N	N	N	+	N	N	N	N	N	+	-	-	-	N	-	92.9	*Corynebacterium capsium*
B9	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	99.5	*Bacillus megaterium*
B10	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	92.5	*Bacillus badius*
B11	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	90.0	*Bacillus mycoides*
B12	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	89.5	*Bacillus amyloliquefaciens*

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

Table 3. Gram negative bacterial isolates from the study

Code	Glucose	ONPG	ADH	LDC	Citrate	H_2S	Urease	Indole	VP	Gelatin	Mannose	Inositol	Sorbitol	% ID	Identified isolate
Carbofuran															
B13	+	+	-	N	+	-	-	-	-	-	+	-	+	89.5	*Enterobacter* species
B14	+	N	N	N	N	N	+	-	-	N	+	N	N	92.8	*Pseudomonas putida*
B15	+	+	-	N	+	-	-	-	-	-	+	-	+	89.0	*Lerclercia adecarboxylata*
B16	+	-	-	-	+	+	+	+	+	-	-	-	+	93.0	*Proteus penneri*
B17	+	N	-	N	+	N	-	-	-	+	+	N	N	89.8	*Pseudomonas aeruginosa*
Paraquat															
B18	-	-	-	-	-	-	+	+	-	+	-	-	-	99.6	*Chryseobacterium indologenes*
B19	+	N	N	N	N	N	+	-	-	N	+	N	N	89.8	*Pseudomonas putida*
B20	+	+	-	N	+	-	-	-	-	-	+	-	+	89.5	*Enterobacter* species
B21	+	N	-	N	+	N	-	-	-	+	+	N	N	89.8	*Pseudomonas aeruginosa*
B22	+	-	-	-	+	-	-	-	-	-	-	-	-	99.9	*Acinetobacter baumannii*
B23	+	+	+	-	+	-	-	-	+	-	+	+	-	98.4	*Enterobacter sakazaki*

Key: H_2S - hydrogen sulphide, VP – Voges Proskauer, ONPG – O-nitrophenyl-β-galactopyranoside, ADH- arginine dehydrolase, LDC- lysine decarboxylase, N – not applicable

Table 4. Yeast isolates from the study

Code	Glucose	Glycine	Arabinose	Galactose	Inositol	Sorbitol	MDG	NAG	Lactose	Maltose	Trehalose	% ID	Identified isolate
Carbofuran													
CY1	+	+	-	-	-	+	+	+	-	+	+	73.0	*Candida krusei*
CY2	+	+	-	+	-	-	-	-	-	+	-	71.0	*Saccharomyces cerevisiae*
Paraquat													
PY1	+	+	-	+	-	-	-	-	-	+	-	71.0	*Saccharomyces cerevisiae*
PY2	+	+	-	-	-	+	+	+	-	+	+	73.0	*Candida stellatoidea*
PY3	+	+	-	-	-	+	+	+	-	+	+	73.0	*Candida krusei*

Key: MDG – methyl-α-D-glucopyranoside, NAG – N-acetyl-glucosamine

Table 5. Actinomycetes isolated from the study

Code	Grams reaction	Glucose	Indole	Urease	Mannose	Lactose	Maltose	Xylose	Arabinose	Gelatin	Esculin	Raffinose	Sorbitol	%ID	Identified isolate
Carbofuran															
A1	+	+	-	-	+	-	+	+	+	-	+	+	-	94.8	*Actinomyces isrealii*
A2	+	+	-	-	-	+	+	-	-	+	-	+	-	94.2	*Actinomyces naeslundii*
A3	+	+	-	-	-	+	+	-	-	-	-	+	-	98.4	*Actinomyces viscosus 1*
Paraquat															
A4	+	+	-	-	-	+	+	+	+	-	-	-	-	99.9	*Actinomyces meyeri*
A5	+	+	-	-	-	+	+	-	-	+	-	+	-	94.2	*Actinomyces naeslundii*
A6	+	+	-	-	-	-	-	-	-	-	-	-	-	99.3	*Actinomyces viscosus 2*

Table 6. Mould isolates from Carbofuran and Paraquat during the study

Reference	Culture characteristics	Microscopic characteristics	Probable genera
I	Light grey-yellowish colonies which developed within 4 days. Reverse was yellowish with furrows. There was dark red pigmentation when culture was old.	Stripes bearing terminal biverticillate or less commonly monoverticillate	*Talaromyces* species
J	Slow-growing white colonies with rug-like aerial mycelia and furrows on surface and reverse side. Reverse was light brown with furrows.	Septate hyphae with dark, branched conidiophores with two or more conidial chains. The conidia formed branching tree-like chains, which as oval and easily dislodged showing dark spots at the point where they are attached to conidiophores.	*Cladosporium carionii*
H	White to brownish wooly surface with raised or higher mycelium colonies. Reverse side of the plate was fairly blue-black.	Branched conidiophores, septate hyphae. The macroconidia was large, slightly curved but had no rostrum	*Curvularia* sp.

4. CONCLUSION

In Nigeria, farmers desire the use of pesticides mixture in the same farm. The isolation of Carbofuran and Paraquat degrading microbes from soils indicated that farm soils harbour pesticide degrading microbial populations and have a widespread genetical diversity and geographical distribution hence, are conceivably worthwhile in environmental biorestoration. The API analysis of the isolated species clustered them into nine different bacterial genera; *Pseudomonas, Proteus, Chryseobacterium, Enterobacter, Kocuria, Bacillus, Lerclercia, Acinetobacter, Paenibacillus, Corynebacterium.* Yeast genera were *Candida, Saccharomyces and mould genera were Talaromyces, Cladosporium carionii* and *Curvularia,* respectively. *Actinomyces isrealii*, *Actinomyces naeslundi*, *Actinomyces viscosus* 1, *Actinomyces meyeri* and *Actinomyces viscosus* 2 were the few actinomyces isolated and identified. All microbial species were pesticides utilizers as observed in the screening which were indicated by increased turbidity of the culture medium. The microbes isolated from the unpolluted soil were capable of growing in the presence of these chemicals suggesting that isolates were potential pesticides degraders.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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