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Full Length Research Article

BIODEGRADATION OF CHLORPYRIFOS ORGANOPHOSPHOROUS PESTICIDE USING AEROMONAS HYDROPHILA ISOLATED FROM SELECTED AGRICULTURAL WASTEWATER IN KADUNA STATE

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ABSTRACT
Chlorpyrifos are broad-spectrum organophosphorus pesticides used excessively for agricultural purposes to fight insects. The presence of such toxic compounds in watercourses exhibits harmful threats to the environment. The technology of bio-removal (or biodegradation) is nowadays the alternate method for environmental curing. The present study aimed to isolate and identify bacterial strains with strong capabilities of biodegrading such pesticides in wastewater from Nassarawa and Hayin Danmani, Kaduna State. *Aeromonas hydrophila* was isolated from agricultural wastewater and identified based on morphological, biochemical tests, and 16S rRNA analysis. The degradation conditions were optimized at 30°C and pH of 7. Preliminary screening assessed growth via optical density. Degradation efficiency was measured in inoculated samples compared to controls, with Chlorpyrifos concentration and degradation rates evaluated. Chlorpyrifos concentrations in water samples NA1-HB1 ranged from 2.71 ± 0.11 to 131.8 ± 0.2. *Aeromonas hydrophila* effectively degraded Chlorpyrifos, with optimal growth observed at an optical density of 0.74 ±0.002. Degradation was faster in inoculated samples, achieving a 73.6 % degradation rate at a Chlorpyrifos concentration of 30 mg/L. The metabolite 3, 5, 6-trichloro-2-pyridinol (TCP), responsible for Chlorpyrifos degradation was detected. *Aeromonas hydrophila* can efficiently degrade Chlorpyrifos in wastewater, utilizing it as a carbon source. Further research should explore more soil microbiota with Chlorpyrifos (CP) utilization capabilities and investigate the metabolic pathway of Chlorpyrifos degradation.

Keywords: Chlorpyrifos, Bioremediation, Wastewater, Organophosphate.

INTRODUCTION

The usage of pesticides in agriculture has greatly aided the production of the country's food supply and its use in the nation amounts to metric tons (Bello, 2021). Over use of pesticides has caused them to be distributed and accumulated in the environment through different channels such as surface runoff and leaching from agricultural lands. It is uncertain how long it will take for these pesticides to be degraded (Deknock *et al.,* 2019). Chlorpyrifos (0, 0-dieethyl 0-(3,5,6- trichloropyridyl) phosphorothioate is one of the most popular insecticides which is efficient against a variety of chewing and sucking insect that are pests to economically significant crops. Exposure to pesticide can cause various physiological and neurological effects on human health, including; respiratory failure, pulmonary edema, cardiac disorders, tremors and coma (David *et al.,* 2015). Of course, a lot of research have been done on how chlorpyrifos affects environment. This can be converted to 3,5, 6- trichloro-2-pyridinol (TCP) being the main breakdown product (Gilani *et al*., 2016). Agricultural wastewater, an excessively water that drains from a field during surface irrigation at the low end of furrows, boundary strips, basins and flooded regions. Both developing and developed nations are increasingly using wastewater for irrigation in agriculture (Raschid-Sally & Jayakody 2010). The emergence of effective treatment methods for the elimination of pesticides in agricultural wastewater is imperative in the current scenario. Several techniques have been developed for the detoxification of organophosphate insecticides, including chemical treatment, incineration, and landfills (Hamad, 2020). However, these methods are associated with certain limitations, such as the generation of sludge, the formation of toxic by-products, high costs, and restricted applicability to a range of pesticides (Ahmed *et al*., 2017). In the environment, microbial degradation is considered to be one of the essential factors to determine the fate of pesticides (Murali *et al.,* 2014). Therefore, the objectives of the study were to isolate and characterize *Aeromonas hydrophila* strains from contaminated agricultural wastewater using conventional and 16S rRNA sequencing as well as to investigate their potential to degrade chlorpyrifos.

MATERIALS AND METHODS

Chemicals and Media

Analytical grade standard of chlorpyrifos (0, 0-diethyl 0-3,5,6-tricloro-2-pyridinyl phosphorothioate)) with a purity of 99% was provided. Solvents, chemicals and culture media were of analytical grade and purchased from standard commercial suppliers.

Study Sites

The study was carried out in Nassarawa and Hayin Danmani communities of Kaduna State. These areas (shown in Figure 1) are located between latitudes 10°46`N and 10°54`N of the equator and longitudes 7° 38`E and 7° 40`E.

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Figure 1: Map of Kaduna metropolis (showing the study area). (Source: KADGIS, 2022)

Collection of Wastewater Samples

Water samples were collected from two drainage canals located at Hayin Danmani and Nassarawa. The samples were randomly collected from two different sites into sterile 15 mL universal bottles. Samples were transported immediately to the microbiology laboratory at Kaduna State University, Kaduna. It was extracted, cleaned up and analyzed as described afterwards.

Isolation and Purification of most common Microbes.

Mineral Salt Medium (MSM) was used for enrichment and isolation. The sole carbon source, Chlorpyrifos was added at a concentration of 1% (w/v) to the MSM after sterilization. Sample preparation was carried by ten-fold serial dilutions from all four wastewater samples and the appropriate dilution of 10^{-3} and 10^{-4} were plated on Nutrient Agar for isolation and purification. Plates were incubated at 30°C for 24 hours and thereafter observed for different colonial traits. Total colony counts of the sample were determined using colony counter (Faria *et al*., 2017). Screening was carried out in 100 mL Mineral Salt Broth containing 5 mL concentration of chlorpyrifos. The flasks were incubated at 37°C for 24 hours. Strains with maximum degradation and minimum incubation time were selected as the most efficient strains, then sub-cultured on Nutrient Agar slant and stored for further analysis (Shabbir *et al.,* 2018).

Optimization of pH and Temperature on Growth of Isolated Strains

For the optimization of pH, 1 mL of freshly grown microbial inoculum was added to MSM containing flasks adjusted to different pH values i.e. 6-8. The CP 10 mgL^{-1} was added aseptically and flasks were then placed on the rotary shaker speed of 130 rpm at 30 °C. After every 24 hours of incubation period, microbial growth of medium was recorded at 600 nm through a period of 1 week.

For temperature optimization, 25 mL MSM in Erlenmeyer flasks containing 10 mgL^{-1} CP were inoculated with 1 mL freshly grown cultures and were kept at different temperatures (20, 30 and 40°C) on rotary shaker through a period of 1 week. After every 24 hours, optical density of medium was recorded at 600 nm.

Preliminary Chlorpyrifos Degradation Assessment

Mineral Salt Broth supplemented with chlorpyrifos (10 mgL^{-1}) were used for biodegradation test. Cells were pre-cultured in broth medium and samples were incubated on rotary shaker at a speed of 150 rpm and 30ºC for 7 days. Medium without inoculation were maintained under the same conditions to serve as controls. Optical density at 600 nm was used for assessing and measuring bacterial growth.

Identification of CP degrading bacteria

Potential chlorpyrifos degrading bacterial were identified using microscopic, biochemical and molecular identification through PCR amplification of 16S rRNA. Genomic DNA extraction of the bacterial isolates was carried out using phenol/chloroform/isoamyl alcohol according to the standard method (Mwaura *et al.,* 2018). The extracted product was subjected to PCR, and the quality of the obtained amplified genomic DNA was analyzed on 1% (w/v) agarose gel in IX TAE buffer. The PCR product was sequenced. The complete sequences of the 16S rRNA isolates were determined and compared using several tree-making algorithms to establish their position within the evolutionary radiation encompassed by similar microorganisms.

Analytical procedure for Extraction

About 50 mL of water sample was extracted with 100 mL of dichloromethane by shaking several times in a separating funnel. The procedure was repeated thrice. The organic layers were collected and passed through anhydrous sodium sulphate for the removal of water content then the collected organic fraction was reduced to 1 mL using rotary evaporator. Samples were then filtered through a membrane filter syringe of 0.2µm Pulsed extraction System (PES) before analysis. Extract was subjected to analysis using Gas Chromatography-Mass Spectrometry (GC-MS).

Biodegradation Studies of Chlorpyrifos in Liquid Media

The biodegradation was evaluated by culturing onto Mineral Salt Broth (MSB) supplemented with chlorpyrifos at three concentrations (20, 30 and 40 mg mL^{-1}) for 28 days. The cultures were incubated at optimum pH and temperature for each isolate on rotary shaker at 150 rpm. Control flasks of equal volume of MSL medium and chlorpyrifos without any microbial population were incubated in parallel at all intervals to assess abiotic loss (Nwaogu *et al.,* 2008). During the experiment, aliquots of the mixtures were collected periodically at 0, 7-, 14-, 21- and 28-days intervals of time for estimation of chlorpyrifos degradation. The optical density (OD) of the aliquots were measured at 600 nm wavelength with spectrophotometer. The cultures from all the flask were pooled together and centrifuged. Residues of CP in the sample were extracted from supernatant using equal volume of dichloromethane.

The chlorpyrifos degradation was calculated using the following equation:

$$X\% = \frac{Cck - Cx}{Cck} \times 100$$

Where X is chlorpyrifos degradation;
Cx is the concentration of chlorpyrifos in the medium containing chlorpyrifos-degrading microbial strain, and;
Cck is the concentration of chlorpyrifos in the medium that does not contain chlorpyrifos-degrading strain.

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Identification of Metabolic Products

The degradation product of chlorpyrifos were identified according to the method of Xu *et al.* (2017) with modification. Investigation was carried out with concentrated extract using Gas Chromatography-Mass Spectrometry (GC-MS) Agilent 6890N/5975, USA. The samples were detected with GC-MS equipped with autosampler, an on-column, spit/spitless capillary injection system, and with HP-5MS capillary column (30.0m × 250µm × 0.25µm) with array detection from 30-500 nm total scan. The operating conditions were as follows: the column was held at 80°C for 5 min, ramped at 8°C`min^{-1}to 200°C (first ramp), held at 200 °C for 5 min, ramped at 15 °Cmin $^{-1}$ to 260 °C (second ramp), and then held at 260 °C for 5 min. The temperatures corresponding to transfer line and the ion trap were 280°C and 230 °C, respectively, and the ionization energy was 70 eV. The injection volume was 1.0 µL with a split ratio of 1:7 at 260°C. Helium was used as a carrier gas at a flow of 1.0 Lmin^{-1}.

Statistical Analysis

The data obtained from the research were subjected to One-way Analysis of variance (ANOVA). Tukey post-hoc comparison test was used to individually compare differences between and within the comparison groups. International Business Machines-Statistical package for Social Sciences (IBM SPSS) software version 23 was used for this analysis. All experiments were done in triplicates.

RESULTS

The result shows the presence of five organophosphorus pesticides in four analysed samples which included ethroprophos, chlorpyrifos, disulfoton, ronnel and phosphorodithioc acid compounds (Table 1).

Concentration of Organophosphorus in Wastewater samples

The quantitative analysis shows that ethroprophos was present in concentration ranging from 0.18 ± 0.05 to 62.11 ± 0.2. Chlorpyrifos with the highest concentration was present in the samples ranging from 2.71± 0.11 to 131.8 ± 0.2. Disulfoton with the least in a concentration ranging from 0.02 ± 0.001 to 0.55 ± 0.03. Ronnel in a concentration ranging from 0.42 ± 0.03 to 104.9 ± 0.2 and phosphorodithioc acid in a concentration ranging from 0.12 ±0.03 to 44.3 ± 0.14 (Table 1).

Table 1: Concentration of Pesticides in Wastewater Samples Collected from Four Agricultural Canals

Sample	Pesticide Concentration (ppm)				
	Ethroprophos	Chlorpyrifos	Disulfoton	Ronnel	Phosphorodithioc acid
NA1	35.1 ± 0.1^{b,c,d}	105.6 ± 0.4 ^{b,c,d}	0.4 ± 0.02 ^{b,c,d}	104.9 ± 0.2 ^{b,c,d}	25.15 ± 0.06 ^{b,c,d}
NB1	32.9 ± 0.2^{a,c,d}	131.8 ± 0.2 ^{a,c,d}	0.02 ± 0.001^{a,c}	0.42 ± 0.03^{a,d}	40.21 ± 0.09 ^{a,c,d}
HA1	0.18 ± 0.05^{a,b,d}	2.71 ± 0.11 ^{a,b,d}	0.55 ± 0.03 ^{a,b,d}	0.57 ± 0.05^{a,d}	0.12 ± 0.03 ^{a,b,d}
HB1	62.11 ± 0.2^{a,b,c}	108.8 ± 0.13 ^{a,b,c}	0.02 ± 0.0 ^{a,c}	1.2 ± 0.04^{a,b,c}	44.3 ± 0.14 ^{a,b,c}

Keys: NA1 = Nassarawa site A, NB1 = Nassarawa site B, HA1 = Hayin Danmani site A, HB1 = Hayin Danmani site B

Physicochemical Parameters of the Various Wastewater Samples

The result of the physicochemical analysis of water sample and its metal concentrations on all the four sites are shown in (Table 2). Results further shows that the variation in the physicochemical properties of the wastewater samples from both sites were statistically significant ($P < 0.05$).

Table 2: Physicochemical Properties and Metal Concentration of the Wastewater Sample from Nasarawa and Hayin Danmani

	Physicochemical parameter				Metal Concentration (mg/l)						
Sample	EC (ppm)	pH	Alkalinity (mg/l)	SAR (meq/l)	NO_3	Cl	Pb	Cr	Ca	Mg	Na
NA1	121 ± 0.2^{b,c,d}	6.78 ± 0.02^{b,c,d}	82.3 ± 0.04 ^{b,c,d}	0.63 ± 0.02^{b,c}	6.29 ± 0.21 ^{b,c,d}	33.9 ± 0.04 ^{b,c,d}	0.91 ± 0.2 ^{b,c,d}	0.08 ± 0.01 ^{b,c,d}	24.8 ± 0.4 ^{b,c,d}	5.6 ± 0.2 ^{b,c,d}	10.5 ± 0.1^{c,d}
NB1	142 ± 0.3^{a,c,d}	6.87 ± 0.12^{a}	112.3 ± 0.1 ^{a,c,d}	0.23 ± 0.11^{a,d}	10.6 ± 0.15 ^{a,c,d}	28.22 ± 0.3 ^{a,c,d}	0.44 ± 0.19^{a,c}	0.01 ± 0.0 ^{a,c,d}	30.6 ± 0.5 ^{a,c,d}	4.7 ± 0.2^{a,c,d}	11.0 ± 0.0^{c,d}
HA1	380 ± 0.25^{a,b,d}	6.81 ± 0.03^{a}	234 ± 0.3 ^{a,b,d}	0.21 ± 0.03^{a,d}	17.13 ± 0.3 ^{a,b,d}	122.3 ± 0.6 ^{a,b,d}	0.14 ± 0.05 ^{a,b,d}	0.06 ± 0.0 ^{a,b,d}	59.1 ± 0.3 ^{a,b,d}	5.8 ± 0.1^{a,b}	17.3 ± 0.3^{a,b}
HB1	385 ± 0.35^{a,b,c}	6.86 ± 0.02^{a}	216 ± 0.25 ^{a,b,c}	0.61 ± 0.07^{b,c}	25.1 ± 0.22 ^{a,b,c}	185.1 ± 0.2 ^{a,b,c}	0.42 ± 0.02^{a,c}	0.03 ± 0.0 ^{a,b,c}	51.0 ± 0.3 ^{a,b,c}	5.9 ± 0.2^{a,b}	17.9 ± 0.4^{a,b}

Keys: NA1 =Nasarawa site A, NB1 =Hayin Danmani site B, HA1 =Hayin Danmani site A, HB1 =Hayin Danmani Site B, EC =Electrical conductivity, SAR =Sodium Adsorption Ratio, NO_3 = Nitrate, Cl = Chlorine, Pb = Lead, Cr = Chromium, Ca = Calcium, Mg =Magnesium, Na = Sodium

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Plate count for the isolated Bacteria

The result showed "NA1" plate count was 52 × 10 ^3 colony forming unit per milliliter of the sample. Site "NB1" had the lowest plate count of 12 × 10 ^3 CFU/ml of the sample. Site "HA1" had the highest with 88× 10 ^3 CFU/ml while Site "HB1" had a total of 35 × 10 ^3 CFU/ml. The colonies were denoted as "MS3" and "MS5".

Morphological Characterization of the Isolated Bacteria

Table 3 shows 2 isolated pure colonies of potential CP degrading bacteria were designated using MS3 and MS5. The isolate (MS3) was observed to be cocci, and non-motile, with colony color yellow. Organism was found to be gram positive. The isolate (MS5) was observed to be rod shaped and motile with colony color grey. Organism was found to be gram negative.

Biochemical Characterization of the Isolated Bacteria

Table 4 shows bacteria characterization where biochemical test revealed the bacterial isolates belonged to the genus *Staphylococcus aureus* and *Aeromonas* species (MS3 and MS5). The comparison of the results obtained were compared with Bergey's manual of bacteriology.).

Table 3: Morphological Characteristics of Bacterial Isolates

Isolate No.	Cell shape	Colony colour	Motility test	Elevation	Gram stain
MS3	Cocci	Yellow	Non-Motile	Convex	Positive
MS5	Rod	Grey	Motile	Convex	Negative

Table 4: Biochemical Characteristics of Bacterial Isolates

Isolate No	Citrate	Urease	Oxidase	Catalase	Indole	MR	VP	H_2S
MS3	+	+	-	+	-	+	+	-
MS5	+	-	+	+	+	-	+	+

Keys: (+) Positive Result, (-) Negative Result, MR: Methyl Red, VP: Voges-Proskauer, H_2S: Hydrogen Sulphide.

Effect of pH on the Growth of Isolate

Growth of isolate was found to be maximum in neutral pH conditions and optimal value was recorded as 7. However, acidic and basic conditions less usage of CP was observed which corresponds to less growth. The effect of pH on utilization of CP by the isolates is shown in (Figure 2).

Effect of Temperature on the Growth of Isolate

Growth of isolate was well supported in temperature range 30°C. Growth at other temperature conditions i.e. 20 and 40°C was comparatively low. The effect of temperature on utilization of CP by the isolates is shown in (Figure 3).

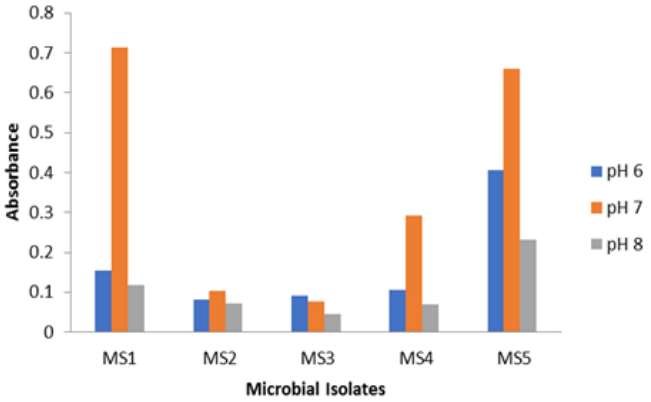

Figure 2.: Effect of different pH values of selected microbial isolates incubated with chlorpyrifos at 10mgL^{-1}

Keys: MS3= *Staphylococcus spp.* MS5= *Aeromonas spp.*

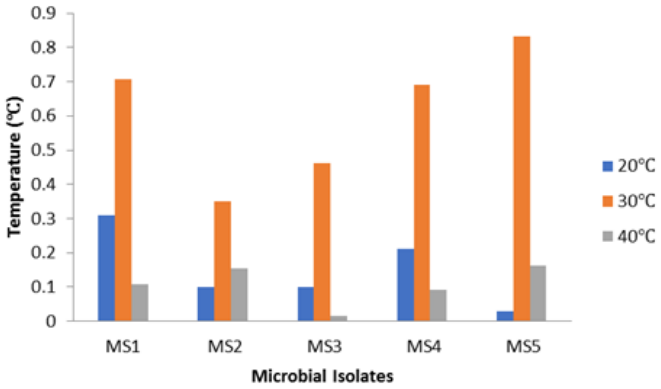

Figure 3.: Effect of different temperature values of selected microbial isolates incubated with chlorpyrifos at 10mgL^{-1}

Keys: MS3= *Staphylococcus spp,* MS5= *Aeromonas spp.*

Preliminary Chlorpyrifos Biodegradation Assessment

After optimization of pH and temperature at different parameters, the best growth of all the parameters was subjected to a preliminary bioremediation assessment and their potential to degrade chlorpyrifos at 10mgL^{-1} was investigated in MSB (Figure 4). After seven days of incubation at 37°C, pH 7, isolates MS5 showed optimal growth with high degradation potential with an optical density of (0.74) when compared with control with an optical density of 0.50 respectively. Isolate MS3 showed low growth and least degradation ability when compared. This shows that there is significant difference between isolates when compared with control at ($p < 0.05$).

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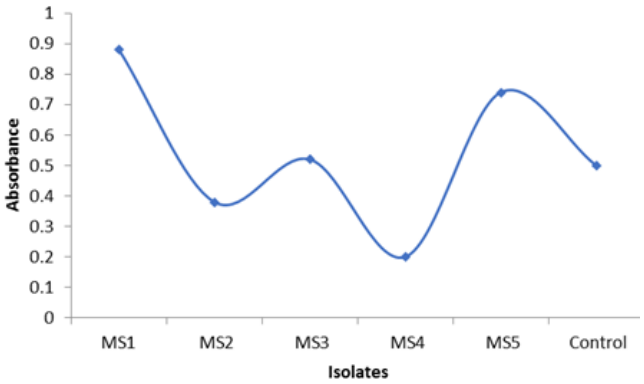

Figure 4: The preliminary biodegradation assessment

Keys: MS3= *Staphylococcus species,* MS5= *Aeromonas species*

Molecular Identification and Phylogenetic Analysis of the Isolates

Amplified Polymerase Chain Reaction (PCR) for 16S rRNA of the CP degrading bacterial strains are shown in Plate 1. Sequence was aligned with already available 16S rRNA genes in the National Centre for Biotechnology information (NCBI) database using Basic Local Alignment Search Tools (BLAST). The BLAST result for isolate MS5 showed sequence of the isolates belonged to *Aeromonas* species. Based on the information in the first ten hits of the BLAST results, all MS5 isolate showed the first ten hits had 96% similarity and were virtually *Aeromonas* species. To further confirm the position of all the isolated strains, the first ten hit sequences of the strains were selected from the BLAST results for the construction of the phylogenetic tree. The result of the phylogenetic tree is shown in Figure 5. Therefore, based on the morphological, biochemical and molecular analysis results, the isolate was confirmed to be *Aeromonas hydrophila* strain.

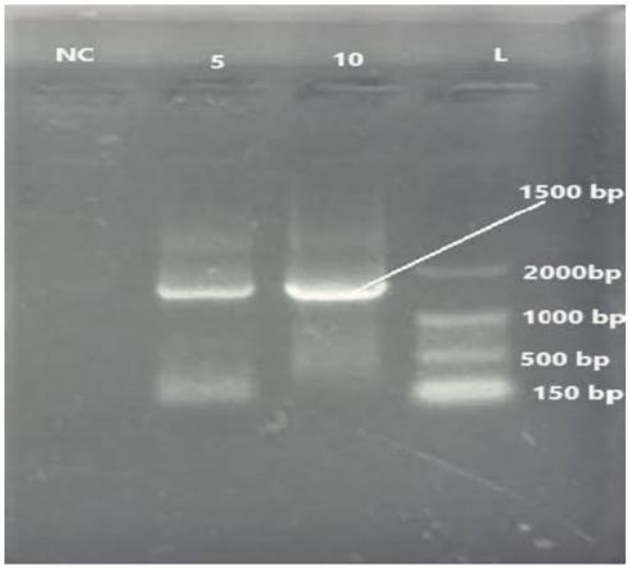

Plate 1: Gel electrophoretogram of the 16S rRNA for isolate MS5.

Keys: NC: Negative control, 5: *Aeromonas species,* L: Ladder (1500bp)

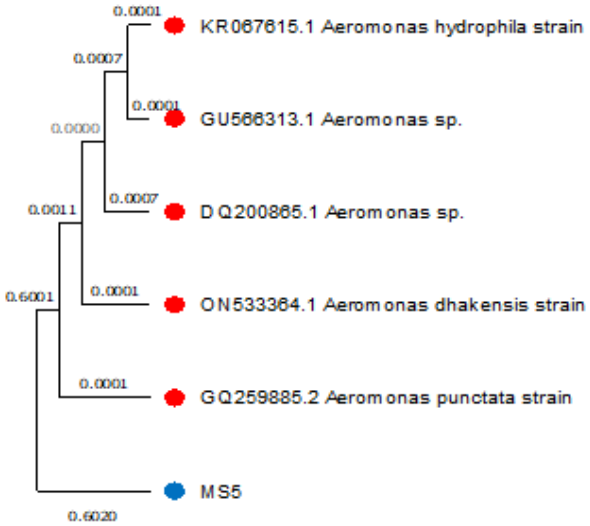

Figure 5 Phylogenetic tree of the isolated strain MS5.

Key: MS5- *Aeromonas hydrophila*

Biodegradation Study in Liquid Media

Isolate MS5 (*Aeromonas hydrophila*) had the highest degradation ability from the start of the treatment to day seven, there has been significant growth of all the isolates except at 20mgL^{-1} when compared with control. The highest growth was observed at a concentration of 30mgL^{1} with an optical density of 0.45 at 600 nm. From day seven to fourteen, there was an absolute increase in optical densities at all the concentrations when compared with control. Furthermore, from day fourteen to day twenty-eight, there was gradual decrease in optical densities in the medium which indicates that the bacterial strains are using chlorpyrifos as a source of carbon (Table 5). A post hoc test reveals that the 95 % confidence level for each treatment is statistically significantly different ($p < 0.05$).

Table 5: Spectrophotometric Degradation Assessment of Chlorpyrifos in Mineral Salt Broth inoculated with *Aeromonas* species at Different Concentrations

	Absorbance value			
Incubation time (Days)	**At 20 mg/L**	**At 30 mg/L**	**At 40 mg/L**	**Control**
0	0.06 ± 0.002	0.24 ± 0.015	0.43 ± 0.03	0.39 ± 0.022
7	0.05 ± 0.001	0.45 ± 0.04	0.13 ± 0.041	0.39 ± 0.004
14	0.13 ± 0.015	0.50 ± 0.036	0.29 ± 0.09	0.35 ± 0.02
21	0.12 ± 0.01	0.13 ± 0.021	0.23 ± 0.023	0.35 ± 0.018
28	0.11 ± 0.02	0.10 ± 0.007	0.20 ± 0.006	0.34 ± 0.00
Percentage (%) Degradation	12.63	73.6	29.6	

Values are presented as Mean ± SD. And $p \leq 0.05$ was considered statistically significant. While Tukey comparison test was used for post hoc analysis.

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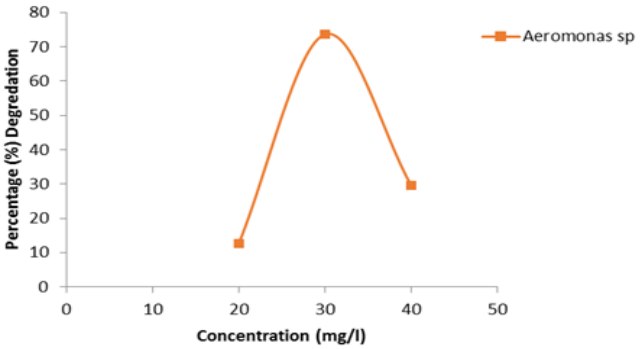

Figure 6: Percentage (%) degradation of microorganism at different concentrations

Investigation of Metabolites after Biodegradation in Liquid Media

After the biodegradation of chlorpyrifos in liquid media. The culture extract was subjected to GC-MS analysis. The GC-MS results showed peak at retention time (RT) 9.150-min representing metabolites. The peak was identified on the basis of its mass spectra and National Institute of Standards and Technology (NIST) library identification process. The peak at retention time corresponded to chlorpyrifos standard. Based on characteristics fragment ion peaks and molecular ion (m/z), the new peak was identified as TCP (Figure 8).

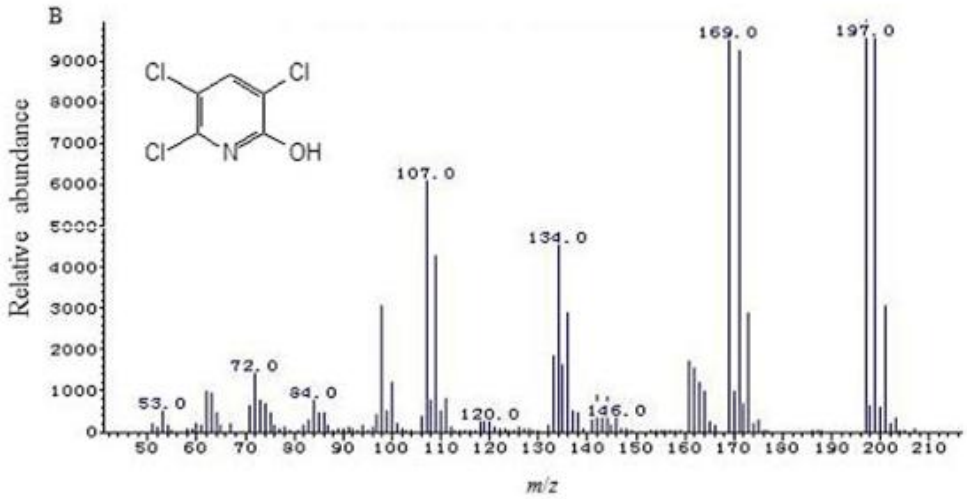

Figure 7: Mass spectra of GCMS analysis of 3,5,6-trichloro-2-pyridinol (TCP) produced from chlorpyrifos degradation by *Aeromonas* strain.

DISCUSSION

In this study, *Aeromonas hydrophila* was isolated from pesticide contaminated wastewater of Hayin Danmani and Nassarawa communities in Kaduna using microbiological methods; these isolates were confirmed by standard and molecular methods. The quantitative analysis shows the presence of five pesticides ethroprophos, chlorpyrifos, ronnel, disulfoton and phosphorodithioc acid. Chlorpyrifos was present in a high concentration ranging from 2.71 ± 0.11 - 131.8 ± 0.2. Similar studies conducted by Fawzy *et al.,* (2014) described chlorpyrifos as the most frequent detected pesticides from four agricultural drainage canals of Egypt. Physicochemical analysis result reveals the pH values of all the samples, electrical conductivity, sodium adsorption ratio, alkalinity and metals were all determined. There was variation in all the samples. According to the guidelines for assessment of irrigation water based on pH, SAR & EC, the acidity or basicity of irrigation water is expressed as pH (<7.0 acidic; >7.0 basic), the normal pH range for irrigation water is from 6.5 to 8 (Bauder *et al.,* 2004). pH of all the samples studied revealed that NA1 (6.78), NB1 (6.87), HA1 (6.81) and HB1 (6.86) were neutral respectively and according to the range. The optimum pH required for bacteria to thrive in wastewater depends on the type of water and soil. Furthermore, the concentrations of electrical conductivity, a measure of electric current which is greatly dependent on the availability of ionic species (Julian *et al*., 2018) and of metals shows that chlorine and calcium were the most abundant in all the samples analyzed.

For the enrichment and isolation of chlorpyrifos degraders, MSM were supplemented with 1% chlorpyrifos as the only carbon source, five morphological distinguishable colonies were observed on mineral salt agar from serially diluted samples that were poured and streaked on different agar plates after five to seven days of incubation at 37°C. Morphological, cultural and biochemical studies were carried out. From the result of gram staining and biochemical identification, it was confirmed that isolates MS5 was gram-negative bacteria while MS3 was gram positive. The isolates were identified as *Staphylococcus aureus* and *Aeromonas species* respectively according to Bergey's Manual of Systematic Bacteriology (Murray et *al*., 1995). The isolation of indigenous bacteria in the studied samples is a strong indication that the isolate might be a good candidate for bioremediation studies.

Notably, abiotic factors like pH and temperature greatly influence the capability of microorganisms to degrade such xenobiotic compounds and different bacterial strains possess varying optimal values of temperature and pH (Liu *et al*., 2013). pH is one of the two main important factors that influence the growth of microorganisms and also the degradation of xenobiotic compounds. Growth of isolates was studied for 7 days in sterilized

MSM supplemented with 10mg^{-1} chlorpyrifos maintained at pH (6-8) conditions. However, in present work bacterial isolates was able to grow at temperature range 20-40°C but 30°C was most suitable for bacterial growth. The quantitative estimation shows that there is gradual increase in the growth of all the isolates from 20°C and a decrease from 40°C in the media. Best growth was observed at 30°C with an optical density of (0.70, 0.34, 0.46, 0.69, and 0.83) nm respectively. Thus, all degradation experiments during present study were carried out 30°C. This is due to the fact that main enzymes responsible for chlorpyrifos degradation have their maximum activity at neutral pH whereas alkaline and acidic pH values impose inhibitory effects. Similar work was carried out by Saunders *et al*., (2012) who reported that growth of bacterial isolates at neutral pH was maximum whereas highly acidic and alkaline pH values impose effects. The quantitative estimation shows that there was a slight increase on the growth from pH 6 and decrease at pH 8. The best growth was observed at pH 7 in all the samples with an optical density (600 nm) of 0.71, 0.10, 0.07, 0.29 and 0.66 respectively after seven days of incubation. After optimization of pH and temperature at different parameters, the best growth of all parameters was subjected to a preliminary biodegradation assessment, and their potential to degrade chlorpyrifos at 10mgL^{-1} was investigated in MSM. After seven days of incubation at 37°C, pH 7, isolates showed optimal growth with degradation potential with an optical density of (0.88, 0.38, 0.52, 0.20 and 0.74). When compared with the control (0.52), isolate MS5 showed high growth with high degradability when compared with all other isolates. This shows that there is significant difference between isolates MS5 compared with the control at $p < 0.05$. The bacterial isolates that grew most rapidly and luxuriously displayed the highest chlorpyrifos degrading capability was selected and further confirmed by partial sequencing of the 16S rRNA gene and BLAST analysis, the BLAST results for the isolates MS5 of which showed 95 % similarity with the species belong to the *Aeromonas* species.

The degradation of chlorpyrifos at different concentrations (20, 30 and 40mgL^{-1}) was examined in MSB on rotary shaker at 150 rpm, 30°C and optimum pH 7 for 28 days. In the first 7 days, there is initial phase of slower degradation in the growth of all the isolates. There was also little degradation of chlorpyrifos in the uninoculated control. Furthermore, it was shown that microbial growth was at 30mgL^{-1} for *Aeromonas spp*. with an optical density of 0.45 ± 0.04. It was also observed that during 14th day of degradation, there was an absolute increase in optical densities at all the concentrations of the isolates. This also showed slow degradation capability. From day 14 to 28, there was drastic reduction in optical densities which resulted in a more rapid rate of chlorpyrifos degradation compared to uninoculated control. The gradual decrease in optical density in the medium supplemented with chlorpyrifos indicates that the bacterial strains is using chlorpyrifos as a source of carbon. According to Yadav *et al*. (2021), bacteria can degrade pollutants efficiently because they have catabolic genes that enable them to survive in different ecological niches in various pH, temperature, and oxygen concentrations. Table 5 shows the degradation rate of chlorpyrifos (30mgL^{-1}) as the most efficient at 71.6 %. 20mgL^{-1} and 40mgL^{-1} showed lower degradation potential as 12.63 % and 29.6 % for *Aeromonas* strain. In another study, it has been reported that 4 strains of *Pseudomonas* isolated from waste water irrigated Agri-soil in India, were able to utilize chlorpyrifos as an exclusive carbon source (Bhagobathy *et al.,* 2008). Also, the work El-Sharkawy *et al*., (2022) showed the degradation ability of *B. cereus* CP6 and *Klebsiella pneumoniae* CP19 isolated from wastewater sediment. The GC-MS results showed peak at retention time (RT) 9.150-min representing metabolite. Peak were identified on the basis of its mass spectra and NIST library identification process. The peak at retention time of 9.150 min corresponded to chlorpyrifos standard. The current results are better compared to those reported in some earlier studies, especially concerning the time needed for maximum degradation. For instance, Shao *et al*. (2015), reported 30.78 % degradation of CP within 14 days by three isolates. Also, the results are more significant than those obtained by Kumar (2011), in which 77 % of CP was degraded in 30 days. The findings corroborate previous studies that isolated bacterial communities with the potential to degrade CP to TCP in the soil and liquid culture. Examples of species isolated in prior studies include *Arthromobacter sp., Enterobacter* strain B-14, *Alcaligenes faecalis*, *Bacillus pumilus*, *Staphylococcus sp*., *Streptococcus sp*., *Achromobactor sp*., *Serratia marcescens*, *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas desmolyticum*, and *Pseudomonas aeruginosa* (Singh *et al*., 2004). These bacterial communities have been shown to catabolize and co-metabolize TCP and CP. Most organophosphate compounds are degraded by microorganisms in the environment as a source of phosphorus, carbon or both. Microbial degradation is an effective and efficient method for eliminating harmful compounds from the environment. Bacteria and fungi are the main transformers during bioremediation, the use of microorganisms to immobilize or degrade wastes, is a prominent process of eliminating them.

Conclusion

This study confirmed the chlorpyrifos-degrading microorganisms *Aeromonas hydrophila* isolated from contaminated agricultural wastewater at Nassarawa and Hayin Danmani communities, Kaduna State. The GC-MS result of the quantitative determination in this study has shown the presence of ronnel, ethroprophos, disulfoton, phosphorodithioc acid and chlorpyrifos which showed to be present in a very high concentration amongst other pesticides. Physicochemical characteristics of the water sample was identified. Bacteria were isolated and were morphologically and biochemically characterized. Screening of the isolates at different pH and temperature concentration indicates pH 7 and 37°C respectively as optimum. The preliminary chlorpyrifos biodegradation allowed the selection of bacterial species with degradation abilities after one week of incubation. The 16s rRNA gene sequence and phylogenetic tree results identified MS5 *Aeromonas species* respectively. The isolate was further tested at three different concentrations of 20, 30 and 40mgL^{-1}. It was found that the isolates showed high biodegradation efficiency of chlorpyrifos pesticide at 30mg^{-1} in *Aeromonas species* (71.6%) after 28 days of treatment. When provided with 20mgL^{-1} and 40mgL^{-1}, the pesticide showed minimum chlorpyrifos degradation in *Aeromonas species* (12.63%) (29.6%) by spectrophotometric analysis. After biodegradation, metabolite was detected by GCMS analysis which showed peak at retention time (RT) of 9.150-min representing metabolite. The two peak was identified on the basis of its mass spectra and NIST library identification process. The peak at retention time corresponded to chlorpyrifos standard. From the present investigations, the results obtained demonstrated the capability of the isolated strains with excellent potentials for future application in bioremediation of chlorpyrifos-contaminated environments.

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