UJMR, Special Conference Edition, Vol. 9 No. 3. June, 2024, pp.30 - 39
E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

https://doi.org/10.47430/ujmr.2493.005

Received: 3rd March, 2024 Accepted: 1st June, 2024

Optimization of Growth Response Parameters, Screening and Molecular Detection of Pesticide Degradation Genes in Bacterial Isolates from Agricultural Soils

[1]Anas, A.* and [2]Shamsudeen, U. and [2]Ibrahim, Y.
[1]Department of Microbiology, Gombe State University, Gombe, Nigeria.
[2]Department of Microbiology, Bayero University, Kano, Nigeria.
*Correspondence author: anasadams77@gmail.com , +234 803 235 7272

Abstract

Pesticides are organic compounds synthesized and used for pest control. The excessive and continuous dispersion of pesticides in the environment results in environmental pollution, necessitating remediation. This study investigated the potential of bacteria isolated from farmland soils in Kano Metropolis, Kano State, Nigeria, with a history of dichlorvos (2,2-dichlorovinyldimethylphosphate) and carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) application, to biodegrade these pesticides. Three sampling sites were involved in sample collection, and the soil physicochemical parameters from each sample were determined. Isolation, identification, and screening of the bacterial isolates capable of utilizing the pesticides as sole sources of carbon were carried out. The following parameters (concentration of the pesticides, pH, temperature, agitation, and incubation time) were optimized to maximize degradation. The potent bacterial isolates were further subjected to molecular analysis for the detection of opd and mcd genes. The pesticide-degrading bacteria were identified as Bacillus sp., Serratia sp., and Pseudomonas sp. Serratia sp. recorded the highest growth in the presence of 1% v/v dichlorvos, while Pseudomonas sp. exhibited maximum growth at a 1% w/v carbofuran concentration. The optimized conditions that yielded the maximum microbial growth are: 100 mg/L pesticide concentration for Serratia sp and 300 mg/L for Pseudomonas sp, a pH of 7.0 and an agitation level of 100 rpm for both organisms, a temperature of 35°C for Serratia sp and 30°C for Pseudomonas sp, and an incubation time of 5 days for both organisms. The opd and mcd genes were identified from Serratia sp. and Pseudomonas sp. respectively. These results suggest that the isolated bacteria have the potential to degrade dichlorvos and carbofuran pesticides from the contaminated soil.

Key words: Biodegradation, carbofuran, dichlorvos, optimization, pesticides, soil.

INTRODUCTION

The use of organophosphate and carbamate pesticides in agricultural activities has raised concerns not only due to their extensive use but also because of their higher toxicity to plants and animals (Chin-Pampillo et al., 2015). However, traditional methods for removing pesticide contamination have proven ineffective and costly due to their volatility and negative impact on the environment (Mohammed et al., 2019). Biodegradation has emerged as an efficient, cost-effective, and environmentally friendly technique, offering a potential alternative to conventional methods (Akbar and Sultan, 2016). This process relies on the ability of microorganisms to convert organic contaminants into simple and harmless compounds in the environment (Akbar and Sultan, 2016). Despite this, there is a lack of research on isolating and optimizing bacteria capable of degrading commonly used pesticides in the Kano region. Furthermore, the isolation and characterization of microorganisms that can degrade pesticides may provide us with new tools to remediate pesticide-contaminated environments or treat waste before final disposal.

MATERIALS AND METHODS

Sampling was conducted at three sites in Kano Metropolis, Kano State. The soil samples were collected from farmlands with a history of dichlorvos and carbofuran pesticide application at depths of 0 to 15 cm using an auger. The samples were air-dried at room temperature, passed through a 2 mm sieve to eliminate unwanted debris, and stored in polyethylene bags in a refrigerator for further experimental analysis (Lakshmi et al., 2015; Soni et al., 2015; Ameh and Kawo, 2017). Soil physicochemical parameters were determined (Beretta et al., 2014; Edori and Iyama, 2017; Lakshmi et al., 2015). The isolation and identification of bacteria were carried out using a modified enrichment culture technique with mineral salt medium (MSM) (Cheesbrough, 2006; Negi et al., 2014; Ravi and Aparna, 2019), followed by screening of the bacterial isolates to evaluate their potential for degrading pesticides (Moneke et al., 2010; Fenner et al., 2013). Additionally, molecular detection of biodegradation genes in the isolates was performed via agarose gel electrophoresis (Miyuki et al., 2013; Ghada et al., 2018; Solà et al., 2018) at the Microbiology Laboratory, Faculty of Life Sciences, Bayero University Kano, Nigeria, following standard methods.

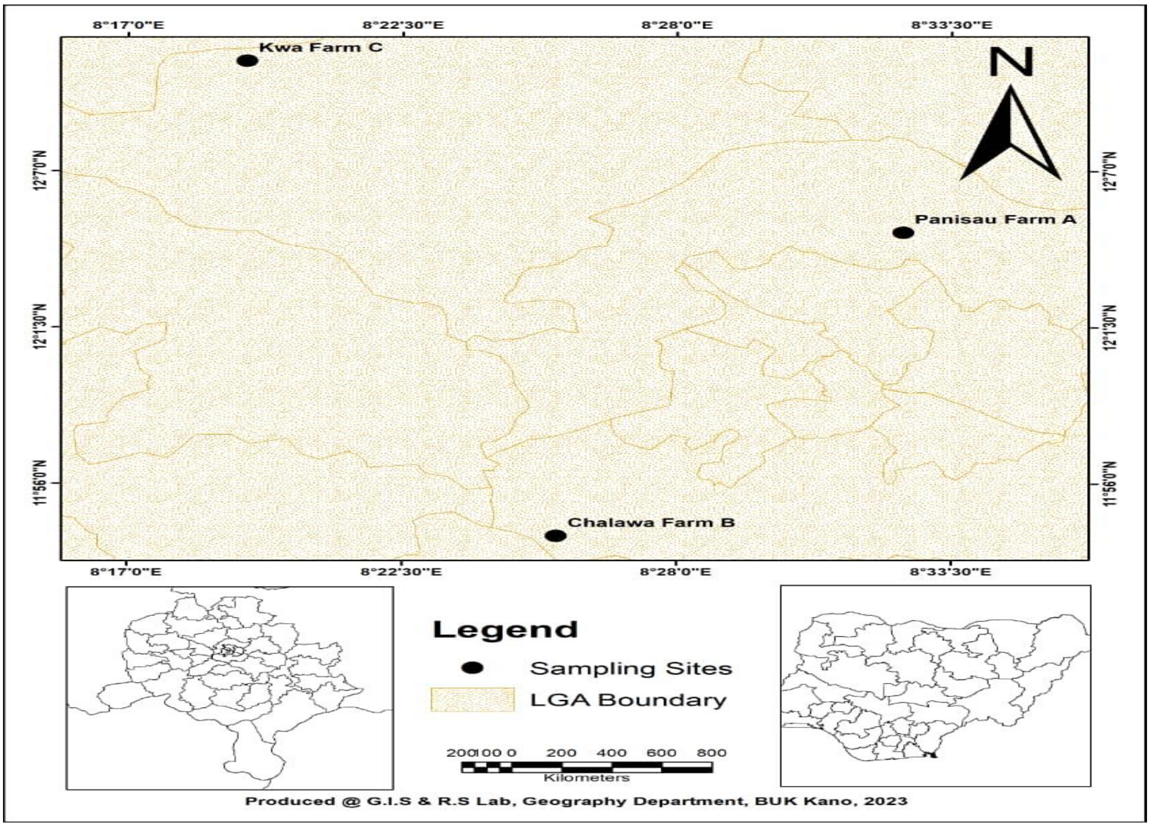

Figure 1: The Locations of the three Sampling Sites

RESULTS

Identification of Dichlorvos and Carbofuran Degrading Bacteria

The isolation was conducted using an enrichment culture technique with mineral salt medium (MSM) as the culture media. Bacterial isolates capable of utilizing Dichlorvos or Carbofuran pesticide as the sole carbon source for growth were obtained from the agricultural soil sample and were presumptively identified based on their morphological and biochemical characteristics, as shown in Table 2 and Table 3, respectively.

Screening for Bacterial Isolate with the Potential to Degrade Dichlorvos/ Carbofuran Pesticides

Figure 2 and 3 show the potential of dichlorvos and carbofuran pesticide-degrading bacteria. The screening was based on the pesticide degrading ability, where higher bacterial growth was achieved after seven days of incubation compared to the initial cell density. This indicates that all the organisms were able to utilize the pesticide as the sole source of carbon for energy and growth, but they varied significantly in their pesticide-degrading abilities. Serratia sp. exhibited higher tolerance to grow in dichlorvos-enriched medium, while Pseudomonas sp. showed higher growth in the carbofuran-enriched medium.

Optimization Studies

Dichlorvos and carbofuran were degraded by Serratia sp. and Pseudomonas sp., respectively, across all the concentrations tested. Higher concentrations resulted in a decreased growth response of Serratia sp. and Pseudomonas sp. The maximum cell density was observed at pesticide concentrations of 100mg/L and 300mg/L for Serratia sp. and Pseudomonas sp., respectively (Figure 4).

The effect of pH on the degradation of dichlorvos and carbofuran by Serratia sp and Pseudomonas sp respectively, is presented in Figure 5. Maximum degradation was observed at pH 7, whereas the least degradation was recorded at pH 9 by the two isolates.

The results for the effect of Temperature on degradation of dichlorvos and carbofuran by Serratia sp. and Pseudomonas sp. are presented in Figure 6. Maximum degradation of dichlorvos by Serratia sp. was recorded at 300C and 350C for carbofuran by Pseudomonas sp.

The maximum degradation was observed in both isolates at 100 rpm after the incubation period of five days. A decrease in degradation efficiency of Serratia sp. and Pseudomonas sp. was observed at higher agitation speeds, as shown in Figure. 7.

The effect of incubation time on the degradation of dichlorvos and carbofuran is depicted in Figure 8. The highest cell density was observed on day 5 for both Serratia sp. and Pseudomonas sp. A decrease in cell density was noted on day 6 of incubation for both strains as well.

Detection of Genes (opd and mcd) Responsible for Dichlorvos and Carbofuran Degradation

The potent bacterial isolates were further analyzed for the presence of the organophosphate-degrading gene (opd) and the methyl carbamate degrading gene (mcd). Amplification was observed with the primers for the opd and mcd genes, resulting in amplification products of approximately 327 bp and 168 bp from Serratia sp. and Pseudomonas sp., respectively. These results were confirmed after gel electrophoresis (Figure 9), indicating the presence of opd and mcd genes in Serratia sp. and Pseudomonas sp., respectively.

Table 1: Soil physicochemical properties of maize (*Zea mays*) farmland

S/N	Parameters	Mean values for soil physicochemical parameters from the three sampling sites			*P-value*
		A	B	C	
1.	Temperature (^{o}C)	42.03±0.46^{a}	40.13±0.25^{a}	40.07±0.34^{a}	0.918
2.	pH	6.447±0.09^{a}	6.477±0.03^{a}	6.227±0.11^{a}	0.914
3.	EC (dS/m)	0.107±0.00^{a}	0.112±0.01^{a}	0.064±0.01^{a}	0.634
4.	OC (%)	0.281±0.04^{a}	0.808±0.03^{a}	0.413±0.03^{a}	0.324
5.	P (mg/kg)	5.466±0.12^{a}	8.383±0.27^{a}	8.072±0.10^{a}	0.926
6.	N (%)	0.117±0.02^{a}	0.152±0.02^{a}	0.175±0.03^{a}	0.161
7.	Ca (cmol/kg)	2.352±0.01^{a}	2.115±0.00^{a}	3.946±0.01^{b}	0.024
8.	Mg (cmol/kg)	0.532±0.03^{a}	0.669±0.02^{b}	0.695±0.08^{b}	0.041
9.	K (cmol/kg)	0.150±0.02^{a}	0.249±0.03^{b}	0.211±0.00^{b}	0.049
10.	Na (cmol/kg)	0.074±0.01^{a}	0.176±0.05^{b}	0.34±0.05^{b}	0.034
11.	% Sand	79.33±2.49^{a}	78.00±1.63^{a}	74.67±0.94^{a}	0.965
12.	% Silt	11.00±1.63^{a}	12.33±0.94^{a}	11.67±0.94^{a}	0.905
13.	% Clay	10.67±0.94^{a}	10.67±0.94^{a}	14.67±0.94^{a}	0.845
14.	Textural class	Loamysand	Loamysand	Loamysand	

Key: EC - Electrical Conductivity, OC - Organic Carbon, P - Phosphorus, N - Nitrogen, Ca - Calcium, Mg - Magnesium, K - Potassium, Na - Sodium

Similar superscripts across the same row indicate no significance difference in the tested parameter across the three sampling sites (p ≥ 0.05). Dissimilar superscripts across the same row indicate a significance difference in the parameter across the three sampling sites (p = ≤ 0.05), when compared using Two-way ANOVA.

Table 2: Morphological and biochemical characteristics of the Dichlorvos degrading bacteria.

CODE	GR	Mor	I	C	O	U	CI	MR	VP	H_2S	MT	Inf.
SDA	+ve	Rod	-	+	+	-	+	-	+	-	+	*Bacillus* sp1
SDB	-ve	Short-rod	+	+	-	+	+	-	+	-	-	*Serratia* sp
SDC	+ve	Rod	-	+	+	-	+	-	+	-	+	*Bacillus* sp2

Key: GR - Gram reaction, Mor - Morphology, I - indole, C - catalase, O - oxidase, U - urease, CI - citrate, MR - methyl red, VP - voges proskeaur, H2S - hydrogen sulphide, MT - motility and Inf - inference.

Table 3: Morphological and biochemical characteristics of Carbofuran degrading bacteria

CODE	GR	Mor	I	C	O	U	CI	MR	VP	H_2S	MT	Inf.
SCA	-ve	Rod	-	+	+	+	-	-	-	-	+	*Pseudomonas* sp
SCB	+ve	Rod	-	+	+	-	+	-	+	-	+	*Bacillus* sp

Key: GR - Gram reaction, Mor - Morphology, I - indole, C - catalase, O - oxidase, U - urease, CI - citrate, MR - methyl red, VP - voges proskeaur, H2S - hydrogen sulphide, MT - motility and Inf - inference.

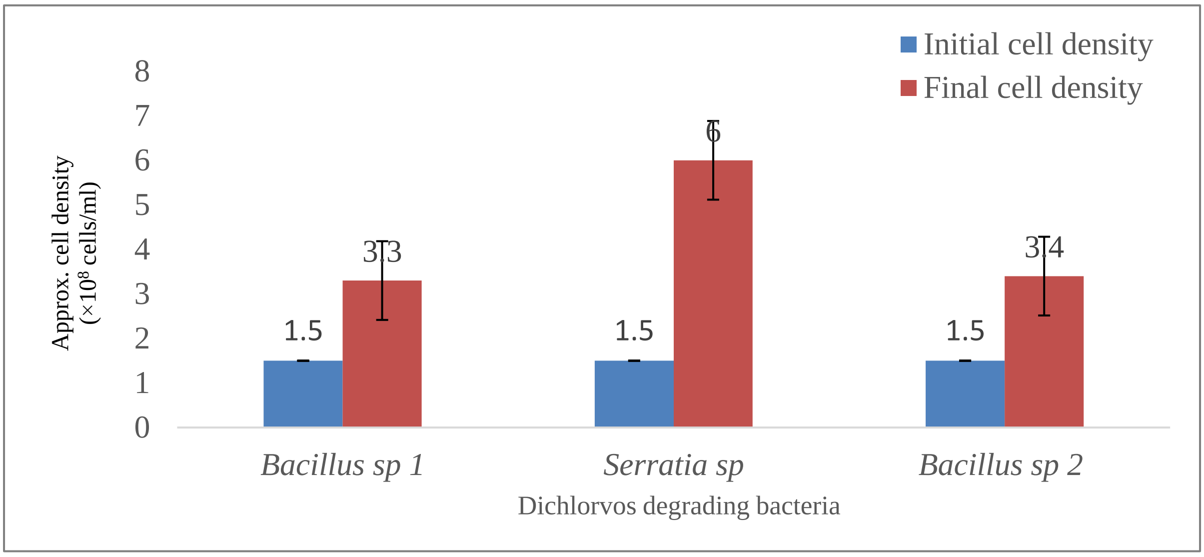

Figure 2: Tolerance to dichlorvos by bacterial isolates from pesticides-contaminated farmlands

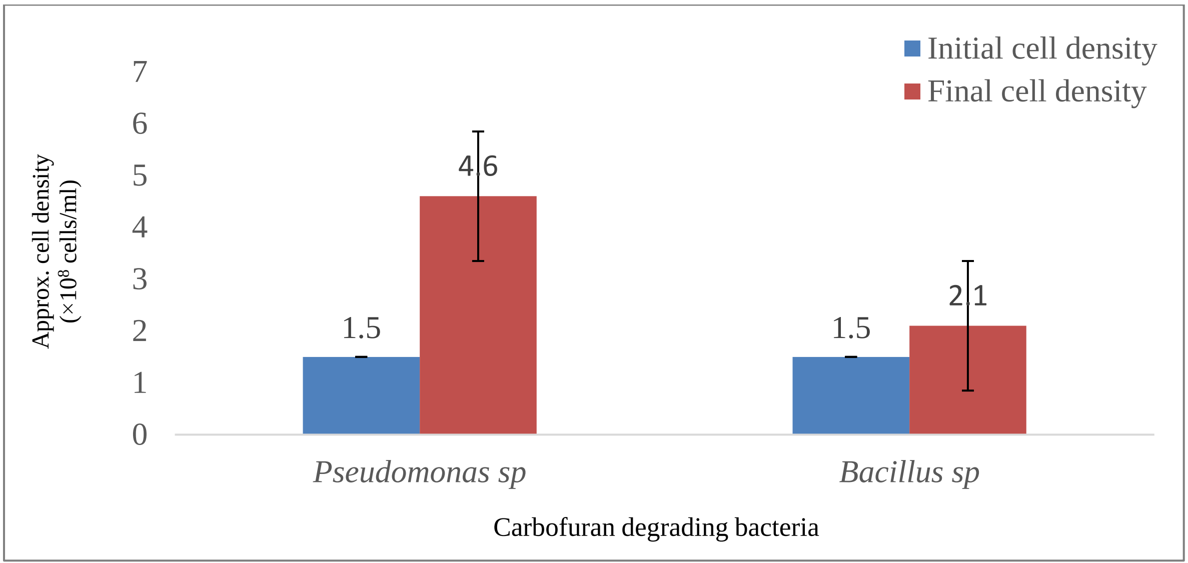

Figure 3: Tolerance to carbofuran by bacterial isolates from pesticides-contaminated farmlands

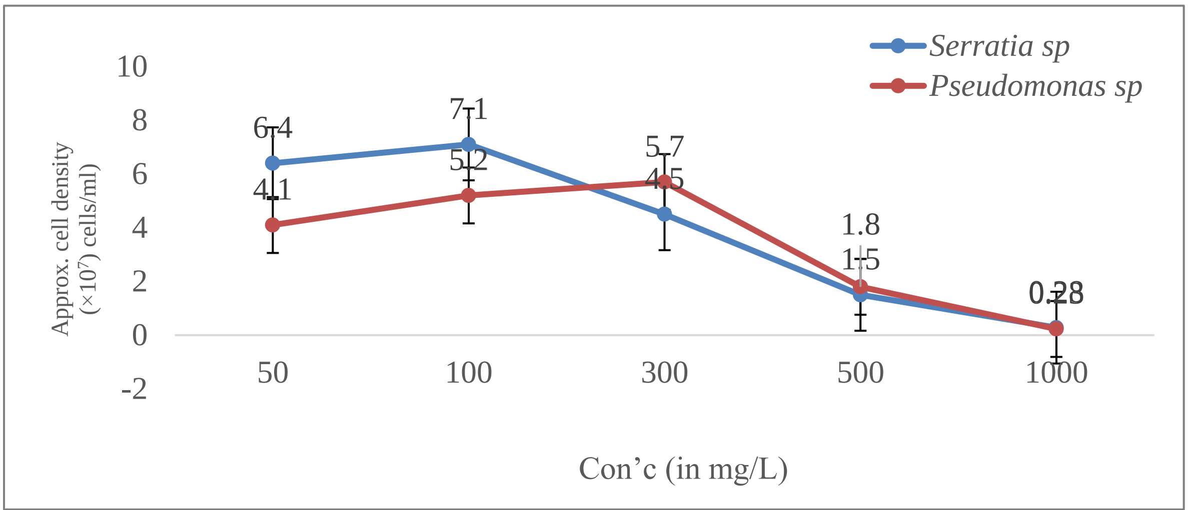

Figure 4: Effect of concentration on the degradation of dichlorvos and carbofuran by *Serratia* sp and *Pseudomonas* sp respectively after five days of incubation

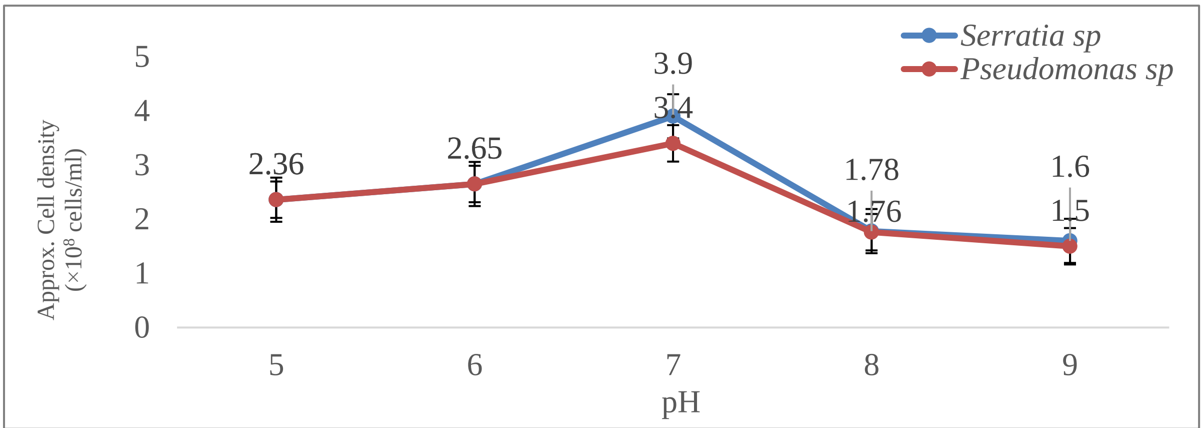

Figure 5: Effect of pH on the degradation of dichlorvos and carbofuran by *Serratia* sp. and *Pseudomonas* sp. respectively after five days of incubation

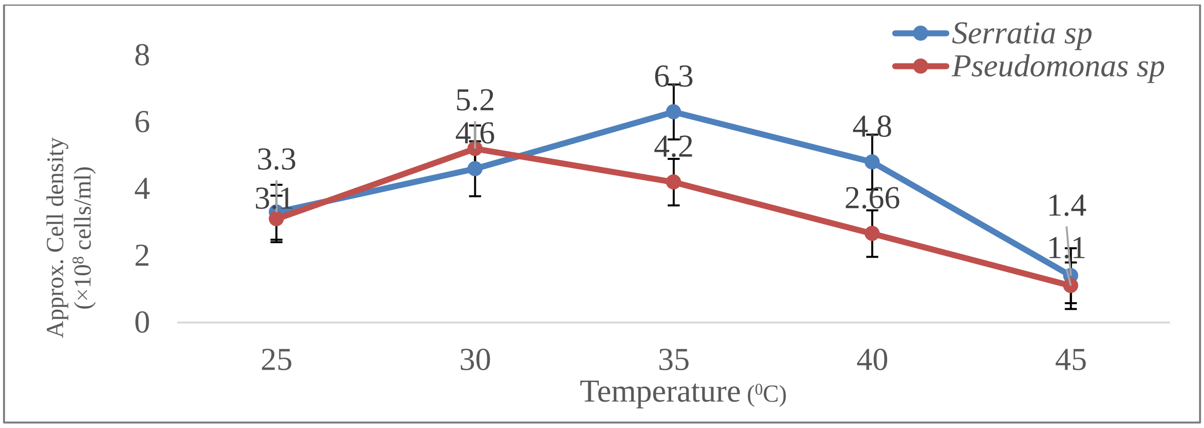

Figure 6: Effect of Temperature on the degradation of dichlorvos and carbofuran by *Serratia* sp. and *Pseudomonas* sp. respectively after five days of incubation

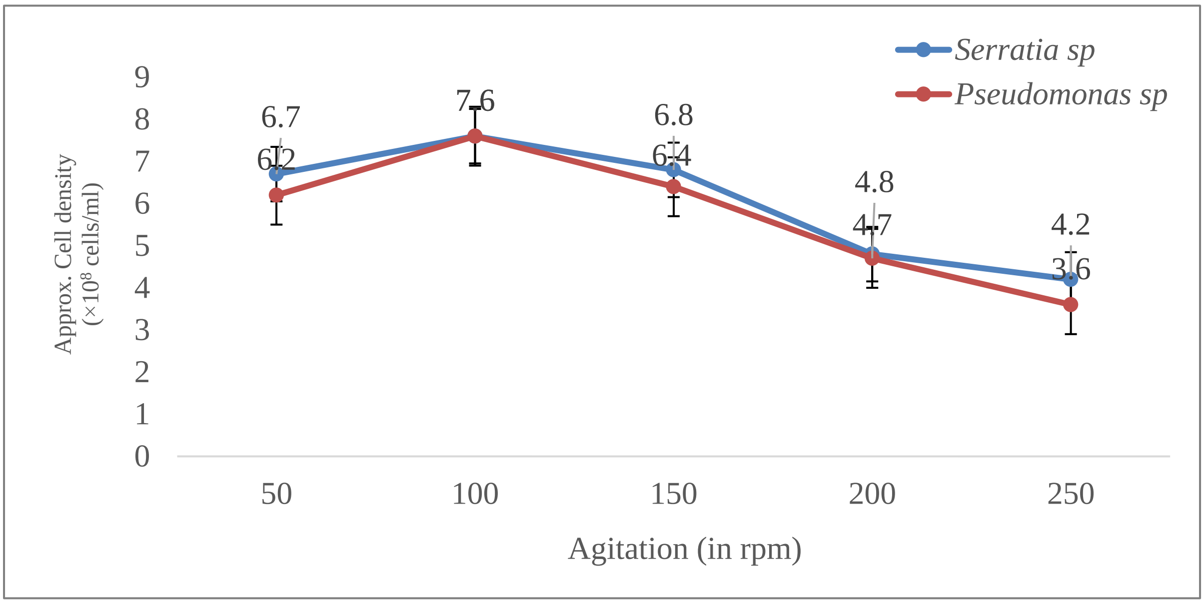

Figure 7: Effect of agitation on the degradation of dichlorvos and carbofuran by *Serratia* sp. and *Pseudomonas* sp. respectively after five days of incubation.

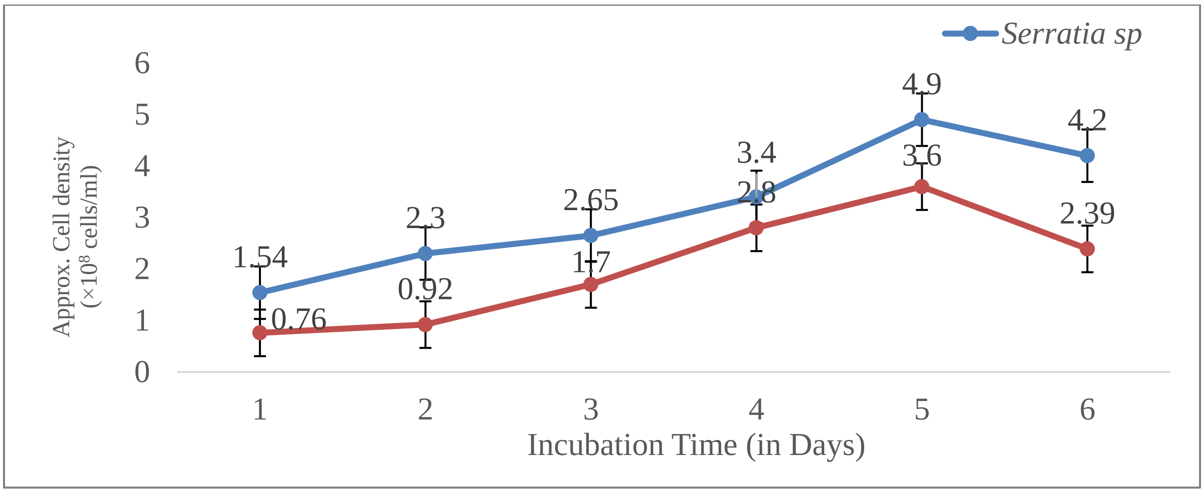

Figure 8: Effect of incubation time on the degradation of dichlorvos and carbofuran by *Serratia* sp. and *Pseudomonas* sp. respectively.

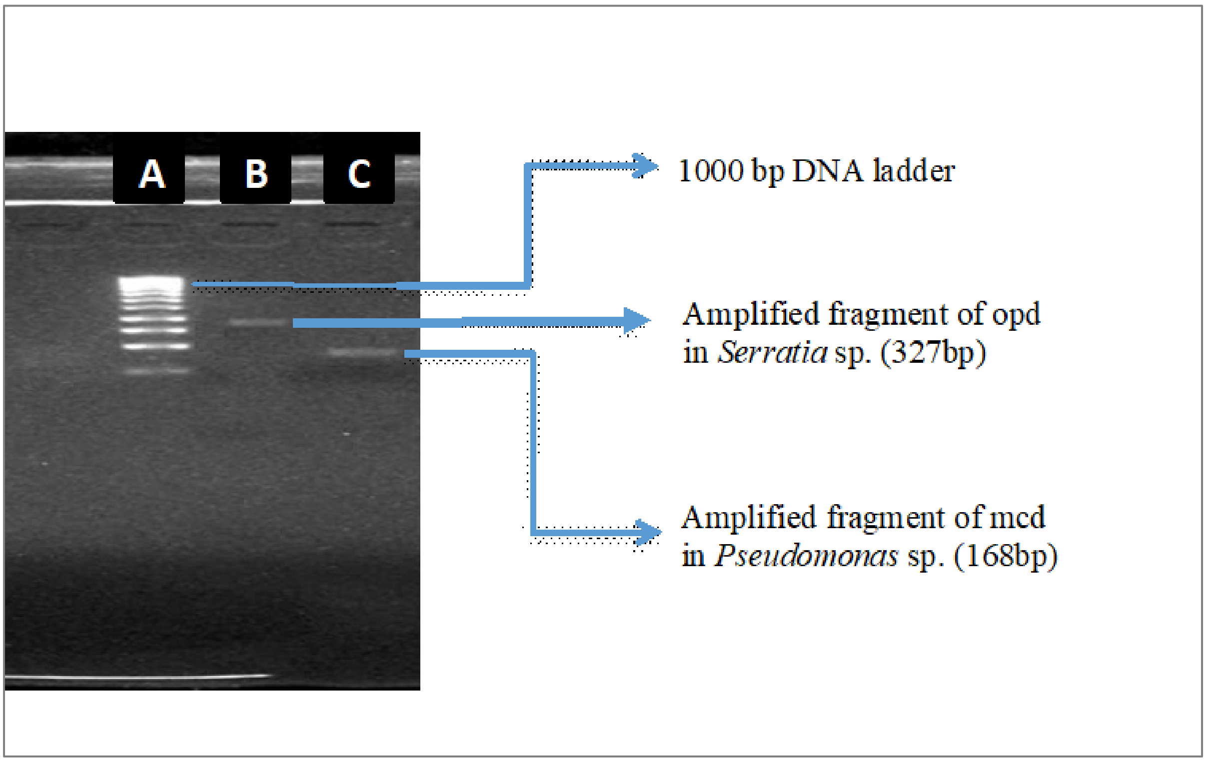

Figure 9: Gel electrophoresis imaging of the PCR products after amplification of the opd and mcd genes

Legend:

Lane A	DNA ladder
Lane B	Amplified fragment of opd gene
Lane C	Amplified fragment of mcd gene

DISCUSSION

The variations in the soil physicochemical analysis values among the three sampling sites could be attributed to the differences in geographical locations and soil types, such as loamy sandy soil, as documented by Edori and Iyama, (2017). The average soil temperature values across the sampling sites ranged from 40.07°C to 42.03°C. Soil temperature is influenced by the absorptive properties of the soil, representing the ratio of energy absorbed to energy emitted by the soil. Edori and Iyama, (2017) highlighted that soil temperature plays a crucial role in regulating seed germination, plant growth, and microbial activity, with variations observed across seasons. The mean soil pH values for the sampling sites were 6.45, 6.48, and 6.23, indicating a slightly acidic nature of the soil. This finding contrasts with observations in dumpsites in Zaria and Enugu, Nigeria, as reported by Uba et al., (2008) and Obasi et al., (2012) respectively. According to Arias et al., (2005), soil pH levels significantly affect nutrient availability to plants and the diversity of soil organisms. The average soil organic carbon values for the sampling sites were 0.281, 0.808, and 0.413, obtained through the decomposition of plants, animals, and anthropogenic sources like organic contaminants, fertilizers, or organic-rich waste (Avramidis et al., 2015). Similar results were reported by Edori and Iyama, (2017) from abattoir soil samples. Phosphorus concentrations across the sites were 5.466±0.12, 8.383±0.27, and 8.072±0.10 respectively, with the higher value in site B possibly due to excessive phosphorus fertilizer use. Phosphorus is a vital macronutrient essential for plant growth and metabolism, supporting microbial activities in the soil, as noted by Wagh et al. (2013). Potassium concentrations ranged from 0.150 to 0.249 cmol/kg across the sites, with potassium playing a key role in nutrient use efficiency and sustainable agriculture. Potassium-solubilizing microorganisms contribute to the solubilization of fixed potassium, aligning with the findings of Muhibbullah et al., (2005).

Dichlorvos and carbofuran-degrading bacteria were isolated from agricultural soil, indicating their presence in farmland with a history of pesticide application, as previously reported by Agarry et al., (2013) and Mohammed et al., (2019). The degradation of Dichlorvos pesticide after seven days of incubation was evaluated, with Serratia sp. showing higher degrading ability than Bacillus sp 1 and Bacillus sp 2 (Figure 2). This difference in degradation ability may be attributed to variations in their metabolic pathways. Agarry et al., (2013) isolated Proteus vulgaris, Acinetobacter sp, Serratia sp, and Vibrio sp, capable of utilizing organophosphate pesticide (Dichlorvos) as the sole carbon source for growth. In the case of carbofuran degradation, Pseudomonas sp. exhibited greater degrading ability than Bacillus sp (Figure 3), possibly due to differences in metabolic pathways. Kevin et al., (2012) also identified Pseudomonas sp. and Alcaligenes as the most potent isolates for the degradation of carbofuran pesticide.

The optimization studies for the biodegradation of dichlorvos revealed that lower concentrations supported bacterial growth, while increased concentrations could be toxic to the bacterium (Figure 4). The cells and enzyme systems may have been hindered by the increasing concentration of the pesticide, resulting in reduced biodegradation. Ning et al. (2012) also found that increased dichlorvos concentration impeded the degradation rate by Flavobacterium YD4. Concerning the impact of pH and temperature, it was determined that pH 7 (Figure 5) was optimal for both isolates, with temperatures of 30°C and 35°C (Figure 6) being ideal for the degradation of Dichlorvos and Carbofuran by Serratia sp. and Pseudomonas sp., respectively. Parte et al., 2019) reported that optimizing pH and temperature could positively affect the bioremediation rate. Deshpande et al., (2004) studied the degradation of the organophosphate (OP) compound dimethoate by Brevundimonas sp. MCM B-427 and noted a significant increase in biodegradation with the optimization of temperature and pH. High degradation efficiency was observed at 100 rpm in Serratia sp. and Pseudomonas sp. A decrease in degradation efficiency in both isolates was noted at higher agitation speeds, as shown in Figure 7. This decrease could be attributed to reduced contact between the pesticide and the culture, or the bacteria's inability to tolerate high levels of oxygen in the medium, as reported by Vijayalakshmi and Usha, (2012). An exponential increase in the degradation efficiency of dichlorvos and carbofuran by Serratia sp. and Pseudomonas sp., respectively, was observed up to Day 5 of incubation (Figure 8). Subsequently, a decline in cell density was noted from Day 6, possibly due to the depletion of the pesticide in the medium, which served as the sole carbon source.

The potent bacterial isolates were found to harbor the organophosphate-degrading gene (opd) and methyl carbamate degrading gene (mcd). These results were supported by previous research by Qiu et al., (2006) and Constantina et al. (2017), confirming that Serratia sp. and Pseudomonas sp. possess the opd and mcd genes, respectively.

CONCLUSION

This study established that Serratia sp. and Pseudomonas sp. can thrive in pesticide-enriched media, with optimal growth observed at 100 mg/L pesticide concentration for Serratia sp. and 300 mg/L for Pseudomonas sp. Both organisms exhibited optimal growth at a pH of 7.0 and an agitation level of 100 rpm. Serratia sp. thrived at a temperature of 35°C, while Pseudomonas sp. demonstrated optimal growth at 30°C. Additionally, both organisms showed peak efficiency after 5 days of incubation, suggesting their potential use in bioremediation efforts for soils and water contaminated by dichlorvos and carbofuran pesticides.

RECOMMENDATIONS

i. Further research is recommended to explore the capacity of the isolates for degrading dichlorvos and carbofuran pesticides, including determining the percentage of degradation, degradation pathways, and other relevant factors.

ii. Further research is recommended to explore the capacity of these isolates for bioremediation in the field, allowing for effective degradation by a genetically diverse consortium of bacteria with versatile metabolism.

iii. It is also recommended that governments should regulate or ban the use of highly toxic and least biodegradable pesticides in agriculture.

REFERENCES

Agarry, S.E., Olu-Arotiowa, O.A. Aremu, M.O. and Jimoda, L.A. (2013). Biodegradation of dichlorovos (organophosphate pesticide) in soil by bacterial isolates. Journal of Natural Sciences Research. 3:8

Akbar, S., and Sultan, S. (2016). Soil Bacteria showing a potential of chlorpyrifos degradation and plant growth enhancement. Brazilian Journal of Microbiology; 47, 563-570. [Crossref]

Ameh, A. A. and Kawo, A. H. (2017). Enumeration, isolation and identification of bacteria and fungi from soil contaminated with petroleum products using layer chicken droppings as an amendment. Bayero Journal of Pure and Applied Sciences, 10, (1) 219 - 225. [Crossref]

Arias, M.E., Gonzalez-Perez, J.A., Gonzalez-Villa, F.J and Ball, A.S (2005). Soil health: A new challenge for microbiologist and chemists. International Journal of Microbiology. 8, 13-21

Avramidisa, P., Nikolaoua, K., and Bekiarib V. (2015). Total organic carbon and total nitrogen in sediments and soils: A comparison of the wet oxidation-titration method with the combustion-infrared method. Agriculture and Agricultural Science Procedia. 4, 425-430. [Crossref]

Beretta, A.N., Silbermann, L., Paladino, D., Torres, D., Bassahun, R., Musselli, A., and García, L. (2014). Soil texture analyses by hydrometer: modifications of the Bouyoucos method. Ciencia e Investigacion Agraria. 41, (2): 263-271. [Crossref]

Cheesbrough, M. (2006). District laboratory practice in tropical countries. Second Edition (2). Cambridge University Press. [Crossref]

Chin-Pampillo, J. S., Ruiz-Hidalgo, K., Masís-Mora, M., Carazo-Rojas, E., and Rodríguez-Rodríguez, C. E. (2015). Adaptation of biomixtures for carbofuran degradation in on-farm biopurification systems in tropical regions. Environmental Science and Pollution Research. 22, (13) 9839-9848. [Crossref]

Constantina, R., Dionysis, K., Despoina, M., Evangelos, K., Panagiotis, A.K., Maria, T., Emmanuel, A.T. and Dimitrios, G.K. (2017). Distribution and function of carbamate hydrolase genes cehA and mcd in soils: the distinct role of soil pH. FEMS Microbiology Ecology, 93, 219. https://doi.org/10.1093/femsec/fiw219

Deshpande, N.M., Sarnaik, S.S., Paranjpe, S.A., and Kanekar, P.P. (2004). Optimization of dimethoate degradation by Brevundimonas sp. MCM B-427 using factorial design: studies on interactive effects of environmental factors. World Journal of Microbiology and Biotechnology; 20, 455-462. [Crossref]

Edori, O. S and Iyama W. A (2017). Assessment of physicochemical parameters of soils from selected abattoirs in Port Harcourt, Rivers State, Nigeria. Journal of Environmental Analytical Chemistry; 4, 194. [Crossref]

Fenner, K., Canonica, S., Wackett, L.P., and Elsner, M. (2013). Evaluating Pesticide Degradation in the Environment: Blind Spots and Emerging Opportunities. Science; 341, 752-758. [Crossref]

Ghada, M. E., Nivien, A. A., Ibrahim, S. A., AbdEl-Razik, A. B., Hammad, M. A. and Fatma, M. H. (2018). Identification of gene encoding organophosphorus hydrolase (oph) enzyme in potent organophosphates-degrading bacterial isolates. Journal of Environmental Science and Technology. 11, (4): 175-189. [Crossref]

Kevin, M.O., Gabriel, M., Kamau, N., and Tsanuo, M. (2012). Characterization of methomyl and carbofuran degrading-bacteria from soils of horticultural farms in Rift Valley and Central Kenya. African Journal of Environmental Science and Technology; 6 (2), 104-114. [Crossref]

Lakshmi, K.B., Madhuri, T., Indrani, V., and Suvarnalatha, D.P. (2015). "Effect of triazophos-an organophosphate insecticide on microbial population in paddy soils". International Journal of Current Research and Review. 7, (4) 63-67.

Miyuki, F., Yoshimitsu, O., and Chowdhury, R.A. (2013). A novel single-step multiplex polymerase chain reaction assay for the detection of diarrheagenic Escherichia coli. Journal of Microbiological Methods; 92, (3) 289-292. [Crossref]

Mohammed, U.M., Normala, H., Wan, Lutfi, W.J., and Mohd, Y.A. (2019). An overview on biodegradation of carbamate pesticides by soil bacteria. Pertanika Journal of Science and Technology. 27, (2) 547 - 563.

Moneke, A.N., Okpala, G.N., and Anyanwu, C.U. (2010). Biodegradation of glyphosate herbicide in vitro using bacterial isolates from four rice fields. African Journal of Biotechnology. 9, (26) 4067-4074.

Muhibbullah, M.D., Nurul-Amin, S.M. and Chowdhury, A.T. (2005). Some physicochemical parameters of soil and water of Sundarban mangrove forest, Bangladesh. Journal of Biological Sciences., 5, (3) 354 - 357. [Crossref]

Negi, G., Pankaj-Srivastava, A., and Sharma, A. (2014). In situ biodegradation of endosulfan, imidacloprid, and carbendazim using indigenous bacterial cultures of agriculture fields of Uttarakhand, India. International Journal of Food, Vaterinary and Agricultural Engineering; 8, (9):935-943.

Ning, J., Gang, G., Bai, Z., Hu, Q., Qi, H., and Ma, A, (2012). In situ enhanced bioremediation dichlorvos by a phyllosphere Flavobacterium strain. Frontiers of Environmental Science and Engineering; 6, 231- 237. [Crossref]

Obasi, N.A., Akubugwo, E.I., Ugbogu, O.C. and Otuchristian, G. (2012). Assessment of physicochemical properties and heavy metals bioavailability in dumpsites along Enugu-Port Harcourt expressway, South East, Nigeria. Asian Journal of Applied Sciences; 5, 342- 356. [Crossref]

Parte, S.G., and Kharat, A.S (2019). Aerobic degradation of clothianidin to 2-chloro-methyl thiazole and methyl 3-(thiazoleyl) methyl guanidine produced by Pseudomonas stutzeri smk. Journal of Environmental and Public Health; 2019:4807913. [Crossref]

Qiu, X. H., Bai, W. Q., Zhong, Q. Z., Li, M., He, F. Q., and Li, B. T. (2006). Isolation and characterization of a bacterial strain of the genus Ochrobactrum with methyl parathion mineralizing activity. Journal of Applied Microbiology; 101, 986-994. [Crossref]

Ravi, P., and Aparna, Y. (2019). Biodegradation of Organophosphorous Pesticide Dichlorvos by bacteria isolated from field sample. International Journal of Scientific Research in Biological Sciences. 6, 41-45. DOI: [Crossref]

Solà, M., Riudavets, J., and Agustí, N. (2018). Detection and Identification of Five Common Internal Grain Insect Pests by Multiplex PCR. Food Control; 84, :246-254. [Crossref]

Soni, Y., Sitansu, K.V., and Hotam, S.C. (2015). Isolation and characterization of Organophosphate pesticides degrading bacteria from contaminated agricultural soil. Online Journal of Biological Sciences. 15, (1):113-125. [Crossref]

Uba, S., Uzairu, A., Harrison, G.F.S., Balarabe, M.L. and Okunolo, O.J (2008). Assessment of heavy metals bioavailability in dumpsites of Zaria metropolis, Nigeria. African Journal of Biotechnology; 7, 122-130.

Vijayalakshmi, P. and Usha, M.S. (2012). Optimization of chlorpyrifos degradation by Pseudomonas putida. Department of Microbiology, Centre for post graduate studies, Jain University, Bangalore-11, Karnataka, India. Journal of Chemical and Pharmaceutical Research; 4, (5) 2532-2539

Wagh, G.S., Chavhan, D.M. and Sayyed, M.R.G (2013). Physicochemical analysis of soils from eastern part of pune city. Universal Journal of Environmental Research and Technology. 3, (1) 93-99