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

Anas, A.* and Shamsudeen, U. and Ibrahim, Y.

1Department of Microbiology, Gombe State University, Gombe, Nigeria.
2Department of Microbiology, Bayero University, Kano, Nigeria.
*Correspondence author: anasadams77@gmail.com, +234 803 235 7272

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.

**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 300°C and 350°C 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

<table>
<thead>
<tr>
<th>S/N</th>
<th>Parameters</th>
<th>Mean values for soil physicochemical parameters from the three sampling sites</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>1.</td>
<td>Temperature (°C)</td>
<td>42.03±0.46</td>
<td>40.13±0.25</td>
</tr>
<tr>
<td>2.</td>
<td>pH</td>
<td>6.447±0.09</td>
<td>6.477±0.03</td>
</tr>
<tr>
<td>3.</td>
<td>EC (dS/m)</td>
<td>0.107±0.00</td>
<td>0.112±0.01</td>
</tr>
<tr>
<td>4.</td>
<td>OC (%)</td>
<td>0.281±0.04</td>
<td>0.808±0.03</td>
</tr>
<tr>
<td>5.</td>
<td>P (mg/kg)</td>
<td>5.466±0.12</td>
<td>8.383±0.27</td>
</tr>
<tr>
<td>6.</td>
<td>N (%)</td>
<td>0.117±0.02</td>
<td>0.152±0.02</td>
</tr>
<tr>
<td>7.</td>
<td>Ca (cmol/kg)</td>
<td>2.352±0.01</td>
<td>2.115±0.00</td>
</tr>
<tr>
<td>8.</td>
<td>Mg (cmol/kg)</td>
<td>0.532±0.03</td>
<td>0.669±0.02</td>
</tr>
<tr>
<td>9.</td>
<td>K (cmol/kg)</td>
<td>0.150±0.02</td>
<td>0.249±0.03</td>
</tr>
<tr>
<td>10.</td>
<td>Na (cmol/kg)</td>
<td>0.074±0.01</td>
<td>0.176±0.05</td>
</tr>
<tr>
<td>11.</td>
<td>% Sand</td>
<td>79.33±2.49</td>
<td>78.00±1.63</td>
</tr>
<tr>
<td>12.</td>
<td>% Silt</td>
<td>11.00±1.63</td>
<td>12.33±0.94</td>
</tr>
<tr>
<td>13.</td>
<td>% Clay</td>
<td>10.67±0.94</td>
<td>10.67±0.94</td>
</tr>
<tr>
<td>14.</td>
<td>Textural class</td>
<td>Loamysand</td>
<td>Loamysand</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>CODE</th>
<th>GR</th>
<th>Mor I</th>
<th>C</th>
<th>O</th>
<th>U</th>
<th>CI</th>
<th>MR</th>
<th>VP</th>
<th>H₂S</th>
<th>MT</th>
<th>Inf.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDA</td>
<td>+ve</td>
<td>Rod</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Bacillus sp1</td>
</tr>
<tr>
<td>SDB</td>
<td>-ve</td>
<td>Short-rod</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Serratia sp</td>
</tr>
<tr>
<td>SDC</td>
<td>+ve</td>
<td>Rod</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Bacillus sp2</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>CODE</th>
<th>GR</th>
<th>Mor I</th>
<th>C</th>
<th>O</th>
<th>U</th>
<th>CI</th>
<th>MR</th>
<th>VP</th>
<th>H₂S</th>
<th>MT</th>
<th>Inf.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCA</td>
<td>-ve</td>
<td>Rod</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Pseudomonas sp</td>
</tr>
<tr>
<td>SCB</td>
<td>+ve</td>
<td>Rod</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Bacillus sp</td>
</tr>
</tbody>
</table>

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.
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 degrading 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 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

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]


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]


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