Trop J Nat Prod Res, March 2024; 8(3):6686-6692 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Biodegradative Activities of Some Indigenous Farm Soil Bacteria on Selected Pesticides

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ARTICLE INFO

Article history:
Received 23 November 2023
Revised 06 March 2024
Accepted 12 March 2024
Published online 01 April 2024

ABSTRACT

Pesticides play a pivotal role in contemporary agriculture, serving as vital tools for safeguarding crops against pest infestations and diseases. Nevertheless, the widespread application of pesticides has raised concerns due to their potential unintended consequences on the environment and human health. This study investigates the variability in bacterial populations in farm soil and their pesticide-degrading potentials. The isolates were characterized using different conventional and molecular methods. The bacterial isolates were tested against organophosphorus pesticides using quantitative and qualitative methods at different concentrations to detect their degradative ability. Three bacterial isolates were selected and molecularly characterized by the 16S rDNA technique. The metabolites produced from the degraded organophosphorous pesticides were analyzed using the Fourier Transform Infrared Spectrophotometer (FTIR). Variations were observed in both Total Bacteria Count (TBC) (10.18 - 11.00 Log_{10} CFU/ml) and Pesticide-degrading Bacteria Count (PDBC) (6.09 - 8.42 Log10 CFU/ml) among different soil samples. The three selected isolates were identified as *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Bacillus thuringiensis* based on 16Sr RNA gene sequences. These bacterial strains exhibited distinct varied susceptibility patterns at varying concentrations of Methomyl and Emamectin Benzoate. This research highlights the role of specific bacterial strains in pesticide degradation and their potential environmental implications.

***Keywords*:** Bioremediation, pesticide-degrading bacteria, FTIR, organophosphorus pesticides

Introduction

Nigeria, as a developing nation, relies significantly on agriculture as a key economic driver, in addition to its oil industry. A significant portion of the population relies on agricultural activities such as food and cash crop farming, fish farming, heliculture, and poultry keeping for their livelihoods.[1] With agriculture contributing 23% to the nation's Gross Domestic Product (GDP) in the first half of 2022,[2] a surplus of marketable agricultural products becomes imperative to sustain the growing population and foster economic development.

Throughout the growth cycle, crops encounter various challenges that can significantly impact agricultural productivity and food security. Pests and diseases pose significant threats to crop production, with far-reaching effects on agricultural productivity, food security, and the economy as a whole.[3] Pesticides, a diverse group of chemicals designed to control pests and enhance crop yields, have well-documented environmental impacts, including soil contamination, water pollution, and harm to non-target organisms.[4] Despite their economic importance in farm pest control, the lingering residues of pesticides contribute to soil and food crop contamination, potentially posing a public health burden through the consumption of contaminated crops.[5]

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Citation: Olowomofe TO, Abdulkareem MA, Molehin OR, Dauda OS. Biodegradative Activities of Some Indigenous Farm Soil Bacteria on Selected Pesticides. Trop J Nat Prod Res. 2024; 8(3):6686-6692. https://doi.org/10.26538/tjnpr/v8i3.31

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The widespread use of pesticides in agriculture, often without due consideration for their adverse impacts, results in the accumulation of residues in the soil, escalating contamination levels and posing a threat to both plant and animal life.[6] Organochlorine pesticides, such as aldrin, heptachlor, endosulphan, and DDT, are particularly concerning due to their tendency to accumulate in the environment.[7]

Pesticides not only alter the physico-chemical and biological properties of the soil but also disrupt microbial activity, raising environmental and human health concerns.[8] Considering these risks, alternative control strategies involving natural biological agents like viruses, insects, beneficial bacteria, and nematodes are worth exploring.

Bioremediation, a natural process utilizing microorganisms like bacteria, fungi, and plants, offers a cost-effective method to eliminate soil contaminants, including pesticides. Its eco-friendly nature has led to widespread use for site cleanup, proving to be a promising alternative to physicochemical remediation methods.[9] Microbes, particularly bacteria, exhibit a remarkable ability to adapt and utilize various pesticides as carbon and energy sources. Indigenous soil bacteria, adapted to local conditions, play a crucial role in pesticide biodegradation and present opportunities for sustainable agriculture.[10]

The focus of this study is on the ability of indigenous soil bacteria to degrade pesticides. These bacteria, naturally present in agricultural soils, have evolved to efficiently break down locally prevalent pesticides. This biodegradation capability holds significant promise for sustainable agriculture, aligning with the principles of integrated pest management (IPM) and organic farming. Harnessing the power of indigenous soil bacteria can reduce the environmental impact of pesticide use, minimize chemical residues in crops, and mitigate the development of pesticide-resistant pests, contributing to more environmentally friendly and sustainable agricultural practices.

Materials and Methods

Collection of Samples

Soil samples were obtained from three distinct agricultural sites situated (7°42'47"N 5°14'47"E)
7°43'23"N 5°15'18"18E) in Ado-Ekiti, Ekiti State in June, 2021. These farms cultivated habanero pepper, maize, okra, cassava, and yam crops and had a documented practice of applying pesticides daily. The samples were collected using sterile polythene bags, properly labeled, and then transported to the laboratory in cooled containers with ice packs for further microbiological analyses.

Total bacteria count

The total bacteria count of the soil sample was determined as described by Olowomofe *et al.*[11]
The colonies were counted using the colony counter.

Isolation of Pesticide-Degrading Bacteria

The enumeration of pesticide-degrading bacteria in the soil samples was done using ten-fold serial dilution. Dilutions 10^{-6} and 10^{-7} were subsequently plated onto Mineral Salt Medium containing Pesticides (Methomyl and Emamectin Benzoate 5) as the sole carbon source, following the methodology described by Trinidade *et al*.[12] The composition of the Mineral Salt Medium consisted of the following components per liter: $MgSO_4$ (0.20 g), NaCl (0.10 g), KH_2PO_4 (1.0 g), K_2HPO_4 (1.00 g), $FeCl_3$ (0.05 g), NH_4Cl (4.00 g), Na_2SO_4 (0.01 g), with a pH of 7, and was made up to a total volume of 1000 ml with distilled water. Subsequently, the plates were incubated at 37°C for 24-48 hours. Colonies that developed on these plates were sub-cultured onto nutrient agar plates until pure cultures were obtained. These pure cultures were then preserved on nutrient agar slants for further screening.

Qualitative Screening of Pesticide-Degrading Bacteria

The qualitative analysis was done by the addition of 20g of agar to 1000ml mineral salt medium. The mixtures were sterilized without the addition of the pesticides, after sterilization the medium was poured into plates. Three pesticides were used for the screening, and filter paper impregnated with 1% and 2% pesticide solution was plugged into the lid of the petri dishes. Each isolate was streaked on the mineral salt medium agar and incubated for 24 hours at 37°C. Uninoculated plates served as the control and the morphology of the growths was examined at intervals.

Quantitative Screening of Pesticide-Degrading Bacteria

The minimal salt broth was prepared using the method outlined by Ijah and Abioye.[12] Different pesticide concentrations (1%, 2%, 3%, and 5%) were introduced individually into separate portions of the sterile mineral salt medium. Each pesticide-enriched medium was inoculated with an 18-hour culture of the bacterial isolates. The inoculated media, each containing a distinct pesticide concentration, were incubated for a standardized duration of 72 hours under controlled temperature conditions. Following the incubation period, bacterial growth in each medium was quantitatively assessed using a spectrophotometer. The optical density (OD) or absorbance of each culture was measured at a specific wavelength (typically 600 nm) using the spectrophotometer. Uninoculated tubes served as the control.

Molecular Identification of Pesticide-Degrading Bacteria Isolates from Farm Soil Samples

DNA Extraction

The bacterial DNA was extracted using the protocol stated by Trindade.[13] After being moved into microcentrifuge tubes, the bacterial cultures were submerged in lysis buffer. To aid with enzymatic digestion and cell lysis, proteinase K was added. To guarantee total lysis, the tube mixture was homogenized and incubated for 15 minutes at 55–65°C.
The same volume of phenol chloroform was added to the tube after the lysis process. Phase separation was ensured by vortexing the liquid strongly. The organic phase and the aqueous phase (containing DNA) were separated by high-speed centrifugation. Carefully avoiding the organic phase, the upper aqueous phase was transferred to a fresh microcentrifuge tube. To the tube holding the DNA, cold ethanol was introduced in an amount that was 2.5 times the volume of the aqueous phase. A slight inversion was used to mix the solution. The tube was incubated at -20°C for a minimum of 30 minutes to precipitate the DNA. The DNA which was apparent as a white mass at the bottom of the tube, was pelleted using high-speed centrifugation. Without affecting the DNA pellet, the supernatant was cautiously removed. To get rid of contaminants, the DNA pellet was cleaned with 70% ethanol. The ethanol was eliminated following brief centrifugation, and the DNA pellet was temporarily allowed to air dry. To achieve the required concentration, the DNA pellet was again suspended in Tris-EDTA (TE) buffer and the spectrophotometry approach was used to evaluate the quality and concentration of DNA. The extracted DNA was kept for a brief period at 4°C.

Polymerase Chain Reaction

Ten microlitres of 5x GoTaq colorless reaction, 3 µl of 25 mM ++Cl2, 1 µl of 10 mM dNTPs mix, 1 µl of 10 pmol each of the primers 27F (5' AGA GTT TGA TCM TGG CTC AG3') and 1525R (5′AAGGAGGTGATCCAGCC3′), and 0.3 units of Taq DNA polymerase are included in the PCR reaction mixture. 8 µl of DNA template was introduced after the total volume was brought up to 42 µl using distilled water. A thermal cycler was used to perform the following PCR outline: a 5-minute initial denaturation at 94°C; 30-cycles of 94°C for 30 s, 50°C for 60 s, and 72°C for 90 s; and a 10-minute final termination at 72°C. At 4°C, the reaction was then maintained.[14]

DNA Integrity

Agarose gel electrophoresis (1.5%) was employed. The gel was prepared using 1XTBE buffer, previously created. The gel mixture was microwaved for five minutes, the homogenized gel was then cooled to 60°C. Three micolitres of 0.5 g/ml ethidium bromide was added for staining, which absorbs UV light and emits visible orange light. The resulting agarose solution was poured into the casting tray following the placement of a comb in its slots. After allowing the gel to solidify for 20 minutes to create wells, 1XTBE buffer was introduced to submerge the agarose gel in the tank. Following this, a 100bp DNA ladder was loaded into well 1. Four microlitres of each amplicon was loaded into subsequent wells. To aid loading and monitor progress, 2 µl of 10X blue gel loading dye was added to the amplicons for color and density. The gel underwent electrolysis for 45 minutes at 120V, was observed under UV light, and captured on camera. The rate of movement of a 100 bp molecular weight ladder ran alongside the experimental samples in the gel was used to calculate the PCR product sizes.

Purification of Amplicons

The ethanol purification process was employed to eliminate PCR reagents from the amplicons. Each 40µl amplicon was transferred to an unused, sterile Eppendorf tube, and 7.6 µl of 3M Na acetate and 240 µl of 95% ethanol were added. After thorough vortexing for complete homogenization, the tube was incubated at 20°C for at least 30 minutes. Subsequently, centrifugation at 13000 g for 10 minutes at 4°C was performed, and the supernatant was removed. The resulting pellets underwent washing by adding 150 µl of 70% ethanol, mixing, and centrifuging at 7500 g for 15 minutes.
Furthermore, the filtrate was decanted, tilted onto a paper towel, and allowed to air dry for ten minutes at room temperature in the fume hood. After that, they were preserved at 20 0C and resuspended in 20 µl of distilled water to be sequenced. A 1.5% Agarose gel was used to confirm the existence of the purified product, and it was run at 110V for approximately 60 minutes. A Thermo Scientific Model 2000 Nanodrop was used to quantify the purified fragment.

Sequencing

This was done using a BigDye Terminator v3.1 cycle sequencing kit using an Applied Biosystems Genetic Analyzer 3130xl sequencer, adhering to the manufacturer's guidelines. Genetic analysis utilized MEGA 6 and Bio Edit tools for all procedures.

Statistical analysis
Microsoft excel and Chi- square of the Statistical Procedure for Social Science version 22.0 (SPSS, Chicago, IL, USA) were used for the data analysis.

Results and Discussion

Table 1 provides data on the bacterial counts in different soil samples, with a focus on both total bacteria and pesticide-degrading bacteria. Sample A has the highest Total Bacteria Count (TBC) of 11.00 CFU/g, while Sample E has the lowest TBC of 10.18 CFU/g. Sample C has the highest Pesticide-degrading Bacteria Count (PDBC) of 8.42 CFU/g, while Sample D has the lowest PDBC of 6.09 CFU/g. The TBC and PDBC values vary slightly among the samples, indicating differences in bacterial populations in different areas of the farm. The range for TBC is from 10.18 CFU/g to 11.00 CFU/g, indicating the variability in total bacterial counts among the samples.
This provides information on the variability or spread of these counts within each sample, which can be important for understanding the microbial composition and potential pesticide degradation capabilities of the farm soil. The soil is a complex ecosystem with a diverse microbial community. The majority of soil bacteria may belong to various taxa that are not specialized in pesticide degradation. They serve different ecological roles, such as nutrient cycling, organic matter decomposition, and symbiotic relationships with plants. These bacteria may not possess the specific enzymes required for pesticide degradation, accounting for the higher TBC observed.
The variation in PDBC among the samples suggests differences in the presence and activity of bacteria capable of degrading pesticides. The history of pesticide use in different areas of the farm may have selected pesticide-degrading bacteria. Bacteria can develop the ability to break down pesticides through repeated exposure.[15]
Table 2 shows the growth pattern of farm soil bacteria in a Mineral Salt Medium (MSM) supplemented with two different pesticides, Methomyl and Emamectin Benzoate, at two different concentrations (1% and 2%). The growth pattern is represented using symbols: NG (No growth), + (Few growth), ++ (Moderate growth), and +++ (Much growth). This data provides an overview of how these bacteria respond to Methomyl and Emamectin Benzoate at different concentrations.
It was observed that the response of soil bacteria to pesticides varied based on the specific bacterial strains present and their susceptibility to the pesticides. The response of soil bacteria to pesticides is closely tied to their susceptibility. Some bacteria may exhibit resilience to specific pesticides, allowing them to thrive even in the presence of these chemicals, while others may be highly sensitive. Susceptibility can depend on the presence of specific detoxification mechanisms or resistance genes within bacterial populations.[16]
Figure 1 shows the growth pattern (OD_{600nm}) of the three selected pesticide-degrading bacteria in MSM supplemented with different concentrations of Methomyl. *Pseudomonas aeruginosa* shows moderate to high growth at 2% concentration and moderate growth at other concentrations, *Bacillus thuringiensis* exhibits moderate to high growth at 2% and 3% concentrations, with high growth at 3% and reduced growth at 5% while *Bacillus subtilis* displays moderate to high growth at 2% and 3% concentrations, with high growth at 3% and reduced growth at 5%. These growth patterns suggest that the bacterial isolates respond differently to various concentrations of the pesticide being tested. Generally, moderate to high growth was observed at 2% and 3% concentrations, while both higher and lower concentrations led to reduced growth for some of the isolates.
Figure 2 represents the optical density (OD_{600nm}) of bacterial growth for each isolate at different concentrations of Emamectin Benzoate 5 for all three isolates, there is a general trend of increased growth (higher OD values) as the concentration of the pesticide increases from 1% to 3%. However, at a 5% concentration, there is a decrease in growth for all three isolates compared to the 3% concentration. This decrease suggests that higher concentrations may have inhibitory effects on bacterial growth. Different bacterial strains may possess distinct genetic and metabolic traits that make them more or less tolerant to specific pesticide concentrations. These strain-specific responses are well-documented in the literature.[17] The toxicity of pesticides can vary significantly based on their chemical properties and mode of action.

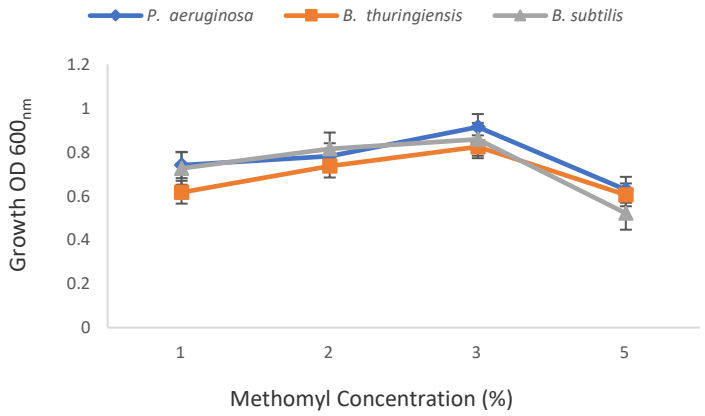

Figure 1: Analysis of Methomyl (Organophosphorous pesticide) degradation by bacteria isolates

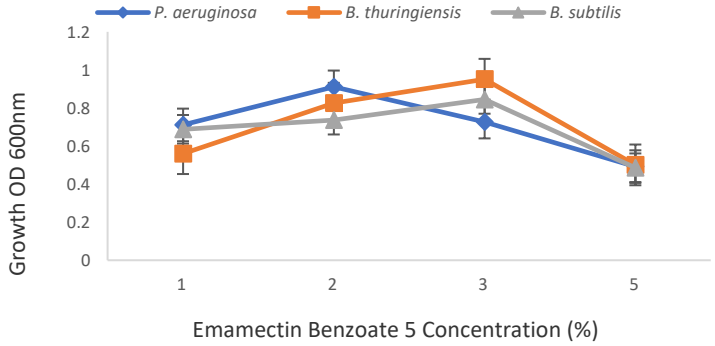

Figure 2: Analysis of Emamectin Benzoate 5 degradation by bacteria isolates

Table 1: Total bacteria count and Pesticide-degrading bacteria count from Farm Soil Samples

Soil samples	Location (GPS)	TBC (CFU/g)	PDBC (CFU/g)
A	7°42'47"N 5°14'47"E)	11.00 ± 0.39	8.14 ± 0.21
B	7°43'23"N 5°15'18"18E	10.67 ± 0.41	7.05 ± 0.35
C	7°43'25"N 5°15'16"E	10.83 ± 0.31	8.42 ± 0.20
D	7°36'57"N 5°18'10"E	10.44 ± 0.20	6.09 ± 0.18
E	7°36'56"N 5°18'7"E	10.18 ± 0.41	7.35 ± 0.11
Range		10.18 - 11.00	6.09 - 8.42

Key: TBC: Total bacteria count; PDBC: Pesticide-degrading bacteria count
NOTE: Values are mean and standard error of three replicates

Table 2: Growth pattern of farm soil bacteria after 48 hours in pesticides supplemented Mineral Salt Medium (MSM)

Isolates code	Methomyl		Emamectin Benzoate 5	
	1%	2%	1%	2%
C-1	NG	+	+	+++
C-2	NG	NG	++	NG
C-3	+	+	+	+
B-1	+	++	++	+
B-2	NG	+	++	NG
B-3	++	++	++	+++
B-4	+	NG	+	NG
B-5	+	+	+	+
B-7	+	++	+	+++
B-8	+	+	+	+
B-9	+	NG	+	+++
B-11	+	+	+	++
D-3	NG	+	+	+++
D-5	++	NG	NG	++
D-11	+	+	++	++
E-2	++	++	++	+++
E-4	+	+	+	NG
E-5	++	++	++	++

KEYS: No growth = NG; Few growth = +; Moderate growth = ++
Much growth = +++

Some pesticides may inhibit bacterial growth at higher concentrations, while others might not show the same effect.[16]

The FT-IR analysis of the non-degraded Methomyl showed O-H stretching observed at 3551 cm^{-1}, indicating the presence of hydroxyl groups (alcohol or phenol) in the compound. C-H stretching at 2311 cm^{-1}, showed the presence of carbon-hydrogen bonds in the compound. C=O peaks at 1449 cm^{-1} and 1406 cm^{-1}, signifies the presence of a ketone functional group in the compound. C=C peak observed at 1406 cm^{-1}, indicates the presence of carbon-carbon double bonds in the compound. C-H bending observed at 1198 cm^{-1}, suggests the presence of carbon-hydrogen bonds that are undergoing bending vibrations. C-O stretching observed, possibly indicates the presence of an alkyl aryl ether functional group. CO-O-CO stretching observed at 1556 cm^{-1}, showed the presence of an anhydride functional group (Figure 3).[18]

The FT-IR analysis of methomyl degraded by *Pseudomonas aeruginosa* showed O-H stretching at 3556 cm-1 and C-H stretching was observed at 2500cm-1. The peaks 1517 cm-1 and 1400 cm-1 showed the presence of alkene and primary alcohol group in the degraded Methomyl. The C-H stretching at 2500 cm-1 and the C-O stretching indicate the presence of benzene derivative in the residual compound as shown in (Figure 4). Many Pseudomonas species are known for their versatility in degrading a wide range of pesticides, including organophosphates, herbicides, and insecticides.[19] The appearance of new peaks associated with alkene and primary alcohol groups, along with the presence of a benzene derivative, suggests that *Pseudomonas aeruginosa* has enzymatically transformed Methomyl into new compounds during the degradation process.

The FT-IR analysis of the Methomyl degraded by *Bacillus thuringiensis* showed O-H stretching at 3468 cm-1 and N-H stretching at 1072 cm-1 and 1008 cm-1 which signifies aliphatic primary amine in the group. The C-H stretching observed at 2223 cm-1 and C=C stretching at 1400 cm-1 indicates the presence of *α, β*- unsaturated ketone group in the compound. C-F and C-Br stretching observed at 925 cm-1 and 1618 cm-1 show the presence of fluoro and halo compounds respectively (Figure 5). The appearance of new peaks associated with aliphatic primary amine, *α, β*-unsaturated ketone, C-F, and C-Br groups indicates that *Bacillus thuringiensis* has enzymatically transformed Methomyl into new compounds during the degradation process. The presence of fluorine and bromine-containing compounds suggests that the degradation process may have involved the substitution of fluorine and bromine atoms in the original Methomyl molecule.

In Figure 6, The FT-IR analysis of the Methomyl degraded by the bacterial strain *Bacillus subtilis* showed O-H stretching at 3468 cm-1 and N-H stretching at 1072 cm-1 indicating the presence of hydroxyl groups (alcohol or phenol) and amine groups respectively in the degraded compound. The C-H stretching observed at 2248cm-1 and 1618cm-1, C-Br stretching suggests the presence of carbon-hydrogen bonds and bromine-containing compounds (halo compound) respectively in the degraded product. The appearance of peaks associated with hydroxyl, amine, and bromine-containing groups suggests that *Bacillus subtilis* has enzymatically transformed Methomyl into new compounds during the degradation process. The presence of a bromine-containing compound indicates that the degradation process may have involved the substitution of bromine atoms in the original Methomyl molecule.

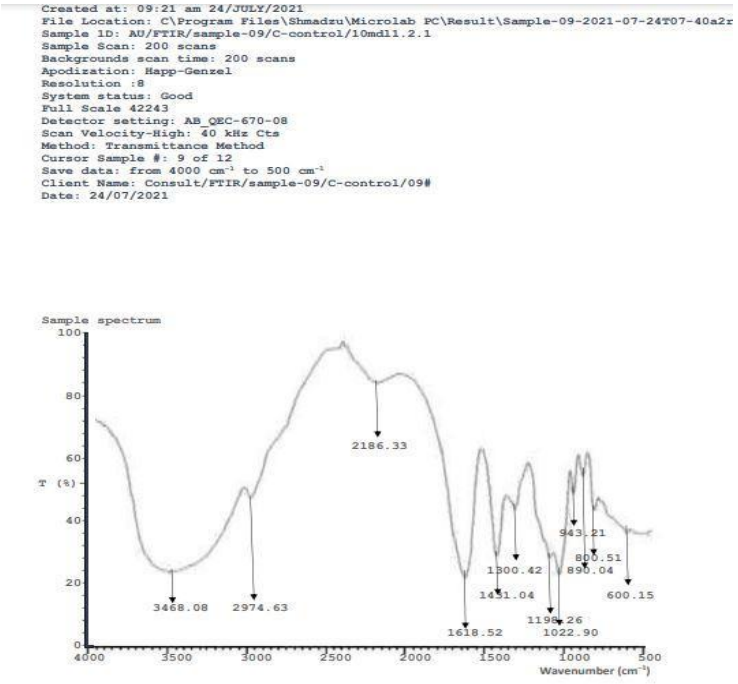

Figure 3: FT-IR Spectrum of Methomyl – Control

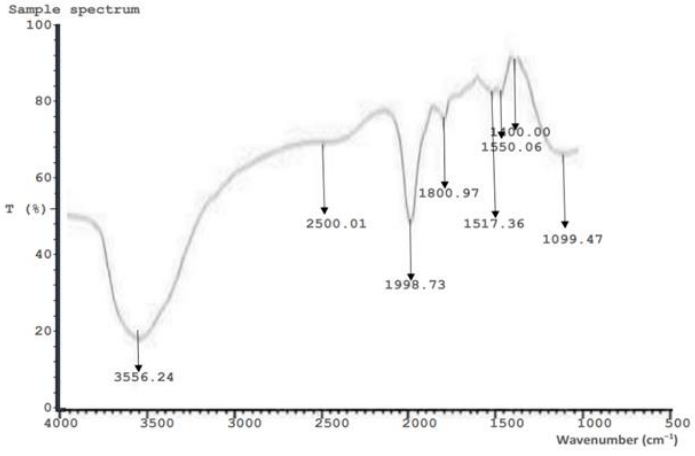

Figure 4: FT-IR Spectrum of Methomyl degraded by *Pseudomonas aeruginosa*

The formation of new functional groups and chemical moieties during the degradation process is common in microbial degradation of pesticides. The transformation of Methomyl into different compounds by *Bacillus subtilis* can have implications for its environmental fate and potential impacts on human health and ecosystems.

The FT-IR analysis of non-degraded Emamectin Benzoate 5 (control sample) revealed the presence of twelve different FTIR spectra which signifies that it is a complex molecule. There is a broad absorption band which indicates hydrogen bond at 3468cm^{-1}, and a narrow band at 2974cm^{-1}, showing aliphatic compounds (Figure 7). There is the presence of an absorption band of C≡C (triple bond region) at 2186cm-1 and double bond regions informing aromatic compounds at 1618cm^{-1}. Identification of fingerprint regions for multiple band absorption at 600, 890, 943, 1022,1198, 1300, and 1431cm^{-1} which also has vinyl terminals (-CH=CH2) at 943cm^{-1} as shown in Figure 7.

The FT-IR of Emamectin Benzoate 5 degraded by *Pseudomonas aeruginosa*, showed O-H stretching at 3100cm^{-1}, C≡C Terminal alkyne (monosubstituted) at 2020cm-1, and the presence of Alcohol and hydroxyl compound (Primary or secondary, OH in-plane bend) at 1350cm^{-1}. At 2000-2500 cm^{-1} there is confirmation of the presence of two triple bond regions (Figure 8).

The FT-IR of Emamectin Benzoate 5 degraded by *Bacillus thuringiensis* shows the presence of 11 FTIR spectrum, there was indication of an aldehydes group at 2725cm^{-1} and also a (C = C) carbonyl group such as ketone group was detected at 1650 and 1746cm^{-1}as shown in Figure 3. The FT-IR of Emamectin Benzoate 5 degraded by bacteria strain *Bacillus thuringiensis* also shows a region of multiple band absorption, and there are also absorption bands at 3900 cm^{-1} which exhibit out-of-plant bending and also indicate unsaturated compounds as shown in Figure 9.

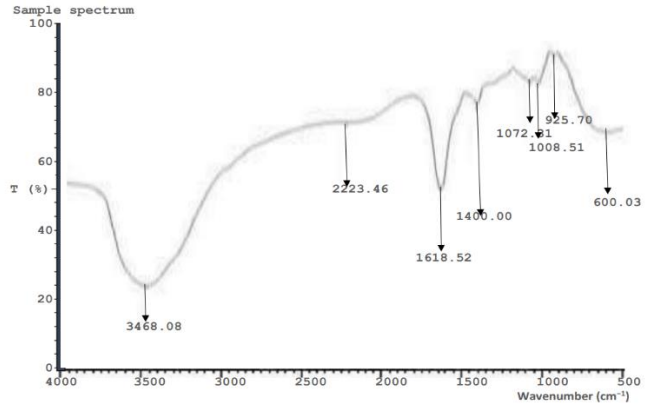

Figure 5: FT-IR Spectrum of Methomyl degraded by *Bacillus thuringiensis*

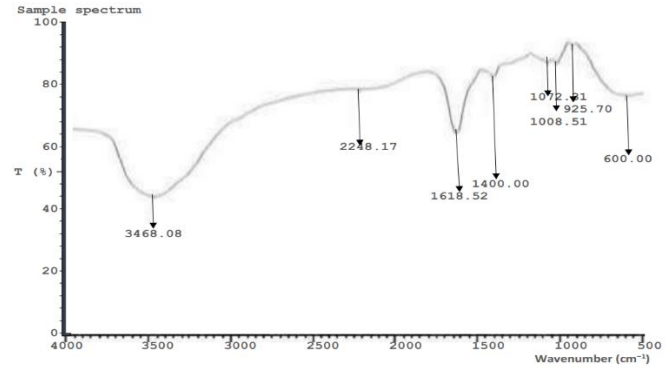

Figure 6: FT-IR Spectrum of Methomyl degraded by *Bacillus subtilis*

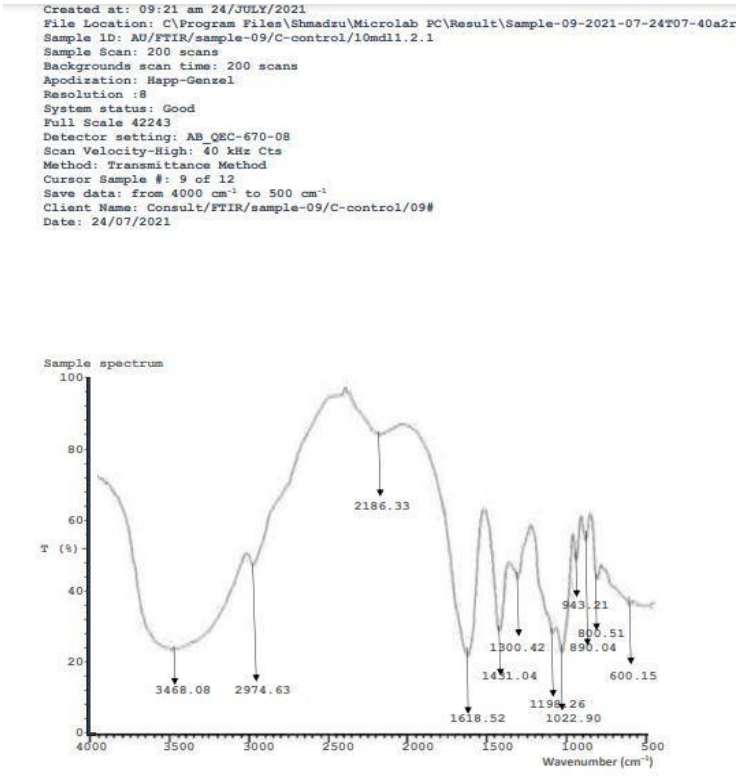

Figure 7: FT-IR spectrum of Emamectin Benzoate 5 – Control

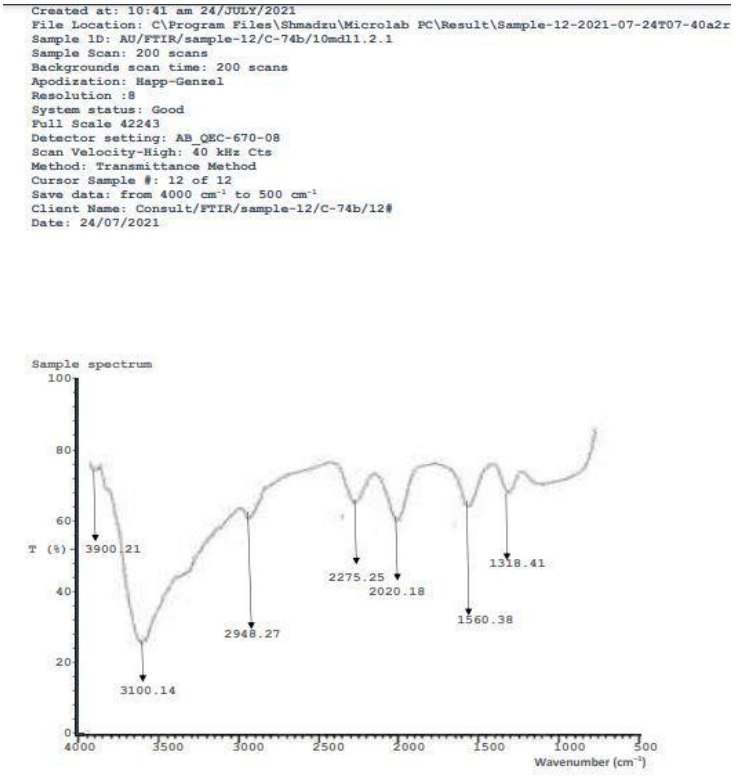

Figure 8: FT-IR spectrum of Emamectin Benzoate 5 degraded by *Pseudomonas aeruginosa*

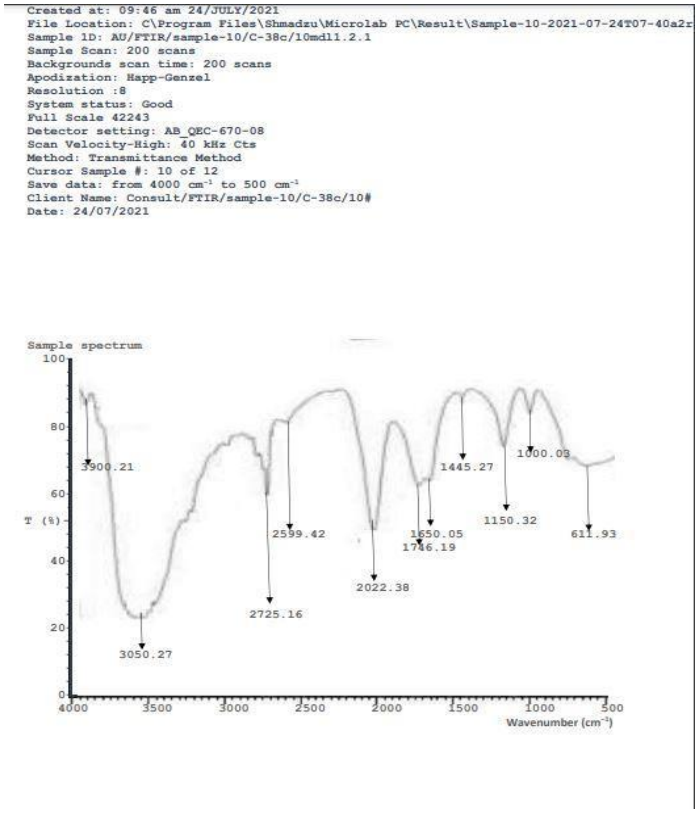

Figure 9: FT-IR spectrum of Emamectin Benzoate 5 degraded by *Bacillus thuringiensis*

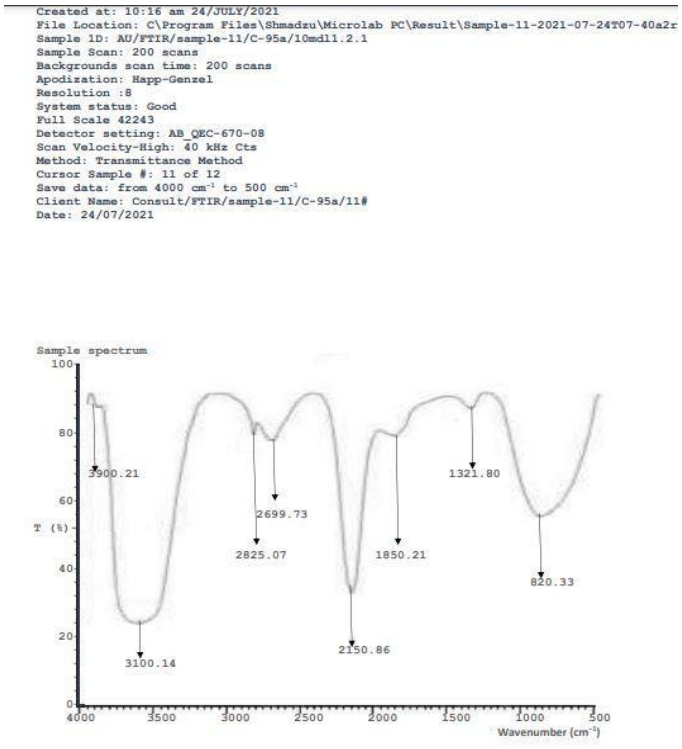

Figure 10: FT-IR spectrum of Emamectin Benzoate 5 degraded by *Bacillus subtilis*

The FT-IR of Emamectin Benzoate 5 degraded by *Bacillus subtilis* shows that there are 8 absorption bands, which makes it a complex molecule. It has two spectra; 3000.14cm^{-1} and 3900.21cm^{-1}i.e (for C-H stretching) which shows that it contains an unsaturated or aromatic ring. 2825.07 shows that it contains a long-chain linear aliphatic compound, while 820.33cm^{-1} and 1850.21 belong to the triple bond region (C≡C). 1321.60cm^{-1} shows multiple band absorption while 820.33cm^{-1} shows to be an aromatic compound with a single and strong absorption band (Para) as shown in Figure 10.

Conclusion

The biodegradative activities of indigenous farm soil bacteria on selected pesticides represent a vital area of research with significant implications for sustainable agriculture and environmental preservation. Harnessing the potential of these microorganisms may lead to a more environmentally friendly and sustainable approach to pesticide management, ultimately benefiting both farmers and the ecosystems in which they operate.

Conflict of Interest

Authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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