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The Role of Microbes Influencing the Degradation of Pesticides by Nitrogen-Fixing Bacteria

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Abstract

Pesticides, although used in large amounts in agriculture for pest and disease control, have a long-term presence in the soil, bringing about negative impacts on the ecosystems and people. However, one of the possibilities for dealing with the issue is through the microbial degradation of the contaminants, which is a safe and sustainable method for environmental remediation. The study was conducted to find out if the soil bacteria which can fix nitrogen, could also degrade pesticides. The soil samples collected from the vicinity of the plants were handled with care to purify and isolate the bacteria, which were later inoculated on Nitrogen-Free Bromothymol Blue (NFB) medium, where the transition from green to blue in color signifying the nitrogen fixation, was monitored. The isolates underwent the identification process, including visual and biochemical tests, and these involved catalase activity tests, among others. The pesticide-degrading capacity of mass cultures cultivated in Luria Bertani hi-veg broth was tested by agar diffusion, and the results were corroborated by HPLC assay. The findings clearly showed the isolation of nitrogen-fixing Gram-positive short bacilli that were able to degrade the Eurolux pesticide by 23.3%. HPLC confirmation supported the degradation, resulting in expressing their potential as bioremediation agents. The study highlighted that nitrogen-fixing bacteria enhance soil fertility through nitrogen fixation and reduce harmful pesticide residues. These microbes are vital for developing eco-friendly, sustainable farming methods that boost productivity and protect the environment.

Keywords: Nitrogen fixing bacteria**,** Pesticides, Biodegradation, Soil fertility, Sustainability, Crop production

Introduction

Contemporary farming methods, such as the intensive use of pesticides, have a long-term influence on the ecology. Pesticides applied in fields often end up in lakes, rivers, and oceans, accumulating in plants and animals. Pesticides can enter the aquatic system via hydrological pathways beneath and on the surface. Drip irrigation, runoff, and drainage water are common causes of this phenomenon (Garcia-Corcoles *et al*., 2019). Inadequate acts such as filling sprayers, cleaning measuring cups, dumping empty pesticide containers, and cleaning spraying apparatus can lead to unnecessary accumulation in the environment. Kumar *et al*. (2010) warn that pesticide buildup could affect the environment. In the intricate tapestry of the environment, a diverse array of microorganisms, including bacteria, fungi, archaea, and beyond, occupy every part of a plant. Soil, which is rich in microbial communities, is ideal for plant growth. To put this into perspective, it is estimated that there are 200 m of fungal hyphae and 1 billion bacterial cells in one gram of soil. (FAO, 2021). These microorganisms form a bond with plants by traveling from soil to plant, resulting in the formation of complex microbial communities known as phytomicrobiomes (Lakshmanan, 2014; Bhatt *et al*., 2020; Chidambaranathan & Culathur, 2022). The composition of these microbial cohorts can affect their host plants in both advantageous and negative ways.

Agriculture is constantly striving to improve productivity. Herbicides are one method utilized to achieve this purpose. Chemical compounds prevent the creation of amino acids, carotenoids, and lipids, or they impair electron flow during photosynthesis. The widespread use of herbicides and pesticides can contaminate agricultural soils, river systems, and groundwater, causing microbial populations to change their structure and function. Herbicides can have unintended consequences, endangering human health (García-Corcoles, 2019; FAO, 2021; Fazeli-Nasab & Rahmani, 2021; Filote, 2021). Pesticides are classified based on their chemical makeup, which includes organochlorines, organophosphates, carbamates, formamidines, thiocyanates, organotins, denitrophenols, synthetic pyrethroids, and antibiotics (Coronado *et al*., 2004; Pavithra *et al*., 2023). Organophosphate pesticides that are regularly employed include parathion and malathion. Monocil is a broad-spectrum insecticide that kills a variety of bugs, including sucking pests, borers, and lepidopteran larvae. The active ingredient is monocrotophos, an organophosphate pesticide. The monocil pesticide has a rapid knockdown effect. Defen insecticide includes Pyraxalt™, a potent nAChR inhibitor that inhibits neurotransmission in insects. Pyraxalt™ is the first insecticide to block nAChRs rather than activate them. Eurolux, another pesticide that kills insects, is also in use.

Microbial breakdown of DDT entails breaking down the compound to less toxic compounds with low molecular weight

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through the enzyme actions of effective microorganisms (Tarfeen *et al*., 2022; Perrine *et al*., 2023; Rizqi *et al*., 2023). It is an ecological and economical approach that involves the use of particular bacteria and environmental conditions in order to clean up properly (Kour *et al*., 2021; Vijayanand *et al*., 2023). DDT is a dangerous toxin that interferes with the growth, reproduction, enzymes, and DNA of microorganisms, which warrants its elimination (Gohil, 2020; Canassa & Baldin, 2022). DDT can also be broken down into less dangerous substances by microorganisms, such as bacteria and fungi, through exudates as their energy sources (Ebsa *et al*., 2021; Fazeli-Nasab *et al*., 2021; Bokade *et al*., 2023). Saprophytic fungal species also acquire resistance and break down different organic pollutants with time (Purnomo *et al*., 2020; Wilhelmy *et al*., 2022). The research paper aims at isolating and characterizing soil microorganisms that can decompose agricultural pesticides, and the efficiency of degrading the pesticides has been established through the use of the HPLC technique **(Table 1)**.

Table 1. Microbial species that can disintegrate pesticides

Pesticides degrading Microbes	Microbial species	References
Bacteria	Arthrobacter globiformis	(Wang *et al*., 2023)
	Ralstonia pickettii bacterium	(Purnomo *et al*., 2011)
	Enterobacter cloacae	(Suman & Tanuja, 2021)
	Staphylococcus hominis	(Nwanguma & Ezeanyika, 2020)
	Staphylococcus equorum Staphylococcus cohnii Kocuria rhizophila	(Nazarova *et al*., 2022)
	Pseudomonas aeruginosa	(Maulianawati *et al*., 2021; Macrì *et al*., 2023)
Algae	Aulosira fertilissima Chlorococcum species Scenedesmus obliquus	(Nie *et al*., 2020)
	Aulosira fertilissima Chlorococcum species	(Woodwell *et al*., 1967; Bugti *et al*., 2024)
	Chlamydomonas species	(Kumar & Shukla, 2023)
	Chlorella vulgaris	(Hussein *et al*., 2017)
	Euglena gracilis	(Priya *et al*., 2014)
	Cylindrotheca species	(Aggarwal & Kumari, 2024)
	Dunaliella salina	(Mondal *et al*., 2019)
	Anabaena species	(Singh *et al*., 2021)
	Cladophora species Cladophora gracilis	([Meeks, 1968)
Fungi	Phanerochaete chrysosporium	(Bumpus & Aust, 1987; Dorn *et al*., 2024)
	Allescheriella species	(Trivedi *et al*., 2022)
	Rhizopus arrhizus Alternaria species	(Mohapatra *et al*., 2021)
	Penicillium species	(Russo *et al*., 2019)
	Aspergillus niger	(Bhalerao & Puranik, 2007)
	Paecilomyces species	(Filote *et al*., 2021)
	Fusarium oxysporum	(Engst & Kujawa, 1968; Shaiba *et al*., 2024)

Materials and Methods

Collection of Samples

Topsoil samples were collected from three non-pesticide-treated areas in the research area **(Figure 1)**. These locations had comparable ecological circumstances. The soils ranged from sandy loam on top to brown loamy sand subsoil that was well-drained. The soil samples were pooled together, homogenized correctly, and transferred to the laboratory for physicochemical and preliminary microbiological investigation (Abdouchakour *et al*., 2015; Aggarwal & Kumari, 2024). Soil samples were collected from Jayanagar, Bengaluru, and Karnataka. To the collected soil samples was added 10 ml of saline solution and vortexed. Several soil samples were naturally dried, powdered, and sieved for analysis of physical and chemical characteristics.

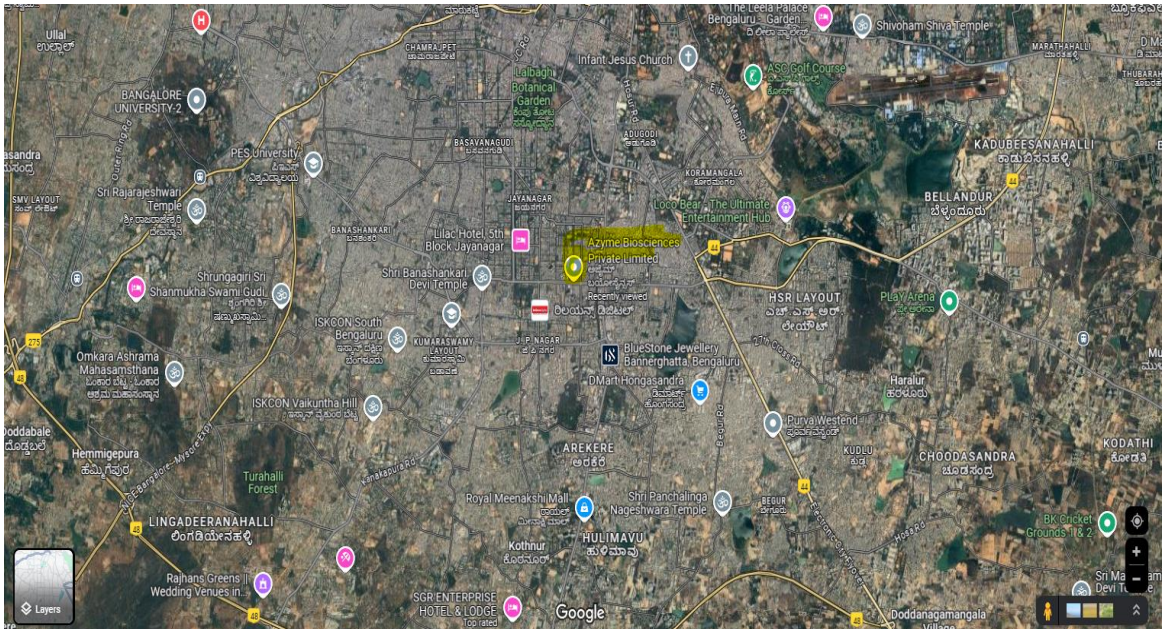


Figure 1. Soil sample collection site in Google Maps.

Isolation of Nitrogen-Fixing Bacteria

Isolated the bacteria by using Yeast extract mannitol agar media and performed the pour plate method with 200 microlitre soil suspension and kept it for incubation at 37°c for 24-48 hours. Subcultured the isolated bacterial colonies by streaking on LB agar media.

Screening and Confirmation

The subcultured bacteria were streaked on NFB media and incubated at 37°c for 24 to 48 hours for screening. The nitrogen-fixing bacteria were confirmed by visualizing the color change of the media from green to blue.

Bioassay Method

Selected four pesticides, Monocil, Malathion, Eurolux, and Defen, and performed spectrum scanning for those pesticides. Observed the highest peak in the spectrophotometer for those pesticides and noted the peaks at their particular wavelengths.

Percentage of Degradation was calculated by using this formula:

$$\text{Percentage of degradation} = \frac{\text{Control} - \text{Test}}{\text{Control} \times 100} \quad (1)$$

Identification of Microbes by Biochemical Tests

Gram staining kit, H2O2 for catalase, oxidase disc, Kovacs reagent for the indole test, MR-VP media with reagents like methyl red indicator for MR and alpha-naphthol, ethanol, and 40% KOH for the VP test, and 1% tryptone for indole. Simon citrate agar for the citrate utilization test, nitrate broth and Durham's tube for the nitrate reduction test, hydrogen sulfate agar for the H_2S test, CMC agar for the cellulose degradation test, and starch agar and iodine indicator for starch hydrolysis. Casein agar for casein hydrolysis, urease broth for the urease test, gelatin agar for gelatin liquefaction, the carbohydrate fermentation test, and Triple Sugar Iron Agar for TSI.

Gram Staining: Crystal violet staining reagent was applied to a heat-fixed, air-dried smear of cells for one minute. Following a 15-second addition of ethanol, a decolorizing agent, the slides were rinsed with tap water. After soaking in safranin for ten minutes, the slides were cleaned with tap water. The hue of gram-negative bacteria will stain pink-purple. Gram-positive lactobacillus is the name given to the bacterial organism that turns blue when exposed to oil.

Indole Production Test: 0.3 mL of 1% tryptone broth was diluted in 30 mL of distilled water, transferred to a test tube, and inoculated with a small amount of pure culture. Incubate for 24-48 hours. To evaluate indole synthesis, apply 2-5 drops of the Kovacs reagent directly to the incubated test tube. After adding the Kovas reagent, a cherry red ring will appear, indicating indole positive; if it remains yellow or slightly murky, it is indole negative. Control is maintained without the inoculum.

MR-VP Text: Peptone (1 g), glucose (5 g), and dipotassium phosphate (5 g) were dissolved in 1000 mL of distilled water. The pH was adjusted to 6.9±0.2. Autoclaved tubes of each MR-VP broth were inoculated and incubated at 37°C for up to 48 hours. For the methyl red test, two drops of methyl red indicator were added, and for the Voges-Proskauer test, 0.6 ml of Barritt's reagent A and 0.2 ml of Barritt's reagent B were used. The tubes were then gently shaken for up to 1 minute to expose the medium to air oxygen and then left to stand for at least 30 minutes. Check for color changes in the tubes. MR: A color change to red indicates that the test is positive for mixed acid fermentation. VP: If the color changes from red to yellow, the bacteria create acetoin. Control is maintained without the inoculum.

Citrate Utilization Test: Ammonium dihydrogen phosphate (1 g), dipotassium phosphate (1 g), and sodium chloride (5 g) are used to

make Simmons Citrate Agar. Agar (15 g), magnesium sulfate (0.2 g), sodium citrate (2 g), and bromothymol blue (0.08 g) are dissolved in 1000 ml of distilled water and then autoclaved. The Simmons citrate slant's surface was striped with inoculum. After that, the tubes were incubated for up to 48 hours at 37°C. Verify the color shift from green to blue, which denotes an alkaline state. The inoculum is kept out of the control.

Gelatin Hydrolysis: Gelatin (120 g), beef extract (2 g), and peptone (5 g) were combined, diluted in 1000 ml of distilled water, and autoclaved to create 1% gelatin agar. After being inoculated for 24 to 48 hours at 25°C, the tubes were submerged in an ice bath for up to 30 minutes, and the gelatin liquefaction was monitored. The organism has created the enzyme gelatinase if the agar has liquefied. The inoculum is kept out of the control.

H2S Test: Prepare Sin agar from beef extract (3.0 g). Peptone (30.0 g), ferrous ammonium sulfate (0.2 g), sodium thiosulfate (0.025 g). Mix 3.0 g agar with 1000 ml of distilled water and adjust pH to 7.3 ± 0.2. Look for a hue change to black, which indicates H_2S presence. A control is maintained in the absence of inoculum.

Nitrate Reduction: Nitrate reduction has been investigated using nitrate broth comprised of 5 g beef extract, 3 g peptone, and 5 g potassium nitrate in 100 mL distilled water, subsequently autoclaved. Following inoculation, the broth was incubated for 24 hours. Reagent A (0.016 g sulfanilic acid in 2 mL of 5N acetic acid) and Reagent B (0.01 g α-naphthyl amide in 2 mL of 5N acetic acid) were formulated. Following a 4-hour incubation, several drops of reagents A and B were introduced, and the mixture was agitated. The cherry red hue signified successful nitrate reduction. If no color was produced, a pinch of ZnCl was included. The appearance of red color with the addition of ZnCl indicated a positive result for nitrate reduction, whereas the absence of color change proved a negative result. A control devoid of inoculum was preserved.

Carbohydrate Fermentation Test: Prepare the fermentation media for carbohydrates by combining a single carbohydrate (glucose, lactose, sucrose, or mannitol) with basal media that contains 10 grams of peptone, 15 grams of sodium chloride, and 0.018 grams of phenol red as an indicator. Autoclave the media, inoculate, and incubate for 24 hours. Check for a color change from reddish orange to yellow; if the color changes to any other color, it is considered negative. A control is kept without the inoculum.

Urease Test: In 1000 ml of distilled water, make urease broth by adding 1 g of peptone, 5 g of sodium chloride, 2 g of potassium dihydrogen orthophosphate, 1 g of glucose, 0.021 g of phenol red, and 20% urea. Then, autoclave on an agar slant, inoculate with the organism, and incubate for 24 hours. If the color changes to bright pink, it indicates a positive reaction. The inoculum is kept out of the control.

Casein Hydrolysis: After being prepared and sterilized, casein agar is transferred into sterile petri dishes. Solidification of the medium is permitted. A center streak of the specified test culture is created using the sterile inoculation loop. After being properly labeled, the plates are incubated at 37°C for 24 to 48 hours. The inoculum is kept out of the control. The plates' zone of clearance is monitored.

Starch Hydrolysis: Starch agar is made by mixing starch (20 g), peptone (5 g), beef extract (3 g), NaCl (5 g), and agar (20 g) in 1000 ml of distilled water and autoclaving. The culture is streaked in a zigzag pattern and incubated for 48 hours at 37 degrees Celsius. The plate was then drenched with iodine solution via a dropper for 30 seconds. Pour out the excess iodine and look for a clear or yellow zone around the growth. If there is a clear or yellow zone around the growth line, it means that the test organism is producing amylase enzyme, which hydrolyzes starch in the surrounding media and turns it into sugars. A control is maintained in the absence of inoculum.

Cellulose Degradation: Carboxymethyl cellulose agar is made, sanitized, and then placed into sterile petri plates. The medium is allowed to solidify. Using the sterile inoculation loop, a central stripe of the test culture is formed. The plates are suitably labeled and incubated for 24-48 hours at 37°C. The plates are checked for a zone of clearance. Control is maintained in the absence of inocula.

Catalase Test: Take one loop of culture that has been incubated for 16-18 hours and place it on a clean glass slide. Then, on the colony-containing slide, apply a single drop of hydrogen peroxide (H_2O_2) at 3% concentration. Watch for the production of oxygen bubbles. Inoculate the organism onto an agar slant and incubate for 24 hours. For a favorable reaction, the color should change to brilliant pink. The control is kept without the inoculum.

Oxidase Test: Using a loop, scrape out any new growth from the culture plate. Place it on the oxidase disc and watch for a color change for ten seconds. It is positive if the color shifts to blue; if it stays the same, it is negative. In the absence of the inoculum, a control is kept.

TSI: TSI agar was formulated with peptone, beef extract, yeast extract, sugars (lactose, sucrose, and dextrose), salts, ferrous sulfate, sodium thiosulfate, phenol red, and agar in distilled water, subsequently autoclaved. The culture was inoculated by stabbing and streaking the agar, followed by incubation at 37°C for 24 hours. A color alteration to yellow, crimson, or black signified sugar fermentation (TSI positive); the absence of change indicated a negative result. A control devoid of inoculum was preserved.

Optimization of Physical Parameters for the Degradation: By adjusting temperature, pH, and substrate concentration, physical factors for degradation were optimized in order to determine the ideal circumstances for optimum degradation efficiency. The temperature was modified within a defined range to evaluate its impact on enzyme activity and bacterial proliferation. pH fluctuations were assessed to identify the optimal acidic or alkaline conditions for maximal substrate degradation. The substrate concentration was optimized to assess the correlation between substrate availability and degradation rate, ensuring that substrate saturation did not impede enzymatic activity. The ideal settings were established based on the maximum degrading efficiency and uniform findings across replicates.

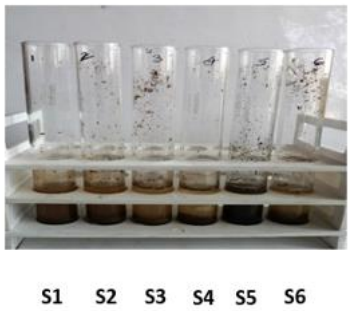
Results and Discussion

Microscopic Observation, Bacterial Isolation, Sample Collection, and Preparation

A total of six unique bacterial isolates were identified based on their distinct colony morphologies (Sousa, 2013). These isolates demonstrated successful growth on selective nitrogen-fixing media, thereby confirming their nitrogen-fixing capabilities, as depicted in **Figures 1a-1d**. The colony-forming units (CFU) per mL were determined using the standard calculation method, yielding a total of 4.1×10^6 CFU/mL. Gram staining analysis indicated that the majority of the isolates displayed a bluish-green coloration, characteristic of Gram-negative bacteria, with the exceptions of S1, S3, and S6, which were identified as Gram-positive (Arifa, 2024). Biochemical characterization led to the preliminary identification of the isolates as follows: Rossellomorea sp. (S1), Pseudomonas sp. (S2), Clostridium sp. (S3), Gluconacetobacter sp. (S4), Scytonema sp. (S5), and Bacillus sp. (S6) (Abdouchakour, 2015; Hackenberg *et al*., 2023). The significance of their role in biological nitrogen fixation (BNF), which is crucial for soil fertility and sustainable agriculture, is demonstrated by the growth of isolates on selective nitrogen-fixing media. The diversity of both Gram-positive and Gram-negative bacteria is believed to be reflected in the variation of colony morphology and Gram staining. The dominance of Gram-negative isolates aligns with the discoveries of nitrogen-fixing strains such as Pseudomonas and Gluconacetobacter, which are known to thrive in various conditions (Sousa, 2013; Skeie & Klock, 2023). Their biochemical profiling (S1-S6) contributes to their roles in promoting plant growth, nitrogen fixation, and bioremediation (Abdouchakour, 2015). The presence of Bacillus and Clostridium species suggests their resilience to environmental factors, warranting further molecular and enzymatic profiling.

Table 2. Characterization of nitrogen-fixing bacterial isolates. This table illustrates the growth performance of bacterial isolates on selective media designed for nitrogen fixation, thereby confirming their nitrogen-fixing abilities. Additionally, Gram staining, catalase activity, and various biochemical tests were employed to evaluate the isolates in order to identify their physiological and metabolic characteristics.

S/N	**Biochemical Tests**						
	Tests	S1	S2	S3	S4	S5	S6
1.	Catalase	Positive	Positive	Negative	Positive	Negative	Positive
2.	Oxidase	Positive	Positive	Negative	Negative	Negative	Negative
3.	Glucose	Positive	Negative	Negative	Negative	Negative	Positive
4.	Lactose	Positive	Negative	Positive	Positive	Negative	Positive
5.	Sucrose	Positive	Negative	Negative	Positive	Negative	Positive
6.	H2S	Positive	Negative	Negative	Negative	Negative	Negative
7.	Urease	Negative	Positive	Negative	Negative	Negative	Negative
8.	Methyl red	Negative	Negative	Negative	Negative	Negative	Negative
9.	Indole	Negative	Negative	Negative	Negative	Negative	Negative
10.	Starch hydrolysis	Positive	Positive	Positive	Positive	Negative	Negative
11.	Nitrate reduction	Positive	Negative	Negative	Negative	Negative	Positive
12.	Citrate	Positive	Positive	Negative	Negative	Negative	Positive
13.	Predicted Species	Rossellomorea sp.	Pseudomonas sp.	Clostridium sp.	Gluconacetobacter sp.	Scytonema sp.	Bacillus pumilus
14.	Strain	Gram positive	Gram negative	Gram positive	Gram negative	Gram negative	Gram positive



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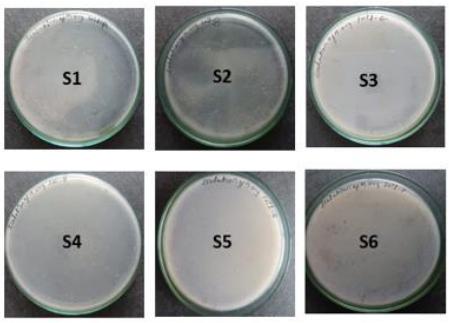

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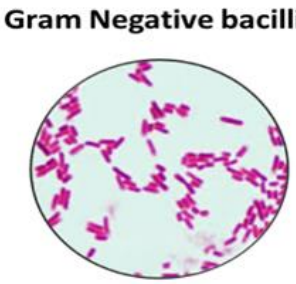

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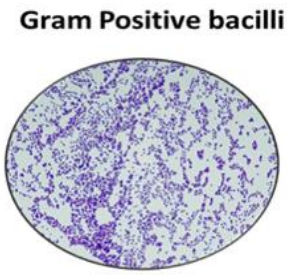

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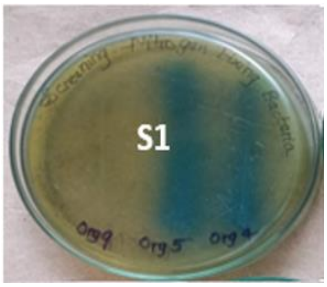
Figure 1. a, b) Isolation of microorganisms from diverse soil samples. c, d) Illustration of Gram-Negative and Gram-Positive Organisms

Nitrogen-fixing bacteria are tested on Nitrogen-Free Broth (NFB) medium, where the growth of diazotrophs is supported without the presence of fixed nitrogen sources. They proliferate to suggest that they are able to transform the nitrogen in the atmosphere into a bioavailable form, such as ammonia. A change of the color of the medium to blue indicates the presence of the nitrogenase enzyme and the fixation of nitrogen. Their ability to fix nitrogen is also confirmed by biochemical and microscopic tests. Therefore, the change in color is a clear indication of active fixation of nitrogen. **(Figure 2)**.



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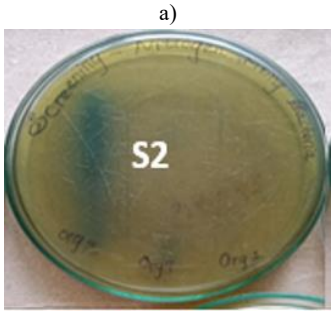

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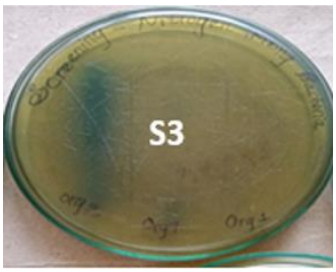

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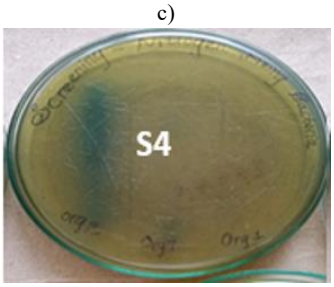

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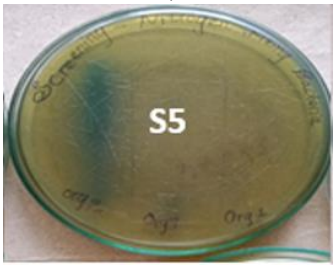

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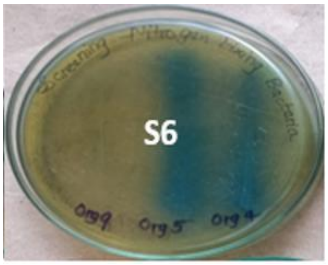

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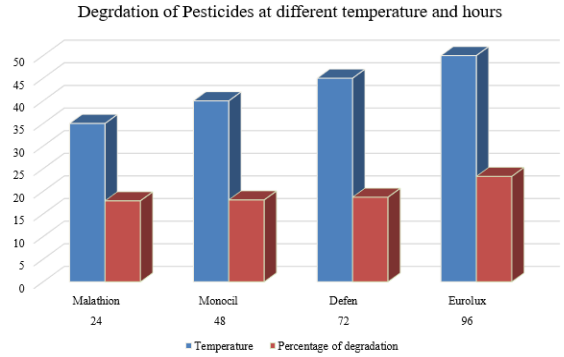
Figure 2. Illustrates the soil samples S1 to S6 exhibiting NFB characteristics through a color transition from green to blue.

Incubation Time and Temperature

The assessment of pesticide degradation by six isolated microorganisms was conducted under different incubation durations (24, 48, 72, and 96 hours) and temperature conditions (room temperature, 30°C, 35°C, 40°C, 45°C, and 50°C) utilizing M9 broth cultures. The absorbance readings demonstrated a consistent increase in the percentage of degradation over time in the experimental samples when compared to the control samples (Engst & Kujawa, 1968; Bumpus & Aust, 1987; Coronado *et al*., 2004; Ebsa *et al*., 2021). The peak degradation of pesticides occurred at the 72-and 96-hour marks, indicating that extended incubation periods enhance the efficiency of microbial degradation. Among the tested temperatures, 40°C and 45°C yielded the highest rates of degradation, whereas room temperature and 50°C showed relatively lower degradation levels, likely due to

diminished microbial activity at these extreme temperatures. Minimal pesticide degradation observed in control flasks confirmed that the breakdown process was predominantly driven by microbial activity. Among the six isolates, several demonstrated enhanced degradation capabilities, underscoring their significance in bioremediation efforts. The most effective degradation was noted at temperatures between 35°C and 45°C, along with extended incubation periods of 72 to 96 hours, indicating the necessity for enzyme induction and metabolic adaptation (Gupta & Verma, 2021; Son & Lee, 2024). The peak degradation observed at 40°C to 45°C corresponds with findings that highlight optimal microbial enzymatic activity within this temperature range (Patel *et al*., 2020). These results underscore the crucial role that microorganisms play in the efficient degradation of pesticides **(Figures 3a and 3b)**.



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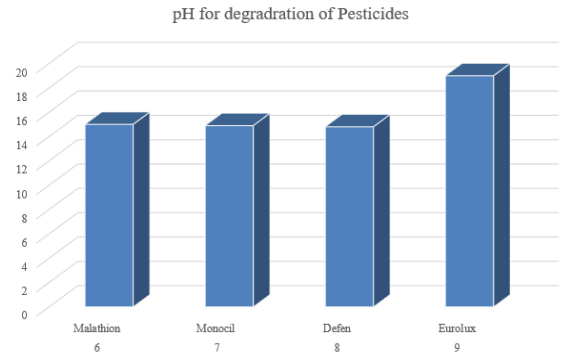


b)

Figure 3. a) The diagram shows the degradation pattern of Monocil, Malathion, Eurolux, and Defen at different temperatures with time. It was noted that the rate of degradation increased with the rise in temperatures, which implies that the breakdown of pesticides is promoted under conditions of higher temperatures. Among the substances, Eurolux showed a faster degradation rate, whereas Monocil showed a higher degree of stability during the considered pH time. b) The illustration depicts the degradation trends of Monocil, Malathion, Eurolux, and Defen across various pH levels. The rates of degradation are presented in relation to changes in pH, highlighting the stability and decomposition of each pesticide over time. Eurolux demonstrated a higher rate of degradation, whereas Monocil displayed increased stability throughout the examined pH ranges.

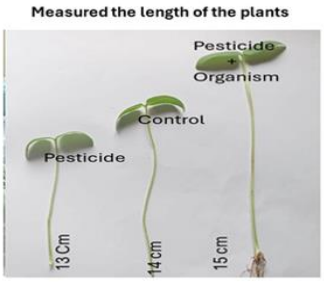
M9 broths were prepared in 12 conical flasks, 6 of which were used as controls and the other 6 as test samples, with pH adjusted to 6, 7, 8, and 9. The samples were then inoculated with the organism and incubated for 72 hours. Absorbance was measured, and the percentage of degradation was calculated.

Enzymatic Assays for Plant Growth and Pesticide Degradation

The detection of Glutamate Dehydrogenase (GDH) and Alkaline Phosphatase (ALP) enzyme activity, as illustrated in the activities discussed, reveals a significant variation in activity across the treatments. The measurement of GDH activity at 340 nm indicates a decreasing trend in the control + Pesticides group, which serves as an indicator of pesticide inhibition. Conversely, a partial recovery is observed in the control + Organism + Pesticides group, suggesting detoxification or acclimatization by the microbes. Likewise, the ALP activity measured at 410 nm was diminished in the presence of pesticides, but it increased slightly in the presence of the organism, indicating that pesticides may promote biodegradation or reduce toxicity (Bhalerao & Puranik, 2007; Bhatt, 2020; Bokade, 2023; Arifa *et al*., 2024). These results demonstrate that pesticides hinder the metabolism of nitrogen and phosphates, while microbial growth alleviates the suppression of enzymes. The noted recovery implies microbial degradation and protection. These findings suggest that microbial bioremediation has the potential to restore an enzymatic balance within systems contaminated with pesticides (Patel, 2022) **(Figure 4)**.

a)

b)



c)

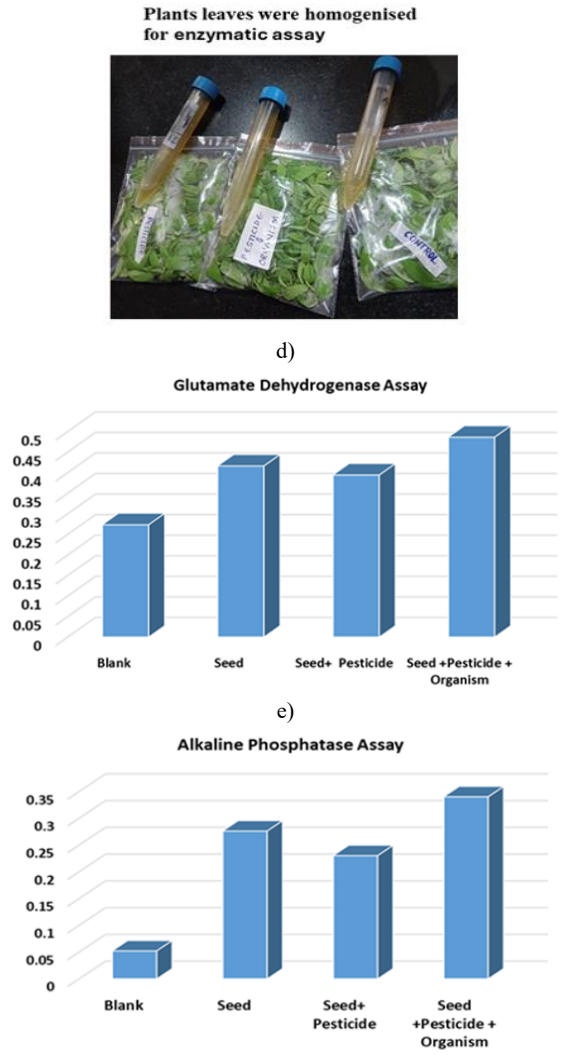


Figure 4. The figure suggests the activity of alkaline phosphatase (ALP) and glutamate dehydrogenase (GDH) in various conditions. The control + Pesticides group showed a decrease in GDH activity (340 nm), which was an indication of enzyme inhibition, whereas partial recovery in the control + Organism + Pesticides group was an indication of microbial detoxification. The same trend was identified in the case of the ALP activity (410 nm), which demonstrates the possibility of involvement of microorganisms in counteracting the inhibitive effects of the pesticides and promoting growth.

UV Visible Spectral Analysis

Malathion, Defen, Eurolux, and Monocil were examined using UV-Vis spectrophotometry, revealing λmax values of 211, 203, 214, and 228 nm, respectively. The analysis indicated that Eurolux (214 nm) demonstrated the most significant degradation during UV-induced degradation, which correlates with its lower persistence regarding molecular structure and photostability (Sharma *et al*., 2021). The reduced absorbance intensity facilitated effective photolytic degradation. In contrast, Monocil (228 nm) exhibited a low degradation rate, suggesting greater environmental persistence. Eurolux is recognized as a rapidly degradable pesticide, typically regarded as safer and less ecotoxic (Kumar & Verma, 2020). UV-based assessments are effective for evaluating pesticide stability and degradation (Gupta *et al*., 2019). It is recommended to perform further research on degradation kinetics under varying environmental conditions (Patel & Mehta, 2022) **(Figure 5)**.

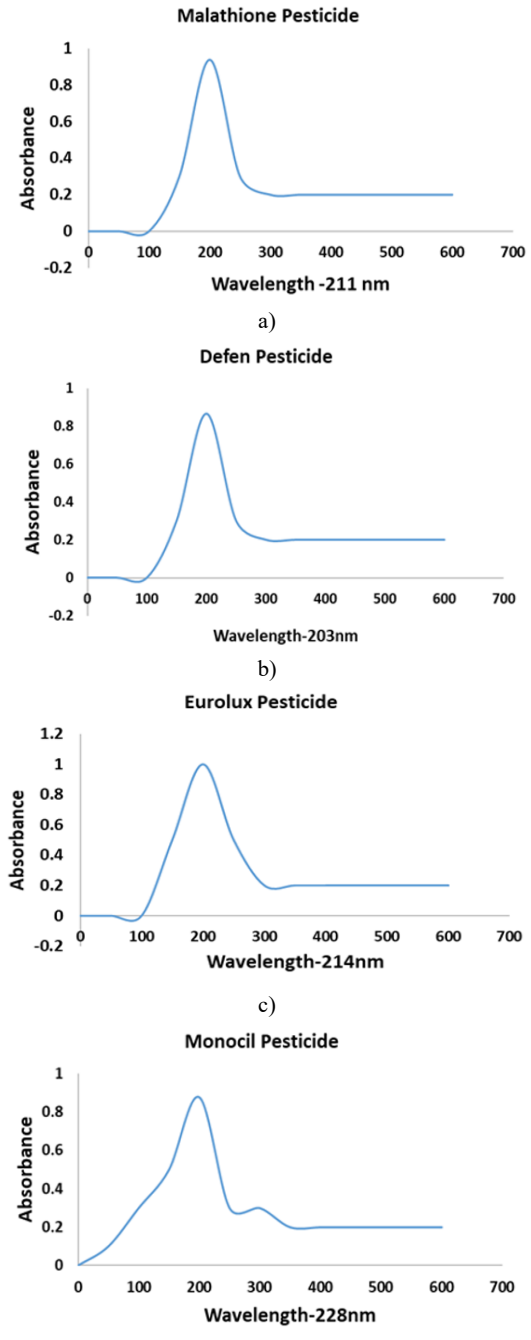
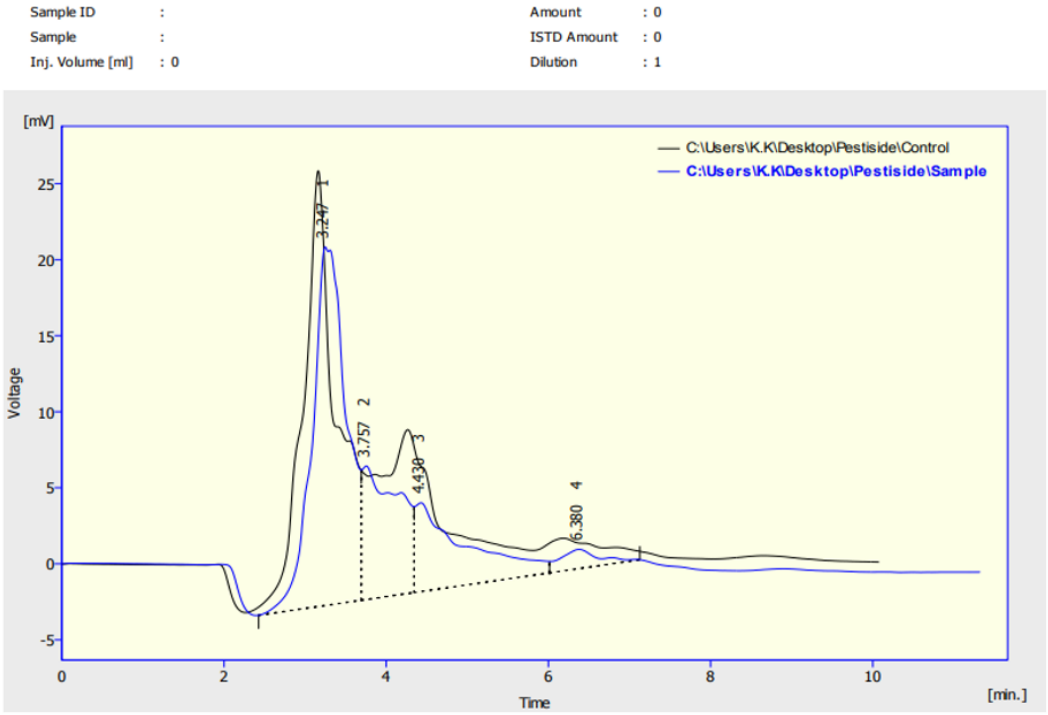


Figure 5. The figure shows UV-Vis absorption spectra of four pesticides with their max absorbance wavelengths (λmax): Malathion 211nm, Defen 203nm, Eurolux 214nm, and Monocil 228nm. Interestingly, Eurolux showed the highest degree of degradation, as shown by spectral response to UV.

HPLC

The study identified and confirmed the presence of microorganisms capable of degrading herbicides, insecticides, and pesticides, with HPLC showing over 70 percent degradation in certain Gram-negative strains within 72 hours. The degradation efficiency was influenced by factors such as soil pH, organic matter content, and microbial diversity, peaking in neutral to slightly alkaline soils (Gupta & Verma, 2021). These findings support the use of microbial bioremediation to reduce pesticide pollution. Further genomic and enzymatic research is necessary to enhance the degradation efficiency (Sharma *et al*., 2019; Patel *et al*., 2020) **(Figure 6)**.



Chromatographic data presents the retention time, peak area, height, and the relative percentage of compounds. Retention time signifies the order of elution, whereas area and height reflect the quantity and intensity of the analyte.

S/No	Retention Time (min)	Area (mV.s)	Height (mV)	Area (%)
1	3.247	687.425	23.580	54.7
2	3.757	275.010	8.775	21.9
3	4.430	252.080	5.833	20.1
4	6.380	41.895	1.256	3.3
	Total	1256.409	39.444	100.0

Figure 6. The microbial degradation of four specific pesticides (Monocil, Malathion, Eurolux, and Defen) over time using chromatograms from High-Performance Liquid Chromatography (HPLC). The retention time, size, and height of the chromatographic peaks correspond to the concentration of each individual constituent. Pesticide degradation is indicated by a progressive drop in peak height and area, with gram-negative bacterial isolates showing the most notable disintegration. The efficiency of microbial bioremediation in reducing pesticide residues was demonstrated by the over 70% degradation that was attained in just 72 hours.

Conclusion

This study found microbial isolates that could effectively degrade pesticides with attention to Eurolux and nitrogen-fixing bacteria (NFB). UV-Vis spectrophotometry and HPLC revealed that Eurolux was most degraded, and it was vulnerable to degradation by microorganisms. The best degradation was at 40-45 °C after 72-96 hours, which indicates the importance of the environmental factors. The presence of NFB in soil implies that it has dual functions to increase soil fertility as well as aid bioremediation. Such isolates as Gluconacetobacter sp. and Pseudomonas sp. had a great potential for pesticide degradation. Results of HPLC showed that microbial activity caused more than 70% of Eurolux destruction, which was better than chemical remediation. There was better degradation of gram-negative bacteria, which is probably because they have the enzyme abilities to degrade complex pesticides. The pH, organic matter, and the diversity of microbes were among the factors that played a vital role in determining the rate of degradation.

Microbial consortia, in particular, NFB, provide sustainable pesticide removal and enhanced soil health. Future researches need

to investigate genetic and enzymatic processes and conduct field experiments to give agricultural applications.

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