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Rejuvenation of Pesticide Polluted Soil from the Isolated Microbial Flora of Agricultural Field

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Abstract - **The widespread use of pesticides leads to imbalances in the qualities of soil, water, and air environments. Pesticides can only be broken down more quickly by combining microbial consortia of native and naturally occurring bacteria isolated from certain polluted environments. To rejuvenate the pesticide polluted soil, the samples was collected and microbial flora were isolated from the soil samples. These floras were introduced into the three pesticides like Carbaryl, Monocrotophos and Malathion (CMM) for the biodegradation activity. Standard microbiological protocols were followed to isolate the microbial flora from the collected soil samples. The isolated microbial strains were confirmed with the isolation of genomic DNA. The biodegradation of pesticides such as Carbaryl, Monocrotophos and Malathion (CMM) were performed with the isolated strains. The bacterial strains such as *Pseudomonas sp, Bacillus sp and Micrococcus sp;* actinomycetes – Azatobacter; fungal species such as *Aspergillus fumigatus, Gliocladium sp and Humicola sp* were isolated from the agricultural field. Among these Bacillus sp showed highest biodegradation activity against the three pesticides. These investigations screened the best microorganism for several pesticides, including carbaryl, Monocrotophos, and Malathion (CMM), the best degradation methods, and the best degradation environment, providing a more practical reference for subsequent study.**
Keywords: ***Aspergillus fumigatus*****,** ***Bacillus sp*****, Carbaryl, Monocrotophos, Malathion,** ***Pseudomonas and Humicola sp.***

I. INTRODUCTION

Due to the issue of natural degradation, the widespread use of pesticides leads to imbalances in the qualities of soil, water, and air environments. These substances cause a variety of environmental issues via biomagnifications. Currently, one of the key methods for removing and degrading pesticide from agricultural soils is microbial degradation. According to certain research, genetically modified microorganisms can break down a particular pesticide, but the difficulty is that they can't be used in the field since they have other negative effects on the environment. Pesticides can only be broken down more quickly by combining microbial consortia of native and naturally occurring bacteria isolated from certain polluted environments.

The bioaugmentation procedures, such as the injection of essential nutrients or organic matter, are necessary to hasten the pace at which a pollutant is broken down by the local bacteria. Environmentally speaking, using native microbial strains with plant development activities is preferable to using chemical approaches. In this review, we have tried to explore the current issue of pesticides in soil environments and their biodegradation with the aid of efficient native microorganisms that degrade pesticides. Furthermore, using powerful native microbial consortia, we emphasized and investigated the molecular mechanism for pesticide breakdown in soil. According to this study, the environmentally benign technique of pesticide-degrading microbial consortia may be suited for the creation of sustainable agriculture [1].

Pesticides can be broken down by microbes from a variety of sources. Pesticide degradation-related bacteria have often been isolated from a number of pesticide-contaminated locales. When these chemicals are used on agricultural crops, they mostly end up in the soil; additionally, the effluent from the pesticide industry, sewage sludge, activated sludge, wastewater, natural waters, sediments, and areas near the manufacture of pesticides are also rich sources of pesticide degrader. There are currently collections of microbes that have been identified and characterized for their capacity to degrade pesticides in various laboratories across the world. The identification and characterization of pesticide-degrading microbes can provide new methods for cleaning up polluted areas or handling trash before it becomes a problem.

Following complete biodegradation of the pesticide, the parent molecule is oxidised to produce carbon dioxide and water, which gives the microorganisms energy for metabolism. Microbes' intracellular or extracellular enzymes are crucial for the breakdown of chemical substances [2]. The entire global grain output has climbed from 500 million tonnes to 700 million tonnes since the turn of the 20th century [3]. Among them, grains make about 80% of the food that people consume. Pests put food in danger during natural growth or storage.

For instance, while being primarily an agricultural nation, China annually loses 40 million tonnes of grain - or 8.8% of its overall output - to a variety of insect pests [4]. India produces 250 million tonnes of grain annually on average, but due to pests and other factors, it loses 11 to 15 percent

of that amount, or 27.5 to 37.5 million tonnes annually [5]. Pesticides are frequently used to manage household and agricultural pests in order to prevent these losses [6]. After pesticides were used, there was a significant decrease in food loss; however, these pesticides are widely dispersed in the soil, water, air, and agricultural goods. Consequently, the widespread use of pesticides poses a serious threat to the ecosystem [7, 8]. They directly endanger human health and the environment by polluting the marine environment and ground water in addition to the land and crops [9].

A significant amount of garbage and pollutants are currently released into the environment as a result of human activity. More than one billion pounds of pollutants are discharged into the atmosphere and water each year. There have been created around 6x106 chemical compounds; every year, 1,000 new goods are created by synthesis, and between 60,000 and 95,000 chemicals are used in industry [10]. Chemical pesticides, which are widely employed in most areas of crop production to reduce pest infestations, safeguard crop yield losses, and prevent lowering product quality, are among these compounds.

We can start with the following two factors in order to resolve the conflict between agricultural products with high yield or stable production and environmental degradation. Finding and developing pesticides with low toxicity, high effectiveness, and low pesticide residues are important, but equally important are methods for decomposing pesticide residues. Studies on the microbial breakdown of pesticide residues began in the 1940s, and as environmental concerns have grown, so has the depth of research on the process and mechanism of organic pollutant degradation. It would not result in secondary contamination and would be inexpensive and environmentally friendly for bacteria found in nature to break down pesticide residues. However, the effectiveness was somewhat low, and the environment was both complicated and unstable, which might have an impact on the viability and effectiveness of pesticides being broken down by microbes. As a result, scientists have carefully studied microbes and have a comprehensive grasp of how organic pesticides degrade.

Numerous microorganisms that could break down and change pesticides have been identified from this group [11, 12, 13]. Additionally, the principal mechanisms by which pesticides degrade were detailed in detail [12, 14]. According to studies, the majority of biodegradable pesticides are concentrated in the soil's microorganisms, such as fungus, bacteria, and actinomycetes [15], of which fungi and bacteria play the primary roles. More in-depth research can be done since the bacteria were simple to produce mutant strains of, with a diversity of biochemical capacity to adapt environment [16]. Insecticide, herbicide, fungicide, and other substances used to manage pests are all examples of pesticides. A pesticide is any substance or mixture of substances designed for preventing, eliminating, repelling, or reducing any pest (insects, mites, nematodes, weeds, rodents, etc). [17]. The term "pesticide" has different meanings in different eras and nations. However, the fundamental nature of a pesticide is essentially unchanged: it is a substance (mixed) that is harmful and effective against the target organisms while being safe for the environment and untargeted organisms [18]. Pesticides are consumed worldwide on a yearly basis in quantities of about two million tonnes, of which the United States consumes 24%, Europe 45%, and the rest of the world (25%). China, Korea, Japan, and India are the Asian nations with the greatest pesticide usage rates, respectively. About 0.5 kg/ha of pesticides are used in India, with organochlorine pesticides making up the majority of that amount. This is due to an increase in insect pest attacks, which are mostly brought on by the current warm, humid climate. Since pesticides have a major impact on both human and animal health due to their biological stability and higher degree of lipophilicity in food commodities [19], their continued usage causes them to accumulate in soil as well as other environmental factors.

The promotion of High Yielding Varieties that characterized the Green Revolution has resulted in widespread usage of chemicals as pesticides, which is the primary reason of the rising need for pesticides. The main reason for the rise in demand for pesticides is the widespread use of pesticides during the green revolution to encourage high yielding varieties. India currently ranks 12th in the world for pesticide use and is the largest producer of pesticides in Asia, producing 90,000 tons of pesticides annually. India's plant protection strategy included the environmentally friendly integrated pest management (IPM) method as one of its guiding principles [20].

Chemical pesticides can be categorized in a variety of ways, but one of the most popular is based on their chemical make-up. This method enables the uniform and scientific grouping of pesticides and enables the establishment of relationships between their structure, activity, toxicity, and degradation mechanisms, among other things. Crop damage is caused by 8,000 species of weeds, 9,000 species of insects and mites, and 50,000 types of plant pathogens worldwide. Different pests, like insects and plants, are thought to be responsible for 14 percent and 13 percent, respectively, of losses. In the production of agricultural products, pesticides are essential. The production of about one-third of agricultural products involves the use of pesticides. Without the use of pesticides, the percentage of fruits, vegetables, and grains lost due to pest damage would be 78%, 54%, and 32%, respectively. Pesticide application reduces crop loss caused by pests to between 35 and 42 percent [4].

Pesticides are mostly used in agriculture, which uses more than 4 million tons of pesticides annually. Since they have been widely utilized for decades, pesticides have significantly enhanced food production [7]. However, because of their degradation, volatilization, and leaching, a significant portion of administered pesticides frequently fail to reach their intended target, posing substantial ecological

issues [21]. Different types of pesticides are frequently sprayed concurrently or successively during actual agricultural practices, interacting with one another [22]. In addition to providing a wealth of data and information for future research on the biodegradation of pesticides like carbaryl, Monocrotophos, and Malathion (CMM), the current study also improved and enhanced the theory underlying the biodegradation of the aforementioned pesticides. The degradation of Carbaryl, Monocrotophos, and Malathion was also examined in this paper. An investigation into the microbial breakdown of CMM was conducted, and resources for bacteria that can break down materials were enriched. As a result, the current study concentrated on important meaning and value to serve as a reference for other studies, such as the biodegradation of other types of pesticides or veterinary drugs, using CMM pesticides to biodegrade environmental pollution, and removing or reducing pesticide residues in agricultural products.

II. MATERIALS AND METHODS

A. Collection and Transportation of Agricultural Field Sample

The current study was conducted in five separate villages in the Vandalur-Kelambakkam main road in Tamil Nadu's Chengalpattu District, 43 KM from Chennai, India (Fig.1 and 2). The following suburban regions were picked to study the biodegradation of pesticides: Mambakkam, Kandigai, Keerapakkam, Kolapakkam, and Vengadamangalam (Figs. 3, 4, 5, 6, and 7). Pesticide-related soil samples were taken at a depth of 1 to 5 cm. In a screw-capped, sterile plastic container, samples were gathered and transported to the lab. The neighbourhood pesticide store provided pesticides including Carbaryl, Monocrotophos, and Malathion (CMM).

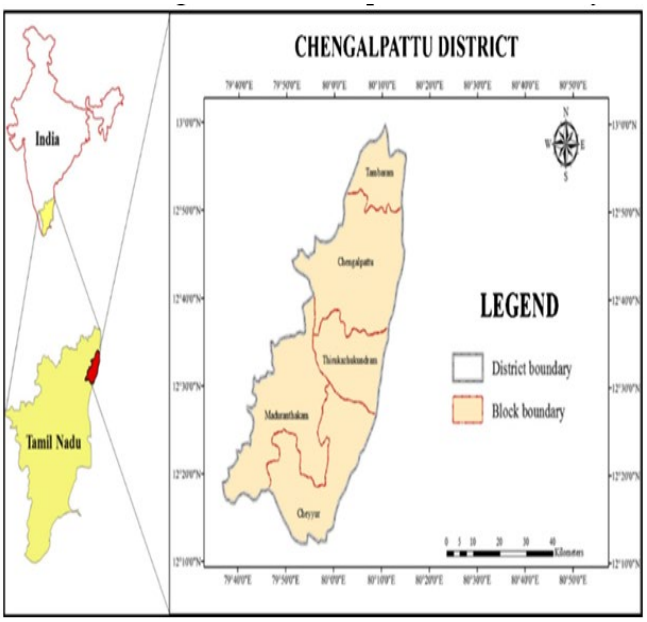


Fig. 1 Map showing the Chengalpattu District

B. Identification and Characterization of Microbes from Contaminated Soil

To isolate bacteria, the collected samples were examined. A sample of soil contaminated with pesticides weighing 1 gram was obtained, and 10 ml of distilled water was added. The combination was mixed before being diluted serially in the range of 10^{-1} to 10^{-7}. By using the spread plate method, 0.1ml of aliquot was put onto the mineral salt medium (MSM). Spread plate approach was used for screening for fungi on Rose Bengal Agar, Potato Dextrose Agar, for Actinomycetes on Actinomycetes Agar.

Three replica plates were retained for the complete sample and kept for the incubation of the bacteria (at 37°C for 24 hours), actinomycetes (for two to five days), and room temperature for fungi, for three to four days. On the culture plate, microorganisms started to proliferate after the incubation. In agar slants, the isolated colonies were subculture and kept at preservation temperature.

Based on the macroscopic and microscopic studies, Determinative Bacteriology Manual by Bergy's was referred to determine the type of bacterial colonies. By using the key characteristics and the lacto phenol cotton blue staining procedure, the fungus was identified. To identify the characteristics of actinomycetes, guidelines were followed. The bacterial isolates were identified according to the key of Bergy's Manual.

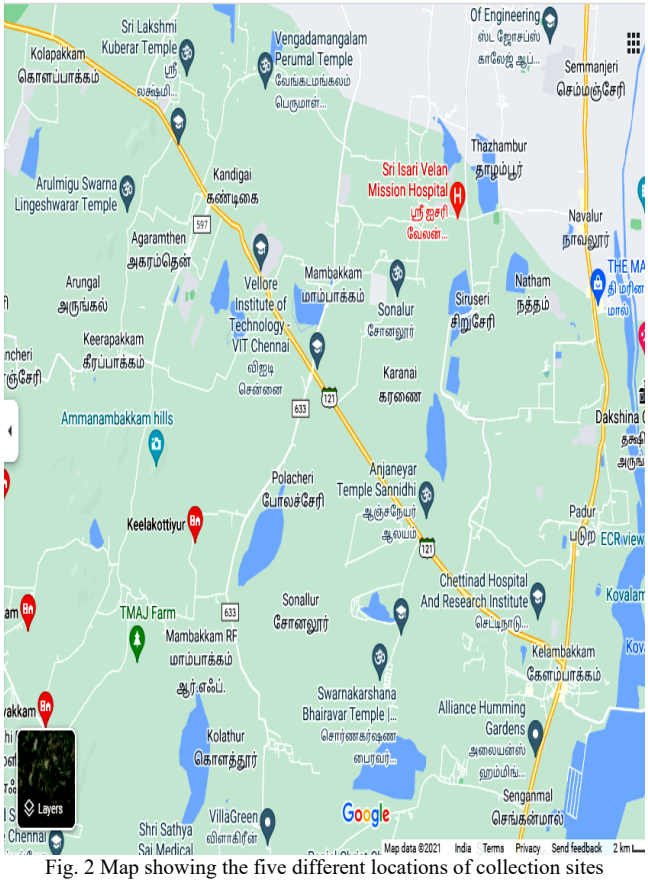


Fig. 2 Map showing the five different locations of collection sites

Fig. 3 Mambakkam collection site

Fig. 4 Kandigai collection site

Fig. 5 Keerapakkam collection site

Fig. 6 Kolapakkam collection site

Fig. 7 Agricultural field of Vengadamangalam collection site

C. Biodegradation Activity

Using little salt broth, the development of isolates that degrade pesticides was assessed. For this, 50 ml broth of mineral salt and pesticide 1 ml of was inoculated with 1 millilitre of the bacterial inoculums. The flasks were then incubated in a microbial shaker at 150 rpm for 20 days at 27°C. Five ml of culture were drawn and centrifuged for ten minutes at 5000 rpm. The particle was thrown away, and the supernatant was gathered to monitor the development of bacteria that break down pesticides. After 0, 5, 10, 15, 20 and 25 days of exposure with the culture media the development of the microorganisms that break down pesticides was measured at 640 nm through the UV spectrophotometer.

D. Thin Layer Chromatography

The plate was evenly covered in silica gel, which was then given time to dry and stabilize. For 30 minutes, activated TLC plates were kept in a hot air oven set at 105°C. The TLC plate was loaded with samples, which were then air dried. A few centimetres above the chamber bottom, the mobile phase was transferred into the TLC chamber. The prepared plate with sample spotting will then be put in the TLC chamber with the sample line side towards the mobile phase. After that, a cover was placed on the chamber. There was enough time for the formation of spots on the TLC plates. The plates were then taken out and left to dry. After adding the developing reagent on the dry plates, the sample spots was observed through UV light.

E. Isolation of Genomic DNA from the Isolated Strains

A micro centrifuge tube was filled with 1.5 ml of an isolated microbial culture, and the tube was spun for 2 minutes to separate the supernatant. Pipetting was used many times to resuspension the pellet in 467 l of TE buffer. The proteinase K was combined with 30 l of 10% SDS and 3 l of 20 mg/ml before being incubated for one hour at 37°C. The phases

were thoroughly mixed by inverting the tube after adding an equal volume of phenol and chloroform. For two minutes, the tubes were spun. A fresh tube was used to transfer the upper aqueous phase and phenol/chloroform was added in equal volume. The mixture was spun for 2 minutes once more. A fresh tube was used to transfer the upper aqueous phase. As the DNA precipitate occurred, 1/10 volume of sodium acetate and 0.6 volume of Isopropanol were added and gently mixed. 1 cc of 70% ethanol was added to the DNA to wash it for 30 seconds. TE buffer containing 100 to 200 l of DNA was resuspended. After being electrophoresis in 1 percent agarose gel, the isolated material was examined under a UV transilluminator to reveal the bands.

III. RESULTS OF THE STUDY

The recent analysis revealed some interesting information. On the culture plate, the isolated microbial colonies could be seen. The two samples with the highest bacterial loads were Vengadamangalam (193X 10^{-7} CFU/ml) and Kolapakkam (219X 10^{-7} CFU/ml). All of the places had colonies that were raised, margined, and coloured white, yellow, and brown. One of the isolated strains yielded positive results in the Gram's staining whereas the other yielded negative results. The endospores of Bacillus sp. were stained. The screened organisms were identified as *Pseudomonas sp, Bacillus sp, Micrococcus sp,* and *Azatobacter sp*. based on the Bergey's manual's reference (Fig. 8, 9 and Table I). Kolapakkam had the highest concentration of actinomycetes colonies ($8X10^{-5}$ CFU/ml), while Mambakkam and Keerapakkam only displayed two colonies ($2X10^{-5}$ CFU/ml). There were no actinomycetes found in Kandigai or Vengadamangalam (Fig. 8 and Table I). In Keerapakkam, Kolapakkam, and Vengadamangalam, two fungal colonies with a density of $2X10^{-4}$CFU/ml were found. In Mambakkam and Kandigai, no fungus colonies were developed (Fig. 8 and Table I).

TABLE I MICROBIAL COUNT OF PESTICIDE CONTAMINATED SOIL IN CFU/ML

Sl. No.	Sample Collection Site	Bacteria	Fungi	Actinomycetes
1	Mambakkam	$132X10^{-7}$	Absent	$2X10^{-5}$
2	Kandigai	$158\ X10^{-7}$	Absent	Absent
3	Keerapakkam	$64\ X10^{-6}$	$2X10^{-4}$	$2\ X\ 10^{-5}$
4	Kolapakkam	$219\ X10^{-6}$	$2\ X\ 10^{-4}$	$8\ X\ 10^{-5}$
5	Vengadamangalam	$193\ X10^{-7}$	$2X10^{-4}$	Absent

The following morphological features were observed in the colonies on the plates such as whitish pale, flat, round, opaque, medium and dull-white, complete, opaque, umbonate or pulvinate, irregular, huge according to the sample taken from the Mambakkam. The white-dull, entire, pulvinate or umbonate, opaque, irregular, huge colonies were seen in the case of Kandigai, as well as convex, entire, white, translucent or opaque, medium, circular. While the colonies in Keerapakkam were white-dull, whole, pulvinate or umbonate, opaque, irregular, and huge, they were creamish-white, elevated, translucent, irregular, and large. The morphological features were observed on the plate colonies was noticed as white, round, elevated, opaque, round, large in the sample taken from the Kolapakkam, and white-dull, whole, pulvinate or umbonate, opaque, irregular, huge. Vengadamangalam had white-dull, whole, pulvinate or umbonate, opaque, irregular, huge colonies as well as white-dull, wavy, umbonate, large, motile, and these characteristics has been represented in (Fig. 8 and Table II). Biochemical studies also disclosed some interesting findings (fig.10 and Table III).

Two distinct shady green and white spongy colonies were seen in the first fungal plate. In contrast, cream-white colored colonies were seen on the second plate. A lacto phenol cotton blue staining experiment produced some intriguing findings. The first plate displayed a stalk with a sterigmata cluster. Conidiophores were seen on culture plate 2's Micronematous, rough-walled conidia with one to three septa (Fig. 11, 12 and Table IV). Additionally, the results of the genomic DNA isolation for the detected organisms were noteworthy. The first lane included a 500 bp ladder, then *Pseudomonas sp*, *Azatobacter sp, Bacillus sp, Micrococcus sp, Aspergillus fumigatus*, *Gliocladium sp* and *Humicola sp*, in that order, and finally Pseudomonas sp. Under a UV transilluminator, the DNA bands were observed in gel plates.

TABLE II CHARACTERIZATION OF BACTERIAL STRAIN FROM THE CONTAMINATED SOIL

Sl. No.	Colony Morphology	Pseudomonas sp	Bacillus sp	Micrococcus sp	Azatobacter sp
1	Colour of the colony	White	Whitish pale	Yellow-dull	White
2	Margin	intact	Spherical	Wavy	whole
3	Elevation	Convex	Smooth	Umbonate	Raised
4	Opaque / Translucent	thick	dense	Opaque	semi-transparent
5	Nature of shape	rounded	around	crooked	uneven
6	Dimension	average	intermediate	huge	large

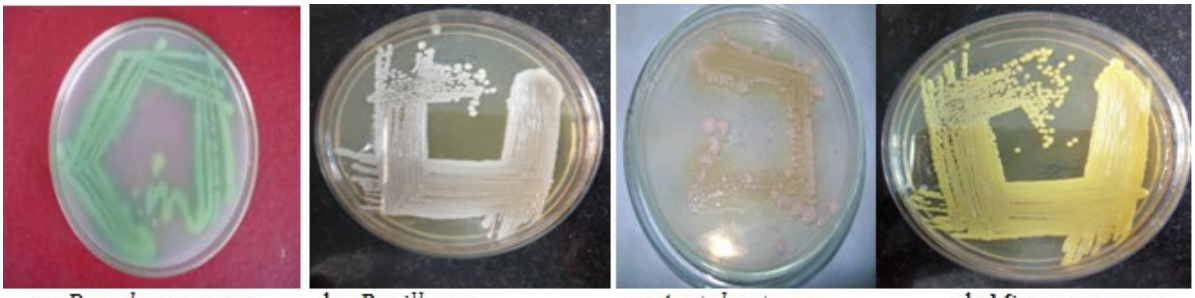


Fig. 8 Colony morphology of isolated strains from five different collection sites

Based on the presence of light blue green colour spots on the silica gel plates, bioactive substances were discovered using thin layer chromatography. The UV transilluminator was used to observe the TLC plates. Calculations were made to determine the distance from the starting point to the finish line. The degraded pesticide sample's Rf value has been used to confirm the existence of degradable chemicals (Fig. 14). The degradation of three different pesticides such as Carbaryl, Monocrotophos and Malathion is concerned the following results has been observed. Among the bacteria *Bacillus sp* had a high efficiency to degrade Carbaryl with rate 76% and rate 65% with Monocrotophos and less efficiency for Malathion with degradation rate 52%. Whereas the *Pseudomonas* degraded the Monocrotophos with high potential 57%, followed by Carbaryl with 47% and Malathion with 26%. Whereas the *Micrococcus sp* degraded the pesticides such as Carbaryl, Monocrotophos and Malathion with the degradation potential of 61%, 74% and 82% respectively. The interesting facts revealed when the Azotobacter was treated with the pesticides with the percentage of 33%, 48% and 37% for Carbaryl, Monocrotophos and Malathion. In the case fungal isolations highest degradation was observed in *Gliocladium sp* with 78% in Carbaryl; 62 % in Monocrotophos and 68% in Malathion. *Aspergillus fumigatus* showed maximum degradation in Malathion with 75%, minimum was observed in Monocrotophos with 29% and moderate 58% was recorded in Carbaryl. As for as the *Humicola sp* is concerned, almost similar degradation was observed in Carbaryl and Malathion with 64% and 65% respectively. The lowest was recorded in Monocrotophos with 43% (Fig. 15 and Table V).

IV. DISCUSSION OF THE STUDY

Pesticides can be bio transformed using various biological systems, such as bacteria. When pesticides are used on a regular basis, some of the soil biota may swiftly develop the capacity to breakdown them. In order to remediate pesticide-contaminated locations, these compounds serve as carbon sources and electron donors for soil microorganisms [23].

TABLE III BIOCHEMICAL TEST FOR PSEUDOMONAS SP, BACILLUS SP MICROCOCCUS SP AND AZATOBACTER SP

Sl. No.	Test	Pseudomonas sp	Bacillus sp	Micrococcus sp	Azatobacter sp
1	Mineral salt medium	Light yellow colonies	Creamy white colour colonies	Yellow	Creamish White
2	Gram staining	Gram negative rod	Gram positive rods	Gram positive rods	Gram positive rods
3	Motility	Motile	Motile	Non-Motile	Motile
4	Endospore staining	-	+	-	-
5	Catalase	+	-	+	+
6	Oxidase	+	-	+	+
7	Indole	-	-	-	+
8	Methyl red	-	-	-	+
9	Voges Proskauer	-	-	-	+
10	Citrate	+	+	-	+
11	Urease	+	-	+	+
12	TSI	-	-	-	+

It is also possible to employ pesticide degradation microbes for bioremediation of other chemical substances for which a degradation pathway by microbes is already recognized. In addition to the presence of microorganisms with degrading enzymes, a wide variety of environmental factors are necessary for the transformation of these substances. In addition, the microbial transformation of contaminants is dependent on a number of physiological, ecological, biochemical, and molecular factors.

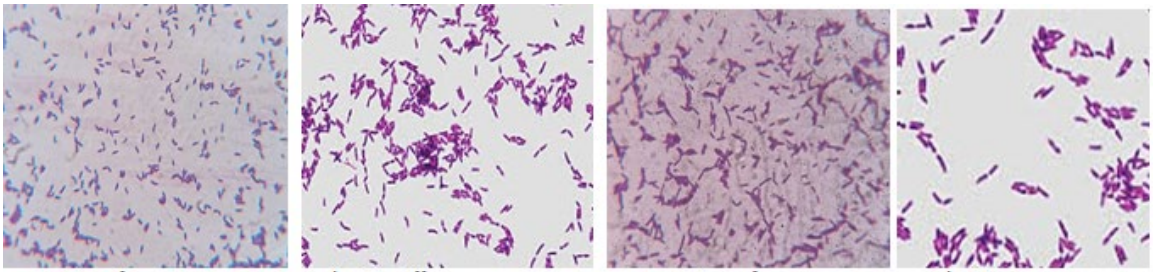


Fig. 9 Photomicrograph of isolated bacterial strains under 100X magnification

Pesticide-degrading microorganisms originate from a range of sources. Besides pesticide industry's effluent, sewage sludge, activated sludge, wastewater, natural waterways, sediments, and regions around the manufacturing of pesticides, soil is the medium that receives the majority of these chemicals since pesticides are usually applied to agricultural crops. A wide range of pesticide-contaminated locations have yielded microbes that have been recognized as pesticide degraders. Microorganisms capable of identifying, growing, and degrading pesticides may be found in laboratories across the world today. Counting on new instruments to repair damaged habitats or treat trash before ultimate disposal is made possible by the identification and characterization of microorganisms that breakdown pesticides [24]. Environmental pollution can be eliminated by microbial activities. Microbiology and geochemistry are the foundations of progress in biodegradation biotechnology. It has become more important to study microorganisms in order to further our understanding of biodegradation (in general) and aromatic-hydrocarbon biodegradation (in particular) in recent years. By using cutting-edge molecular and analytical techniques (such as sequencing microorganisms' DNA), we've gained a better understanding of the processes (how), products (what), and individuals (who) involved in the biodegradation of organic contaminants. *Pseudomonas* is the most effective bacterial genus for the breakdown of hazardous substances, according to Abo-Amer, 2012. The bacteria's capacity to breakdown these substances is influenced by the amount of time they spend in touch with them, as well as the circumstances in which they grow and evolve. According to the degradation percentages of 99.8, 89.4, and 98.4 in another research by Pseudomonas species in the biodegradation of the herbicide aroclor 1242, these bacteria have a greater ability to breakdown it. Diverse fungus species were identified from Algerian pesticide-contaminated soils. *Aspergillus fumigatus*, *Aspergillus terreus*, *Absidia*, *Aspergillus niger* and *Corymberifera microsporis, Rhizopus microsporus* were found to be the most common isolates. The herbicide metribuzin was shown to be degraded by 53 of the isolated species in this investigation. Furthermore, it has been shown that this herbicide stimulated the growth of Absidia and Fusarium genera; these genera were able to remove 50% of the chemical in just 5 days. In addition, *Botrytis cinerea* and 31 additional isolated species were able to eradicate metroburon and linuron herbicides almost completely. Endosulfan and methyl parathion insecticides can be degraded by the fungus *Trichoderma viridae*, according to [25].

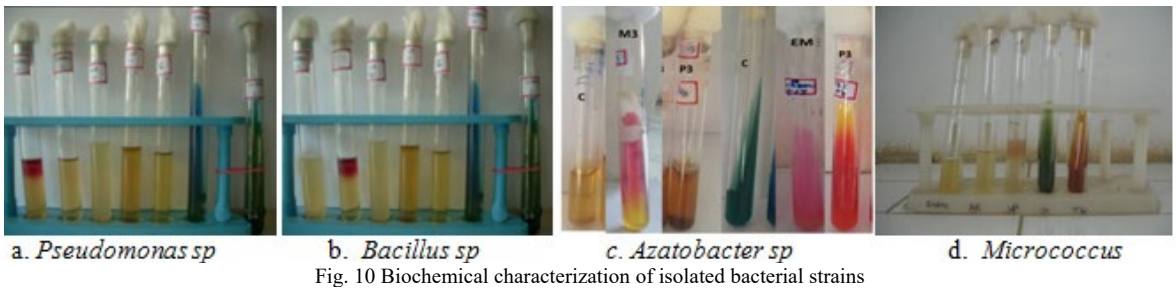
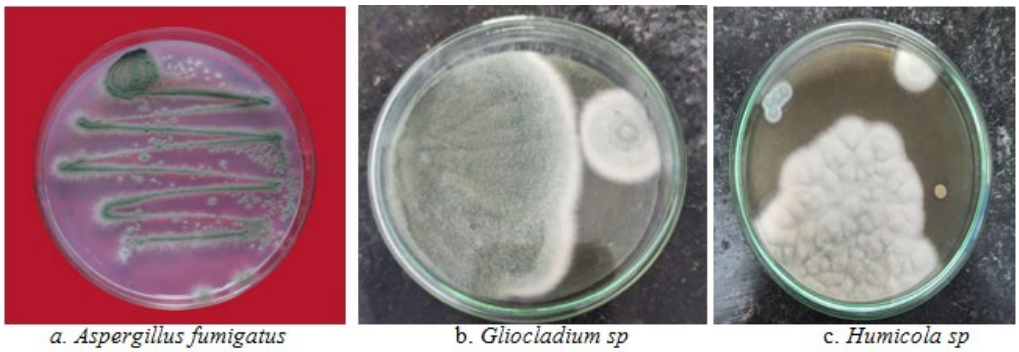


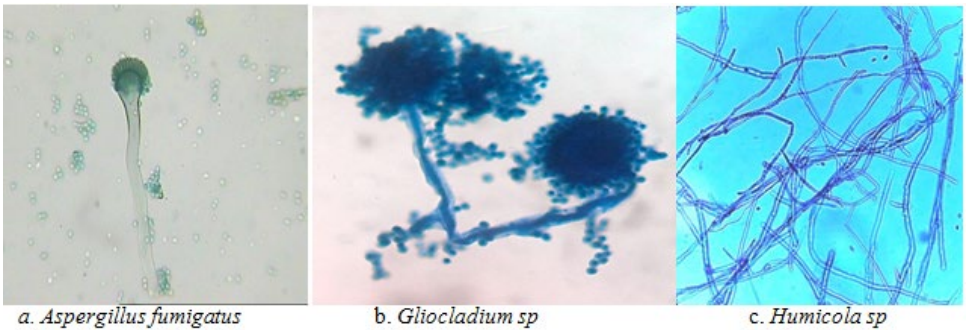
Fig. 10 Biochemical characterization of isolated bacterial strains

Many previous studies proved that *Rhodococcus sp*. is an efficient degrader of triazines to nitrate. Nitrite (30%), nitrous oxide (3.2%), ammonia (10%), and formaldehyde (30%) were generated as a result of microbial activity (27 percent). To thrive in pesticide-contaminated soils, a plethora of bacterium genera have evolved. Many organophosphorus insecticides are hydrolysed by enzymes in these microbes, including those present in P-O, P-F, P-S, and P-C bonds[6]. Pesticides such as ethyl-parathion and methyl-parathion can be degraded by soil microbes. The most promising, cost-effective, and efficient approach is using microorganisms to remove contaminants through biodegradation. Biodegradation refers to the breakdown of organic compounds into their inorganic components. There are a variety of reasons why microbial transformations may occur, including energy requirements or the need to remove contaminants (co-metabolism). In light of the fact that microorganisms are everywhere, plentiful, diverse, and capable of functioning even in the absence of oxygen and under other extreme conditions, as well as their ability to degrade pollutants even in the absence of oxygen, the search for pollutants-degrading microorganisms, understanding their genetics and biochemistry, and developing methods for their application is an important one [26].



a. *Aspergillus fumigatus* b. *Gliocladium sp* c. *Humicola sp*

Fig. 11 Colony morphology of fungal strains isolated from the contaminated soil



a. *Aspergillus fumigatus* b. *Gliocladium sp* c. *Humicola sp*

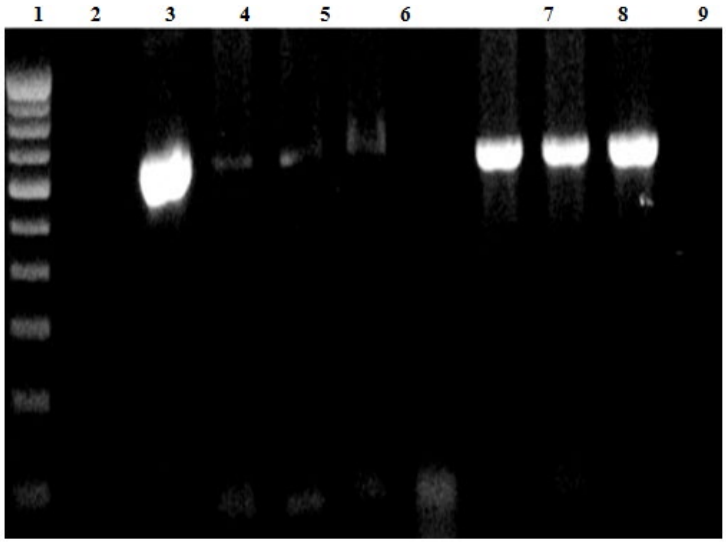
Fig. 12 Photomicrograph of fungal strains

There is a wide range of organic contaminants, but the diversity of microbial members and their ability to generate or breakdown organic substances is probably higher. Instead of a single strain, soil and aquatic microbial populations are made up of various, synergistic or antagonistic communities. Metabolic collaboration refers to the transfer of substrates and products among a well-coordinated microbial population in natural settings where biodegradation takes place. As a result of chemical and physical interactions, microorganisms can alter the structure of a material, or even degrade it completely, depending on the target molecule. Bacteria, fungus, and actinomycetes are the primary microbial communities that convert and breakdown pesticides. Pesticides and other xenobiotic can be biodegraded by fungi by adding tiny structural modifications to the molecule, making it non-toxic. It is then discharged into the environment, where microbes can further degrade the biodegraded herbicide [27].

TABLE IV MACRO AND MICROSCOPIC CHARACTERS OF FUNGAL STRAINS FROM THE CONTAMINATED SOIL SAMPLES

Sl. No.	Colony Morphology & Staining	Aspergillus fumigatus	Gliocladium sp	Humicola sp
1	Colony colour	Dark green	White to cream	White
2	Size	280-580 µm	240-460 µm	210-390 µm
3	Surface	Filamentous, elevated	Umbonate, elevated	Umbonate, elevated
4	Vesicle Serration	Biseriate	The terminal branches give rise to flask-shaped phialides.	Septate-forming densely coiled
5	Shape	Globose, ellipsoid	Rapidly growing, spreading and cottony colonies	Granule, chain
6	Medulla Covering	Entirely	Erect and branch repeatedly at their apices	Partially
7	Conidia Surface	Smooth, finely roughened	Slimy ball of one-celled	Micronematous, the conidia rough-walled with one to three septate

Extracellular enzyme-producing microorganisms such as fungi and bacteria are considered as excellent sources. It has been suggested that white rot fungus might be useful bioremediation agents, particularly for chemicals that are not easily destroyed by bacteria. An enzyme that can break down a wide range of organic molecules is responsible for this capacity. Lignoperoxidases and manganese peroxidases are among the extracellular enzymes that are involved in the breakdown of lignin, as are laccases and oxidases. Pesticide-degrading bacteria have been found in a growing number of samples, and the list continues to grow. Esterases, glutathione S-transferases (GSTs), and cytochrome P450 are the three major enzyme groups involved in degradation [27].



Lane 1 – 500bp ladder; Lane 2 –*Pseudomonas sp*; Lane 3 – *Bacillus sp*; Lane 4 & 5 – *Micrococcus sp*; Lane 6 – *Azatobacter sp*; Lane 7 – *Aspergillus fumigatus*; Lane 8 – *Gliocladium sp*; Lane 9 – *Humicola sp*
Fig. 13 Genomic DNA Isolation from the identified microbes

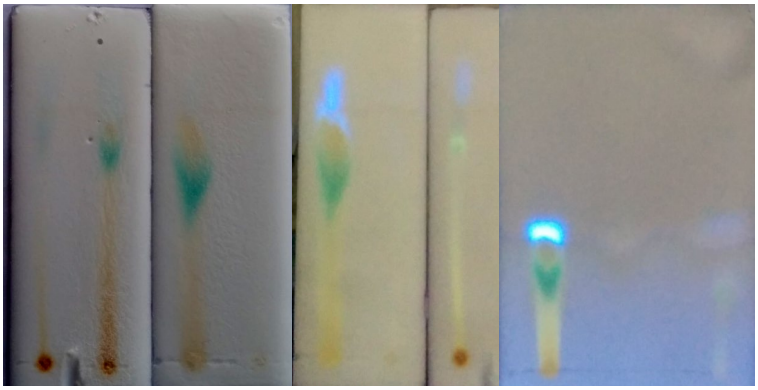
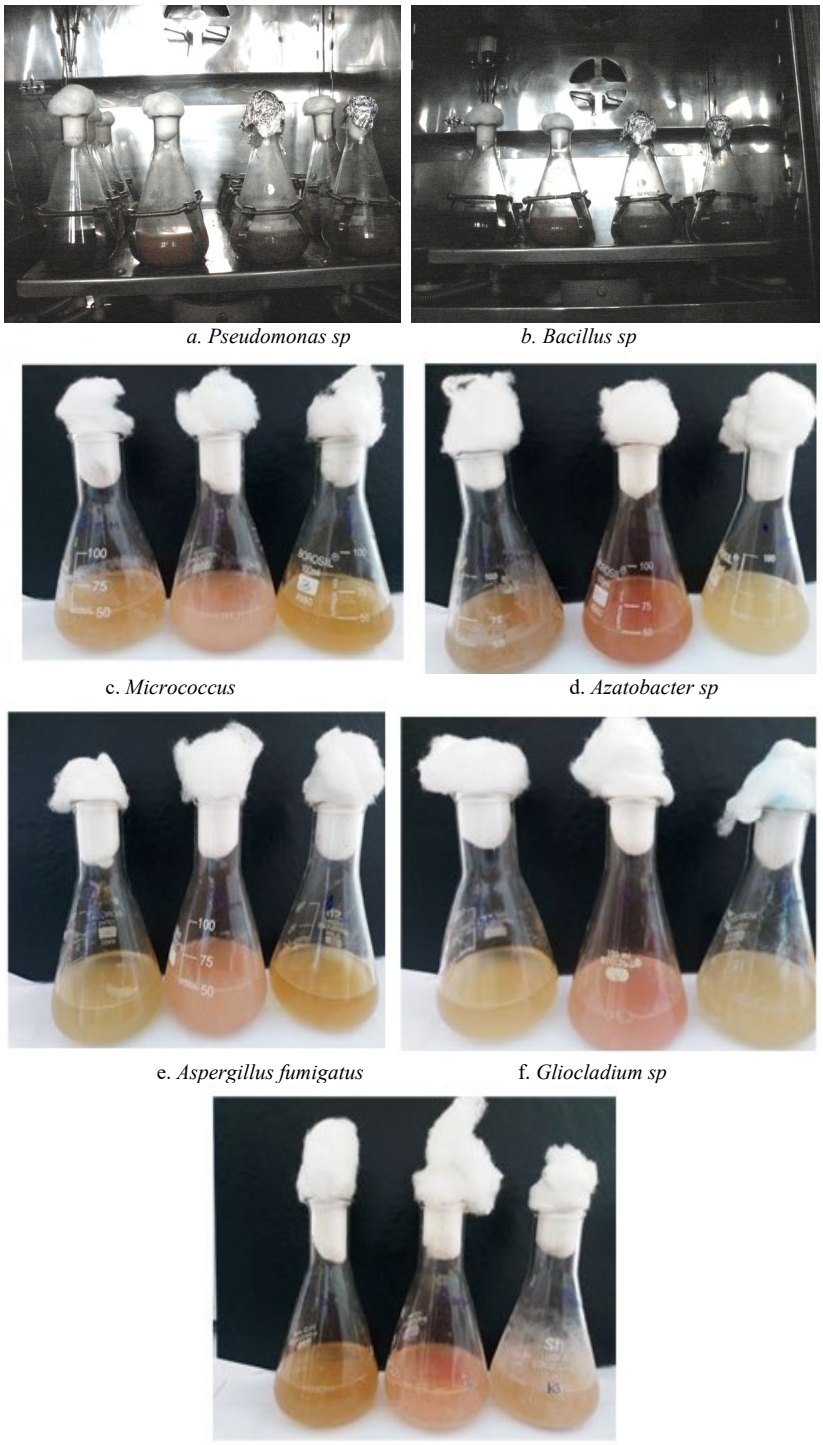


Fig. 14 The degraded compound of pesticides on TLC plates

Several insecticides' biology relies heavily on enzymes. It is a new treatment method that uses enzymes to convert or destroy pesticides in contaminated settings. Pesticide breakdown mediated by enzymes may be more efficient than current chemical techniques. Many pesticides work by targeting certain enzymes that play important physiological roles. Some pesticides are activated in situ by enzymatic activity, and many pesticides work by targeting these specific enzymes. Enzymes are involved in many different types of biochemical reactions, such as hydrolysis (the breaking down of organic molecules), oxidation (the creation of nitrogen groups), hydroxylation (the addition of an oxygen group to another compound), dehalogenation (the reduction of nitro groups to amino groups), sulphur substitution with oxygen (the metabolism of side chains), and ring cleavage (the breaking up of rings). A pollutant's capacity to be detoxified or transformed by microbes depends on their metabolic capability, which in turn is reliant on the pollutant's accessibility and bioavailability [28].

Organophosphonate herbicides readily support the growth of phytopathogenic fungus, and the fungus quickly destroys the herbicides it consumes. Pirimicarb, a pesticide, may be decomposed by *Trichoderma viride* and *T. harzianum*. Activated charcoal enhances the degradation capability. *Sphingomonas yanoikuyaecan* breakdown carbamate and pyrethrin (OPs) with excellent efficiency under hard circumstances thanks to the enrichment culture technique. This strain was evaluated using gas chromatography. A salt-resistant actinomycete degrades the carbofuran. The *S. alanosinicus* is capable of degrading up to 95% of carbafuran. carbofuran is the only carbon source, thus it's suitable for use in salty soils. Pesticides may be degraded by more than 30 microorganisms; however, the Gliocladium genus has the greatest capacity to degrade carbofuran. Trametes sp. and *Polyporus sp*. were also shown to be able to breakdown a wide range of compounds, including pesticides. *A. fumigatus*, *A. sydowii*, *A. terreus*, *A. flavus*, *Fusarium oxysporum*, and *Penicillium chrysogenum* were also shown to be capable of degrading pesticides. The old techniques of pesticide degradation, such as physical degradation, chemical degradation, and physical-chemical degradation, all resulted in secondary contamination as they decomposed pesticides in the soil [29]. It has been more common in recent years to utilise pesticides as the primary nutrition for microbes, which eventually decomposes into tiny molecules like CO_2 and H_2O.



a. *Pseudomonas sp* b. *Bacillus sp*

c. *Micrococcus* d. *Azatobacter sp*

e. *Aspergillus fumigatus* f. *Gliocladium sp*

g. *Humicola sp*

Fig. 15 Biodegradation Potential of isolated microbes on Carbaryl, Monocrotophos and Malathion

The process was known as an enzymatic reaction, which meant that the compound entered the microorganism's body in a specific way, and then under the influence of various enzymes, the pesticide would be degraded or broken down into smaller molecular compounds that were either nontoxic or had a lower toxicity [30, 31]. For instance, the biodegradation of 2, 4-D was regulated by a gene on the plasmid [31, 32]. Many studies have shown that the plasmid is able to control most of these enzymes. Bacterial expression of plasmid and chromosomal genes degraded pesticides. Oxidation, reduction, hydrolysis, and reductive dehalogenation were among the methods for degradation of the compounds studied. To minimize secondary pollution, bacteria would break down organic macromolecules into smaller, non-toxic compounds. Mineralization and co-metabolism have been shown to be the primary processes for pesticide degradation and the subsequent degradation of their intermediates, according to research [33].

TABLE V POTENTIAL OF BIODEGRADATION BY ISOLATED MICROBIAL STRAINS ON CARBARYL, MONOCROTOPHOS AND MALATHION

Sl. No.	Degradation of Pesticides	Carbaryl				Monocrotophos				Malathion			
	Isolated Organisms	Initial (ppm)	Final (ppm)	Difference	Degradation (in %)	Initial (ppm)	Final (ppm)	Difference	Degradation (in %)	Initial (ppm)	Final (ppm)	Difference	Degradation (in %)
1	Pseudomonas sp	100	53	47	47%	100	43	57	57%	100	74	26	26%
2	Bacillus sp	100	24	76	76%	100	35	65	65%	100	48	52	52%
3	Micrococcus	100	39	61	61%	100	26	74	74%	100	18	82	82%
4	Azatobacter sp	100	67	33	33%	100	52	48	48%	100	63	37	37%
5	Aspergillus fumigatus	100	42	58	58%	100	71	29	29%	100	25	75	75%
6	Gliocladium sp	100	22	78	78%	100	38	62	62%	100	32	68	68%
7	Humicola sp	100	36	64	64%	100	57	43	43%	100	35	65	65%

Parts of the deterioration process were broken down into three categories: In the first place, target adsorption took place on the cell membrane's surface and was an important dynamic equilibrium process. It was shown that the chemical structure of the target isomerism had a direct effect on how quickly and efficiently the target entered cells. Enzymatic reactions in membranes of xenobiotic targets proceeded quickly [7]. Organic molecules are converted into inorganic compounds by soil bacteria, which is known as mineralization. As a result, certain microbes were able to breakdown several of the synthetic pesticide chemicals because they possessed the enzymes to do so. To begin with, they may be utilized by microorganisms to get nutrients and subsequently decomposed into inorganic matter, CO_2, and water. Pesticides were totally converted into a non-toxic inorganic material by mineralization, making it a suitable method of decomposition. Biomass or exogenous organic matter can be used as the major energy source to decompose some chemical chemicals such as insecticides, fungicides, and herbicides that aren't found in nature. The term "co-metabolic" refers to this fact [18].

Depending on the amount of sunshine and the depth of the water, Endosulfan has a half-life of 2 to 820 years in water under mid-European settings. Groundwater and surface waters have both been implicated in its presence. When ingested or applied to injured skin, Carbofuron is very acutely hazardous and enters the body mostly through the gastrointestinal tract. Anxiety, dizziness, giddiness, nausea, vomiting, diarrhoea, burns to the lips and mouth and shortness of breath and fast heartbeat are all common side effects of exposure.

Nose bleeding, skin splinters, peeling, blistering, and discoloration of the nails are all possible side effects. Environmental Protection Agency: "very biologically active and harmful to plants and animals"; New Zealand's Environmental Risk Management Authority: "highly ecotoxic to the aquatic environment". In Anomuran crab *Emerita asiatica*, fish and amphibians, it has produced teratogenic deformities, disturbed frog hormones, and is genotoxic to tadpoles. Pesticide microbial degradation is a hot topic right now, with several studies being conducted in this area [34, 35, 36, 37, 41, 42].

The minimal pollution and effective usage of two strains were the hallmarks of this method. The creation of a system of several bacteria solved the problem of a single strain's incomplete transformation [16]. Finally, the foreign protein was incorporated into the cell membrane and carried out its intended function. The direct interaction between bacteria and pesticide residues facilitated the purifying process of proteins, as well as increased the degradation rate [38, 39, 40].

The isolated *Bacillus sp*. from the pesticide-polluted agricultural area has the maximum degradation capacity against all three pesticides, including Carbaryl, Monocrotophos, and Malathion, as shown in the preceding report. *Pseudomonas sp* also performed better in Malathion and Monocrotophos than any other bacterial species. Early studies focused on the breakdown of Carbofuron and Endosulfan. According to the findings of this latest study, the *Micrococcus* had reduced ability to breakdown Malathion. All three herbicides were significantly degraded by *Azatobacter sp*. *Gliocladium sp*, followed by Monocrotophos and Malathion is the most effective fungal isolate in degrading Carbaryl.

Studies were conducted to identify the best microorganisms for pesticide degradation, including Carbaryl, Monocrotophos and Malathion (CMM), as well as the best ways for degrading these pesticides. The isolated *Bacillus sp* from the pesticide-contaminated agricultural field had the maximum degradation potential against all three pesticides, including Carbaryl, Monocrotophos, and Malathion, as shown by the aforementioned story. Additionally, Pseudomonas sp displayed superior outcomes in Malathion and Monocrotophos. The early researches focused on the breakdown of Carbafuran and Endosulfan. This most recent

research has further demonstrated how little ability Micrococcus has to break down Malathion. All three herbicides were moderately degraded by *Azatobacter sp*, *Gliocladium sp*, followed by Monocrotophos and Malathion has the highest capacity for degradation against carbaryl among the fungal isolates. These investigations screened the best microorganism for several pesticides, including Carbaryl, Monocrotophos and Malathion (CMM), the best degradation methods, and the best degradation environment, providing a more practical reference for subsequent study.

V. CONCLUSION

The microbial degradation method was frequently employed in pesticide degradation in addition to the conventional approaches, such as the physical degradation method, chemical degradation method, and so on. This technique provided a good degrading effect, was inexpensive, and was highly efficient. Some pesticide ingredients are taken as nutrition by microorganisms, which then break them down into small molecules mostly by mineralization and co-metabolism. Numerous variables, including the kind of pesticide, the kind of microbe, as well as the environment's temperature, humidity, acidity, and air composition, had an impact on the impacts of degradation. These investigations screened the best microorganism for several pesticides, including carbaryl, Monocrotophos, and Malathion (CMM), the best degradation methods, and the best degradation environment, providing a more practical reference for subsequent study.

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