

Analysis of Microbial Degradation of Pesticides

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ABSTRACT

The use of pesticides has been increased and it is hazardous to the environment, it affects the fertility of the soil and is toxic for the living things. Excessive use of pesticides destroys the natural flora of the soil. The chemicals present in the pesticides remain in the soil and cause pollution. Different methods of physical, biological and chemicals are used to remove these pesticides. Microbial pesticide degradation is the best method that is being used as it is cost-effective and does not disturb the indigenous microbes. Different techniques of microbial degradation are used which include biodegradation, biostimulation, phytoremediation, and bioaugmentation. Different strains from bacteria are used for the degradation of these pollutants. Fungi and plants are also used for the degradation of pesticides. Microbes, bacteria, fungi, and plants have been found to help in the degradation of organophosphorus, organochlorine, chloroacetanilide, triazine, and many other pesticides. Genetically modified organisms have also been used. Microbes used these pesticides as the sole source of carbon, sulfur, and phosphorus under favorable conditions for growth. This review provides descriptive information on the strain of bacteria and fungi from various microorganisms and plants are involved in the degradation of different toxins. They degrade these pesticides into different compounds. Some metabolites are produced during degradation.

Keywords: Organophosphate, Organochlorine, Bioaugmentation, Biostimulation, Phytoremediation, DDT.

INTRODUCTION

Pesticides are the chemical compounds that use to kill pests including fungi, insect, and weeds. Pesticides are of many types such as bactericides, fungicides, insecticides, rodenticides, larvicides, virucides, nematicides. Pesticides are being used worldwide to protect food and commercial products. ¹It is also being used in agriculture sites to control unwanted plants and preventing the plants from an attack of fungi, insects, rodents. The loss of food has been decreased largely by the use of pesticides.² They are also being used in public health to control the mosquitoes that cause diseases like malaria and typhus.³ Two million tons of pesticides are using worldwide each year. Recently almost over 500 pesticides have been registered to use worldwide. Pesticides should kill pests only but unfortunately, they are lethal to non-target species also. ⁴Due to their unplanned and undetermined target, only 10% of pesticides reach to their target and remaining effect on soil, water, sediments. we cannot ignore the hazardous effects that these pesticides cause on the environment including soil and water, on humans and even on the animals. In humans, it causes acute cancer, neurological disorder, and immunodeficiency and also causes estrogenic activity. Some of the pesticides are water-insoluble and they cannot be degraded⁵. These pesticides remain in soil and water and act as toxic chemical residue. This toxic chemical residue becomes a major cause of pollution and also affects the health of organisms by reaching in drinking water and food resources. Therefore degradation of these pesticides becomes necessary. Instead of applying the chemical and physical methods to degrade the pesticides, the best way is to biodegrade them. Because the method of biodegradation of pesticides is cost-effective, environment-friendly and reliable.⁶ Degradation of pesticides depends upon the micronutrients present in the soil, temperature, pH, moisture content and number of microbes present in the soil. The microbes that we can use in biodegradation could be bacteria, fungi, and actinomycetes.⁷ The enzymatic activity of these bacteria causes degradation of toxic compounds and their use is economically and environment-friendly Because bacteria are capable of producing mutant strains and they can adapt themselves rapidly according to the changes in the environment and it doesn't form secondary pollutants. Studies on microbial degradation of pesticides were firstly



started in the 1940s. Today biotechnology is emphasizing the use of genetically modified organisms for bioremediation.⁸ The treatment of these pesticides should be done this way that the degradation of the compound will not generate intermediates. The existing technologies are using physical or chemical ways to treat the pesticides, and according to FAO, they could cost up to 3000-4000 USD/tons. So these methods are not cost-effective, environment- friendly, and have many other disadvantages. So there is a need to develop biological treatment over these conventional methods. The objective of this review article is to research the literature and finding out different methods of biological degradation of pesticides and to find out different types of microorganisms that we can use for the removal of these toxic pesticides from our environment.⁹

PHYTOREMEDIATION

The phytoremediation method is effective for degradation because it does not affect the topsoil. It can convert complex toxic organic compounds into a non-toxic simpler form by synergistic relationship with microbes.⁷ A Suitable combination of plants and the endophytes present in them can increase the rate of degradation of compounds. According to earlier studies, *Kochia sp* tends to degrade atrazine, metolachlor, and trifluralin. Poplar tree also tends to remediate the nitrate, atrazine, alachlor present in contaminated groundwater.¹⁰ Phytoremediation can be divided into the following process.

Phytoaccumulation is the process that extracts the contaminants from soil and accumulates them into plant organs. Phytodegradation is the degradation of organic compounds. Phytovolatilization in which Plants volatile the organic compounds and Phytostabilization is the process in which Plants convert the molecule into non-toxic or less toxic substances.¹¹

But the process of phytoremediation is slow and some of its techniques such as phytoaccumulation does not degrade the substances but stores these toxic substances into its organ, in leaves, stem... Whenever herbivores eat these plants, the pesticidalorganic compound can move into their body and can affect them.¹²

BIOSTIMULATION

In biostimulation, we provide nutrients to the indigenous microbes and enhance their ability to degrade certain harmful chemicals. Microbes are the native flora of the area and are well suited so it is very eco-friendly,³ but they need certain nutrients for their growth. Different bacterial strains degrade different compounds. Some fungi also degrade the harmful compounds. Nutrients we provide to the microbes may be carbon, oxygen, phosphorus, and nitrogen. Some may also need inorganic compounds as well. As it stimulates microbes to form enzymes that breakdown contaminants.⁷ Oxygen supply affects the rate of stimulation. Mostly the carbon to nitrogen is 10:1 and carbon to phosphorus ratio is 30:1. Various species of bacteria do degradation by forming metabolites. Some fungi also do degradation as candida sp. degrade lindane by the process of biostimulation.

BIOAUGMENTATION

The soil is polluted with different aromatic compounds which include halogenated aromatic compounds, polycyclic aromatic compounds, benzene, ethylbenzene and toluene which are toxic and carcinogenic. The presence of these chemicals in the environment affect human health.¹³ Most of the chemicals compounds are





recalcitrant and persistent they remain in the environment for a long period. They cause pollution in the environment so their degradation from the environment is necessary. Different techniques are used for the degradation of these chemicals from the environment one is bioaugmentation it is the type in-situ bioremediation.¹⁴ In bioaugmentation, different microorganisms and consortia of microorganisms are introduced at the site of contamination. Genetically engineered microorganism is also used. In bioaugmentation growth of indigenous microbes is enhance by adding nutrients to the contaminated site. The microorganism is selected for bioaugmentation. Bacteria are isolated from the contaminated soil and culture in the laboratory under optimized conditions the optimal conditions. Different bacteria are used for the process of bioaugmentation. The use of consortia of microorganisms for the degradation of aromatic compounds is more effective as compared by using the single strain of bacteria. Not only bacteria fungi are also used in bioaugmentation.

MICROBIAL DEGRADATION OF CHLORPYRIFOS AND PHORATE

Organophosphate pesticides are a class of organophosphorus compounds and are used as insecticides for killing insects. Organophosphate pesticide chlorpyrifos has been used for pest control.¹⁵ But it is toxic and causes environmental pollution; therefore, bacterial strains are used to degrade pesticides.³ Two bacterial strains that are present in soil are used to degrade chlorpyrifos. One strain JCp4 which is identified as *Achromobacter xylosoxidans* and the other strain FCp1 identified as *Ochrobactrum sp.*³ They can degrade pesticides in liquid media and as well as in soil. These bacteria can degrade the pesticides in sterile as well as nonsterile soil and they can degrade chlorpyrifos 93-100% off within 42 days. Chlorpyrifos affects plant growth. ¹⁶The bacterial strain used for the degradation of chlorpyrifos enhances the growth of the plant. Different colonies were isolated and grown in a nutrient-containing media with chlorpyrifos supplement. These bacteria used the chlorpyrifos as the source of carbon.²

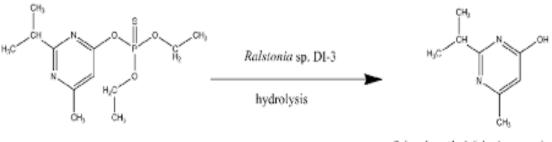
Two organophosphate pesticides chlorpyrifos and phorate were detected in the corn and they cause pollution in processed food.² Lactic acid bacteria (LAB) is used in silage it is an important microorganism that is present in food. LAB can degrade organophosphate pesticides. *L. Plantarum* present in the silage can accelerate organophosphorus degradation.¹⁷ Three strains of LAB including *L. Plantarum* 1.0622, *L. Plantarum* 1.0315 and *L. Plantarum* 1.0624 was used for the degradation of chlorpyrifos and phorate which are obtained from LAB in the laboratory. Three strains of bacteria were inoculated in fresh whole corn silage and the degradation of organophosphate was observed.

MICROBIAL DEGRADATION OF DIAZINON

This diazinon is an organophosphate insecticide used in agriculture. It is contact insecticide it kills the insect by inhibiting the action of an enzyme in the nervous system. It is applied directly on the soil to kill insect pest but it remains in the soil and becomes a pollutant. It also causes water pollution because from the soil they can be washed off into nearby streams. Most of the pesticides are toxic and they inhibit the enzyme acetylcholinesterase which is involved in neurotransmission in the form of acetylcholine. ¹⁸Four bacteria that can degrade the diazinon were isolated from the soil. By 16S rDNA sequencing, it is indicated that the bacterial strain D1101 which belongs to *Serratia marcesans* group can degrade diazinon they used the diazinon as the sole source of carbon and



phosphorous. This strain can degrade diazinon completely within four days in MSM media. The *diazinon was* degraded in sterilized and unsterilized soil within 14 and 16 days.



Diazinon

2-iso-4-methyl-6-hydroxypyrimidine

Fig 1: Degradation of diazinon by *Ralstonia sp.* The compound 2-iso-4-methyl-6-hydroxypyrimidine is obtained by degradation of diazinon which is less toxic. This strain of bacteria belongs to the *Serratia marcesans* group.¹⁹

Another bacterial strain *Ralstonia sp.* D1-3 which is present in agricultural soil can degrade diazinon. Its degradation rate is higher and it is not spore-forming bacteria. The bacteria used the diazinon as a source of carbon for growth. The compound obtained by the degradation of diazinon is 2-iso-4-methyl-6-hydroxypyrimidine (IMHP) it is a persistent and less toxic product.²⁰¹⁵

MICROBIAL DEGRADATION OF QUINALPHOS

Quinalphos is an organophosphate pesticide used for controlling the pest. Quinalphos affect the activity of a testicular steroidogenic enzyme which results in the degeneration of germ cells. The degradation of quinalphos is done by using bacteria. An *Ochrobactrum sp.* strain HZM can degrade quinalphos (QS) and identify its metabolite. This bacterial strain was isolated and identified in the laboratory from contaminated soil.³ The bacteria used this as a source of carbon for growth and it was determined by Seubert's mineral salt medium (MSM) which contains 2mmol 1⁻ of quinalphos as a source of carbon. Two metabolite products are obtained from the degradation of quinalphos one is 2-hydroxyquinoline and the other is diethyl phosphate which is further used as a carbon source.

Another bacteria which can degrade quinalphos was isolated from the soil. By rRNA gene sequence analysis the bacteria were identified as *Bacillus thuringiensis*. The quinalphos degrade at the optimum condition at temperature $35-37^{\circ}$ C and pH 6.5-7.5. MSM media containing 20μ g mL⁻ of quinalphos.

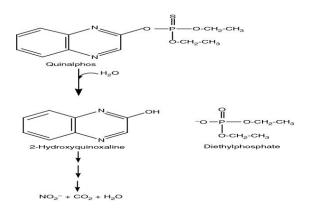


Fig 2: Degradation of quinalphos by *Ocrabactrum sp.* Strain HZM the Quinalphos is degraded into 2-hydroxyquinoline by *Ocrabactrum sp.* And further converted into water CO_2 and NO_2^-



Sodium acetate is added additionally as carbon source and yeast is added as a nitrogen source in culture medium for bacterial growth. Bacillus thuringiensis is the best source of quinalphos degradation.²¹

ORGANOCHLORINE

It includes DDT, Methoxychlor, dieldrin, chlordane, toxaphene, kepone, polychlorophenol, lindane. In the 20th century, organochlorine pesticides were used widely during the second world war to control the mosquito that spread malaria and typhus, but now they are banned in most of countries due to their high toxicity and persistence in the environment.²² If the soil gets treated by pesticides, their residue remains in the soil and they become hazardous for the environment as well as for health. Therefore effective methods are required for the removal of these contaminants from the environment.²³

PENTACHLOROPHENOL

Pentachlorophenol used as a preservative of leather and wood. But it is highly toxic to all kinds of life and also causes mutations and different types of diseases in humans. But it is a recalcitrant organic pesticide that cannot be degraded because it has a highly stable aromatic ring and high content of chlorine.²⁴

Martin et al. stated that *janibacter sp.* strains isolated from the saline and arid environment can be used for the biodegradation of pentachlorophenol. PCP is a hydrophobic compound and therefore has low bioavailability and low solubility. But they used tween (80) that act as a surfactant and it increased bioavailability and solubility of PCP in water.²¹

LINDANE, CHLORDANE, METHOXYCHLOR

Lindane, Chlordane, and methoxychlorane are also organochlorine pesticides that are persistent in the environment and their half-life is 980, 365, 120 days respectively. All of these are insecticides and they are affecting the water floating bodies and agricultural soil. These were observed in the edible fish of Arginteen Patagonian. Due to an increase in biomass by glucose that provides them NADH+, the removal of PCP increases.²⁵

Streptomyces genus of Actinobacteria can be used for removal of these pesticides because they have mycelial growth, ability to colonize the semi-solid substrate and also produce surfactants that increase the solubility and bioavailability of pesticides.³

Determining factors that affected the degradation rate of Lindane, Methoxychlor, Chlordane is the texture of the soil, assay conditions (sterility, slurry formation), moisture, availability of micro-organisms, pH, temperature, O₂availabilty, the concentration of pesticides, and micronutrient content.²⁶ For treating the Lindane, methoxychlor, and Chlordane effectively, different strains of Streptomyces consortium have been used in different soil systems. A₂, A5, A11, M₇ of *Streptomyces sp.* as actinobacterium consortium. The experiment was done for the removal of mixed Pesticides, by adding the isolated Streptomyces strain on different soil systems such as in liquid medium, in sterile soil microcosm, in non-sterile silty clay loam soil microcosms, in a slurry system. Consortium growth was observed with no significant difference. The order of removal of the OPs was methoxychlor 26% lindane 12.5% chlordane 10%.²⁷



DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane) used in 2nd world war. It has also been used to control the insects in agriculture. But it causes various diseases like cancer, endocrine disruption. There are many species have been identified that can be used to degrade the DDT such as *Sphingobacterium sp. D6, Clostridium sp. Alcaligenes sp. Bacillus sp. White rot fungus, serretiamarcescenes.* DDT also can be degraded by *Pleuro ostreatus* and *Pseudomonas aeruginosa.* Therefore, P.aeuroginosa is used along with white-rot fungus, which is acting as biosurfactant.⁷

Name of pesticide	Strains of microbes that degrades pesticides	Structures
Chlorpyrifos	Achromabacter xylosoxidans JCp4'	
	Strains of Ochrobacterum Sp. FCp1	
Phorate	Lactic acid bacteria strains	
	L.plantrum 1.0622, L.plantrum 1.6024	CH CH SCH D-CH
	L.plantrum 1.0315	
Diazinone	Strain of Ralstonia sp.D1-3	
	Strain of Serretia Marcesans D1101	Hoc Cons
Quinalfos	Strain of Ocrabacterum sp. HZM	
	Bacillus thuringiensis	N S O S S S S S S S S S S S S S S S S S
Lindane	Strains of Streptomyces consortium A2, A5, A11, M7	
Methoxychlor	Strains of Streptomyces consortium	
		Loto-
DDT	Streptomyces consortium	
Chlordane	P.ostratus P aeuroginosa.	
РСР	Strain of Janibacter sp. FA523	



Atrazine	Arthrobacter histidinolovorans	
Glyphosate	Bacillus cereus CB4	HOLN CH
Chlorimuron ethyl	Pseudomonas sp. LWS Aspergillus niger	
Alachlor	Actinomycetes, Pseudomonas sp. strains	
Tricyclazole	Blue-green Algae sp.	EH.
Tricyclazole Metalaxyl	Blue-green Algae sp. Brevibacillus brevis sp.	
		Files Files

Bacteria can degrade the DDT aerobically or anaerobically. If we degrade DDT anaerobically by *P.aeruginosa* several metabolic products such as DDMU, DDMS, DDOH, DDA, 2-Chlorobiphenyl, 4-chlorophenyl, 4-Chlorobenzoic acid Catchecol, and hydroquinone. On aerobic degradation, by providing O₂, DDE is detected as the end product of DDT.while*P.ostreatus* transform the DDE to further metabolic products such as DDD, DDMU, DDA, DDMS.²⁸

When *P.aeruginosa* and *P. ostreatus* used in a mixed culture they degraded DDT to DDE, DD, DDMU. In aerobic conditions, Oxygenase and peroxidase play an important role. While white-rot fungus produces oxidative lipolytic enzymes such as MnP, LiP that degrades lignin and other pollutants. But an intracellular enzyme also produces named as Cytochrome P450 monooxygenase that causes dehalogenation, dehydrogenation, hydroxylation, reduction, oxidation of organic pesticides.

When *P.aeruginosa* and *P.ostreatus used* in the mixture, the degradation rate of DDT was higher as compared to when both of them were used for degradation, individually.

MICROBIAL DEGRADATION OF HERBICIDES

Herbicides are used to kill unwanted weeds and protect or keep our desired plant unharmed but some herbicides are very toxic and persistent .as they persist in soil and also move to an aquatic system and also harm it.to remove this persistent herbicides microbial degradation is being used nowadays.²⁹



One of the herbicides as atrazine is being degraded by microbes. A KU001 strain of bacterium was isolated from a sugarcane field which was degrading atrazine. Biolog carbon source analysis shows that that the isolated bacterium was *Arthrobacter histidinolovorans* .the pathway used by the strain of bacteria for the degradation of atrazine consists of catabolic genes *as trim,atzB*, and *atzC* .the bacterial strain was using the atrazine as its only nitrogen source .the strain KU001 was degrading atrazine at a rate 4-5 fold faster than indigenous microbial community.³⁰

Glyphosate is itself not an herbicide but a toxic ingredient of herbicide produced by China. During the preparation of herbicide, the wastewater containing glyphosate is discharged. It is very toxic so it should be treated before its release. So microbes are now being used for its degradation. ³¹ There are two pathways for its degradation one involves breakage of C-N bond while the second pathway involves the breakage of the C-P bond. It is being degraded by the bacterial strain CB4 (*Bacillus cereus*). CB4 is gram-positive and rod-shaped. The optimal conditions for the degradation of glyphosate were PH 6, incubation temperature 35 and 6 gL concentration. In 5 days CB4 utilizes 94.47% of the glyphosate. But the growth rate of microbes was affected by its concentration due to its toxicity.

Sulfonylurea is being throughout the world against weeds in rice, barley, cotton, potato, corn, and wheat. Bacteria were isolated and identified by the rRNA gene sequence as pseudomonas sp.it was used by Pseudomonas sp. As the sole nitrogen source. The rate of degradation was 81% in 7 days. The strain LW3 was degrading at optimal temperature 30 and PH 6.5. by using metabolic³²

Chlorimuron-ethyl is primarily degraded by the ph and temperature-dependent chemical hydrolysis but the microbial is also being done. The hydrolysis was faster in the moist soil than the dry soil. Some fungi such as *Alternaria* and *Fusarium* were unable to survive in media as chlorimuron ethyl can show drastic effects on some species and inhibit their growth. *Aspergillus niger* survived in it, at 25 and in dark this fungi can degrade chlorimuron ethyl. Aspergillus *niger* degrades it by releasing the intracellular enzymes which convert it into simple forms which could be used by the microbes as an energy source. ³³

Alachlor another toxic and mobile herbicide its persistence is also high in the soil. It is a carcinogen and causing some serious threats.it could be degraded by nature as by precipitation and volatilization .though many studies show that degradation of alachlor could be done better on aerobic conditions. From a cornfield, a bacterial consortium was separated which could degrade alachlor. In early cultures, the degradation rate of alachlor was less. due to the toxicity of alachlor, the growth rate of microbes was effected. Later repeated subculturing increases the rate of degradation. Optimal pH for degradation is 7.5. in the inoculated media the rate of degradation was also high.³⁴

DEGRADATION OF FUNGICIDES

The Agricultural product has a forcible contact with surrounding as fungicides contaminate the soil and it tending to cause harm all around surrounding by affecting soil makeup. There are factors such as humidity, light, the intensity of heat that makes easier the process of degrading the harmful residue.³⁵



In India, rice is known by many people because its harvest rate all over the world is high but a disease "BLAST" caused by "Pyricularia oryzae Savara" deteriorates rice crop especially from the seedling stage but symptoms produced on all section of plants. After a prolonged period of

study, it was found that fungicide "Tricyclazole" can be used to make rice crop resist against "BLAST" because this fungicide introduces rice crop in a superior position effectively than other fungicides as this fungicide engrossed by the root to all other parts of the plant. Tricyclazole can be drawn by a person to function by its continuous usage without interruption but a microbial species that is Blue-green algae that are used to convert this Tricyclazole in a non-toxic form.

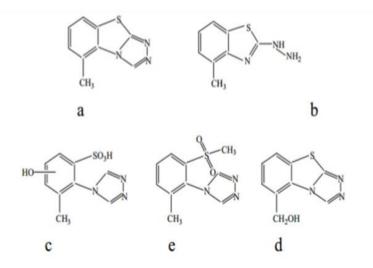


Fig 3: (a)Tricyclazole (b)Hydrazinyl-4-methyl-1,3-benzothiazole(c)Metabolite(d) Tricyclazole alcohol

The effects of organic matter which bar into all-around places should be immune. We have to confer our full attention and all probable way for pesticides remediation. Many distinct categories of organic matter can be used to engross and deteriorate harmful substances residue. six different types of fungicides that we put on the surface of the vineyard during trials and outcome proved that this fungicides mixture has enough power to deteriorate harmful residue.

Two other types of fungicides (metalaxyl and furalaxyl) are also helpful to alienate many disorders of structure and function in plants i.e mildews, late blight, etc. This state of confusion caused by *Albugo Candida, Fusarium Solani*, *Peronospora parasitica, Phytophthora sp.* And *pythium sp.* The destiny of both racemic metalaxyl and racemic furalaxyl rely on many distinct measurable factors such as pH, humidity, the intensity of heat, etc and the deterioration rate of harmful substances residue are different in different soil. There is *Brevibacillus brevis sp.* that is also used to degrade these two fungicides³⁴

Carbendazim(MBC) is another fungicide which also helps to eliminate many fungal disorder structurally and functionally in many plants but after elimination the state of confusion MBC remaining attached instead of falling off in the environment and hurts the many parts of plants of animals even at a small amount. There are different microbes which accountable to eliminate this residue gently through different processes as the growth rate of microbes i.e Actinobacterium Rhodococcus sp. is highly affected to this fungicides

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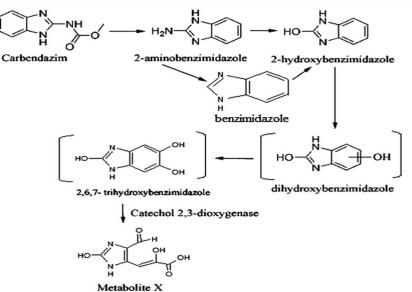


Fig 4: Proposed degradation pathway of MBC by strain djl-6-2. Carbendazim converts into 2-aminobenzimidazole which on further degradation form dihydroxybenzimidazole and at the end, it converts into a metabolite.

CONCLUSION

This Review highlights the mechanism by which ,different strain of different types of microbes to degrade the different types of pesticides, fungicides, herbicides as strain of Agrobacterium sp. as well as acetobacter xylosoxidans JCp4 used to degrade chlorpyrifos, lactic acid bacterial strains, *L.plantrum*1.0622, *L.plantrum* 1.6024 and *L. Plantarum* 1.0315 converts phorate into simplest form that is immune to environment. Strain of *Ralstonia sp*.D1-3 and strain of *Serratia marcesans* D1101 degrades diazinon, strain of *Ocrabacterium sp*.HZM, as well as *Bacillus thuringiensis* used to convert to quinaphlos into harmless compound, strain of *Streptomyces consortium* A2, A5, A11.M7, degrade Lindane, methoxychlor, and chlordane. *Blue-green algae sp*. also help to breakdown tricyclazole into simpler one, *Brevibacillus brevis sp*. degrades fungicides like metalaxyl and furalaxyl. *Actinobacterium Rhodococcus sp*. Helps to make the environment safe by regarding harmful fungicides like carbendazim and pseudomonas sp. helps to convert chlorimuron ethyl that is hazardous for all living organisms.

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