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Impact of Pesticide (Chlorpyriphos) on Soil Microbial Diversity

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Abstract

The paper studied the impact of soil contamination on microbe population. The microbes mineralise and biotransform organic compounds and associated pesticides. Pesticides, extensively used in agriculture for pest control strategies reduce soil enzymatic activities that act as a ''biological index'' of soil fertility and biological processes in the soil environment. For the study, soil samples were serially diluted, inoculated on NA and PDA medium by using spread plate technique under aseptic conditions and incubated at 37°C and 26°C temperature for optimum growth. 3 selected microbial strains were cultured onto MSL medium supplemented with different chlorpyrifos concentrations (0, 50, 100, 150, 200 µg ml-1) for 12 days. In the non-contaminated soil, microbe population was found to be significantly higher.

Keywords: Chlorpyriphos, Xenobiotic Characteristics, Microbial Characterisation

1. Introduction

The microbial mineralisation of organic compounds and associated biotransformations such as nutrient dynamics and their bioavailability are adversely affected by the pesticides. The applied pesticides reduce soil enzymatic activities that act as a ''biological index'' of soil fertility and biological processes in the soil

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environment. The microbial biomass is an important indicator of microbial activities, and provides a direct assessment of the linkage between microbial activities and nutrient transformations and other ecological processes. Pesticides are extensively used in agriculture as a part of pest control strategies. Owing to their xenobiotic characteristics, pesticides may adversely affect the proliferation of beneficial soil microorganisms and their associated bio-transformation in the soil. Inactivation of nitrogen-fixing and phosphorus-solubilising microorganisms is often observed in pesticide-contaminated soils. The biodegradation of organic pollutants is a natural process whereby bacteria and other organisms alter and break down organic molecules into substances, eventually producing carbon dioxide and water or methane. Although the ultimate aim of the biodegradation is to degrade the organic contaminants completely into harmless constituents such as carbon dioxide and water, many intermediate metabolites can also be formed in the process. What makes bioremediation so desirable is that it is a permanent solution; it destroys the contaminant, focuses on detoxification rather than waste translocation [1]. The literature survey findings, in the present study was taken up with the following objective of examining the biological dissipation of pesticide in the Chlorpyriphos contaminated soil and effect of pesticide on soil microflora and pesticide degradation by isolate/ consortium obtained from contaminated soil. Chlorpyriphos (0,0-diethyl-3,5,6-trichloro-2-pyridyl phosphorothioate) is a insecticide/acaricide for treatments of crops, lawns, and ornamental plants. It is a widely used insecticide and is effective against a broad spectrum of insect pests of economically important crops. It persists in the soil for 60-120 days and has very low solubility in water (2 mg/l-1) but is readily soluble in most organic solvents. Chlorpyriphos undergoes a transformation in the soil by the abiotic hydrolysis and microbial degradation. One of the major factors contributing to the net impact of applied pesticides on soil microbes is its bioavailability in the soil environment. Adsorption and desorption processes regulate the concentration of a contaminant in soil. In addition to soil texture, the presence of organic matter and vegetation also influences pesticide toxicity to the microbes in the soil environment. Degradation of the pesticide depends upon the type

of the soil, soil property, the moisture content of the soil and pH [2].

The amount of applied pesticides reaching the target organism is about 0.1% while the remaining bulk contaminates the soil environment [3-4]. With the growing use of pesticides in contemporary agriculture, the issue of the impact of these chemicals on the composition of soil microorganisms and the processes they direct has received more attention [5-7]. The applied pesticides may harm the indigenous microorganisms, disturb soil ecosystem, and thus, may affect human health by entering the food chain.

2. Materials and Methods

2.1 Soil Sample Collection

The soil samples were collected from rhizospheric and non-rhizospheric region from the organic garden; both without the contamination of any chemical pesticide and with the contamination of Chlorpyriphos from field soil.

2.2 Isolation of Microorganisms

Collected soil samples were serially diluted up to 10^{-7} dilution. The diluted soil sample was inoculated on NA and PDA medium by using spread plate technique under aseptic conditions and incubated at 37°C and 26°C temperature for optimum growth. Colony characteristics were analysed. The gram staining and the cotton blue mount were done for their characterisation as bacteria and fungi. The different strains were identified by gram staining method [8].

2.3 Characterisation and Identification of Bacteria

The characterisation was done on the basis of the cultural appearance of the organism, colonial morphology, differential and selective media, and also by biochemical tests [9].

In the case of fungi, the number of the colony was simply counted on potato dextrose agar plates. For the identification of fungi, Lactophenol-cotton blue mounting was done, examined under a microscope (40X), and the results were noted down.

2.4 Biodegradation of Chlorpyriphos by the Selected Isolates

Mineral Salt Liquid (MSL) medium supplemented with Chlorpyrifos (10 mg l-1) was used for biodegradation test**.** Cells were pre-cultured in broth medium which was harvested by centrifugation and washed three times with sterilised distilled water. For all preliminary experimental tests, the cells were used at a concentration of 10^6 cells ml^{-1} and samples were incubated on a rotary shaker at 150 rpm and 30 ^oC for 3, 5, 7, 10 and 12 days for bacteria and fungi respectively. Medium without inoculation was maintained under the same conditions and served as controls. Further tests were carried out to select the microbes that have Chlorpyriphos-degrading capability; dry weights of each microbial isolate and turbidity were measured. Afterwards, three selected microbial strains were cultured onto MSL medium supplemented with Chlorpyriphos at five concentrations (0, 50, 100, 150, 200 µgml-1) for 12 days. The cultures were incubated at optimum pH and temperature for each isolate on a rotary shaker at 150 rpm. Control flasks of an equal volume of MSL medium and Chlorpyrifos without any microbial population were incubated in parallel at all intervals to assess abiotic loss. During the experiment, samples were collected periodically at 0, 1, 2, 4, 6, 8, 10 and 12 days intervals of time for estimation of the growth of pesticide-degrading bacteria and the optical density was taken at 520, 540, 560, 590 and 620 nm using UV – spectrophotometer [10]

3. Result and Discussion

3.1 Isolation of Microorganism from Organic Soil

An average number of bacterial colonies were 6.2 X 10 ^{6} CFU/gm in organic soil.

Eighteen bacterial strains and five fungal strains (Fig 2 and 3) were isolated from the rhizospheric and non-rhizospheric region of organic garden soil.

Table 1: Total number of isolated bacterial pure strains

S.no. (S)	Identification	Gram's Reaction
1	*Coccus*	positive
2	*Staphyllococcus*	positive
3	*Staphyllococcus*	negative
4	*Bacillus*	negative
5	*Coccobacillus*	positive
6	*Coccus*	negative
7	*Bacillus*	positive
8	*Bacillus*	positive
9	*Pleomorphic*	Positive
10	*Streptobacillus*	positive
11	*Coccus*	negative
12	*Coccus*	positive
13	*Coccus*	negative
14	*Staphyllococcus*	negative
15	*Bacillus*	negative
16	*Streptobacillus*	positive
17	*Bacillus*	positive

Table 2: Total number of isolated fungal strains

Strain no.	Colony morphology	Microscopic characterstics	Identification on microscopic view
I	Grassy green with white margin	The conidial head is typically radiate, biseriate; Conidia are globase to subglobase.	*Aspergillus flavus*
II	Light green colony	Sporangiophore is simple branched with column-shaped columella.	*Mucor*
III	A white colony with dense cottony growth	Sporangiophores are smooth-walled, non-septate, simple or branched, sporangia greyish black, powdery in appearance.	*Rhizopus*
IV	Black colony	Conidiophores are smooth walled hyaline, conidial head are biseriate.	*Aspergillus niger*
V	Grey colony	Columnar, uniseriate conidial heads, short smooth walled conidiophores.	*Aspergillus fumigatus*

3.2 Microbial Characterisation

On the basis of the various biochemical tests performed the seventeen bacterial strains (Fig 1) isolated were further identified.

Table 3: Result of the fermentation test

Isolate no.	Glucose				Sucrose				Lactose			
	24 hrs		48 hrs		24 hrs		48 hrs		24 hrs		48 hrs	
	colour	gas production	colour	gas production	colour	gas production	colour	gas production	colour	gas production	colour	gas production
1	+	−	++	−	Dark red	_	Dark red	_	Dark red	_	Dark red	_
2	−	−	-	−	_	_	_	_	Dark red	_	Dark red	_
3	−	−	+	−	++	+	+++	+	Dark red	_	Dark red	_
4	+	−	++	−	−	−	−	−	Dark red	_	Dark red	_
5	−	−	−	−	−	−	−	−	Dark red	_	Dark red	_
6	++	−	++	−	−	−	−	−	Dark red	_	Dark red	_
7	_	_	_	_	_	_	_	_	_	_	_	_
8	_	_	_	_	_	_	_	_	_	_	_	_
9	_	_	_	_	_	_	_	_	_	_	_	_
10	_	_	+	+	+++	_	+++	_	_		_	
11	++	−	+++	−	++	+	+++	+	Dark red	_	Dark red	_
12	+++	+	+++	+	Dark red	_	Dark red	_	Dark red	_	Dark red	_
13	+++	+	+++	+	+++	+	+++	+	−	−	Dark red	_
14	+++	−	+++	+	+++	+	+++	+	−	−	Dark red	_
15	−	−	−	−	++	−	+++	−	Dark red	_	Dark red	_
16	+++	+	+++	+	+++	+	+++	+	_	_	_	_
17	_		+	+	_	_	_	_	_	_	_	_

+ve = positive result ; -ve = negative result

Table 4: Result of another different biochemical test

Isolate no.	Amylase test	Cellulase test	Casein hydrolysis test	H2S production test	Catalase test
1	+	-	+	++	-
2	-	-	+	++	-
3	+	-	+	++	-
4	+	-	+	++	-
5	-	-	+	+	-
6	+	-	+	++	-
7	-	-	-	+	-
8	-	-	-	+	+
9	+	-	-	+	-
10	+	-	+	+	-
11	+	-	-	+	-
12	+	-	+	+	-
13	+	-	+	+	-
14	-	-	+	+	-
15	-	-	+	+	+
16	-	-	+	+	-
17	+	-	+	+	-

+ve = positive result ; -ve = negative result

3.3 Isolation from the Chlorpyriphos Exposed Wheat Soil

Average total number of bacterial colonies was 3.8 X 10^3 CFU/ gm

Ten bacterial strains and three fungal strain (Fig 2) were isolated from wheat soil.

Table 5: Total number of bacterial isolates

S.no.	Identification	Gram's Reaction
1	*Coccus*	negative
2	*Small rods*	negative
3	*Staphyllococcus*	negative
4	*Bacillus*	positive
5	Coccus	positive
6	*Coccus*	negative
7	*Streptobacillus*	negative
8	*Bacillus*	negative
9	*Coccus*	negative
10	*Staphylococcus*	positive

Table 6: Total number of fungal isolates

Strain no.	Colony morphology	Microscopic characterstics	Identification on microscopic view
I	Grassy green with white margin	Conidial head are typically radiate, brushlike	*Enicillium*
II	A black colony with dense cottony growth	Beak-like, alternate septation	*Alternaria*
III	Black colony	Conidiophores are smooth walled hyaline, conidial head are biseriate.	*Aspergillus Niger*

3.4 Microbial Characterisation

On the basis of the various biochemical tests performed, the ten bacterial strains isolated were further identified.

Table 7: Result of biochemical test

Strain number	Fermentation test (glucose)		Amylase test	Cellulose test	Casein hydrolysis test	H_2S production test	Catalase test
	Gas production	Acid production					
S1	+	-	-	-	-	-	-
S2	+	-	-	-	-	-	+
S3	+	-	-	-	+	+	+
S4	+	-	+	+	-	+	+
S5	+	-	+	-	-	+	-
S6	+	-	-	-	-	-	+
S7	+	-	-	-	-	-	+
S8	+	-	+	+	+	-	+
S9	+	-	-	-	-	+	+
S10	+	-	+	+	-	+	+

+ve = positive result ; -ve = negative result

In the non-contaminated soil, both bacterial population and fungal population were higher. In contrast, in pesticide-contaminated soil, both populations were greatly suppressed. The bacterial population in general is not able to survive and multiply well in the presence of pesticide. It has been reported that one of the primary metabolites of (3, 5, 6-trichloro-2-pyridinol) possesses antibacterial properties [11]. A significant decline in bacterial populations observed in the present study could be attributed to the generation of such antibacterial metabolites. Similar observations were reported regarding the utilisation of Chlorpyriphos as a carbon source by bacteria isolated using an enrichment procedure [12]. Some organophosphorus insecticides such as Diazinon, Ethion, Parathion, Fonofos, Malathion, and Gusathion are susceptible to microbial hydrolysis and serve as carbon sources for the growth of pure and mixed cultures of *Flavobacterium sp., Pseudomonas sp. and Arthrobacter sp.*[13 -14].

4. Conclusion

Chlorpyriphos has a harmful effect on soil microorganisms and their biodiversity, as well as enzymatic activity. The microbial and

biochemical soil indices identified in the study provided necessary information about soil quality and fertility. The calculation of Colony Forming Unit (CFU) of soil confirms the fact that the use of this fungicide in contaminating doses creates a risk to living organisms. These findings suggest that use of Chlorpyriphos designed for the control of diseases in crops and vegetables should be used carefully and according to the manufacturer's recommendations. Uncontrolled doses distort the homeostasis of soil, which can have a strong impact on plant growth and yield.

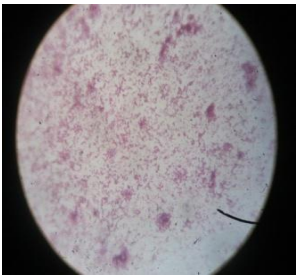


Figure 1a

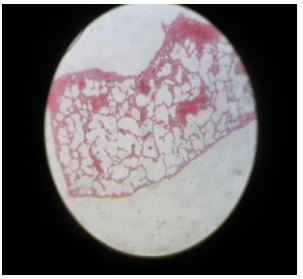


Figure 1b

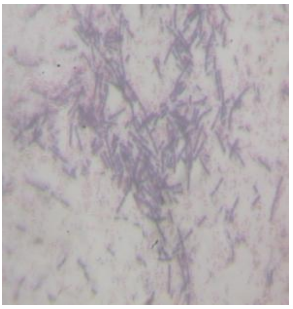
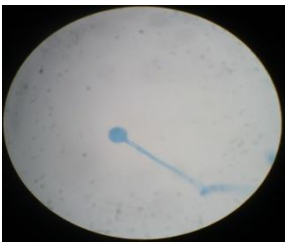
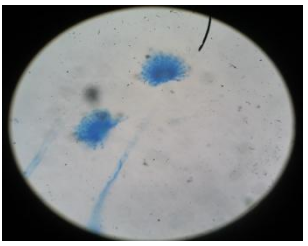


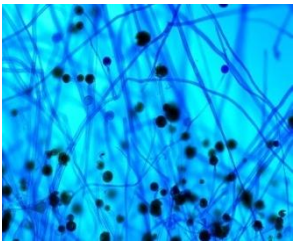
Figure 1c

Figure 1: Microscopic view of gram negative *Bacillus* (1a), gram negative *Staphyllococcus* (1b) and gram positive *Bacillus* (1c).



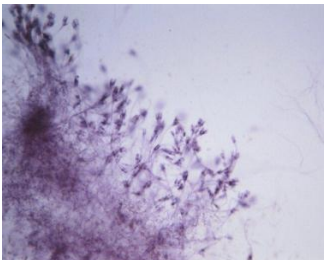


2a **2b**



2c

Figure 2: Showing a microscopic view of Mucor (2a) Aspergillus (2b) and Rhizopus (2c)



3a 3b

Figure 3: Microscopic view of Alternaria (3a) and Penicillium (3b)

References

[1] B. K. Singh. "Organophosphorus-degrading bacteria: ecology and industrial applications," Nat Rev Microbiol. Vol 7, pp. 156-164, 2009.

[2] G. Xu, W. Zheng W, Y. Li, S. Wang, and J. Zhang. "Biodegradation of chlorpyrifos and 3,5,6-Trichloro-2-Pyridinol by a newly isolated *Paracoccus sp*. strain TRP," Int. Biodeterior. Biodegradation Vol. 62, pp 51–56, 2008.

[3] J. F. Carriger, G.M. Rand, P.R. Gardinali, W.B. Perry, M.S. Tompkins, and A.M. Fernandez. "Pesticides of potential ecological concern in sediment from South Florida Canals: An ecological risk prioritization for aquatic arthropods. Soil Sed", Contam. Vol 15, pp. 21–45, 2006.

[4] D.Pimentel, "Amounts of pesticides reaching target pests: Environmental impacts and ethics". J. Agric. Environ. Ethics 8, pp 17–29, 1995.

[5] M. Andrea, T.B. Peres, L.C. Luchini, S. Bazarin, S. Papini, M.B. Matallo, and V.L.T. Savoy. "Influence of repeated applications of glyphosate on its persistence and soil bioactivity", Pesq. Agropec. Bras. Vol 38, pp. 1329–1335, 2003.

[6] J. Baxter and S.P. Cummings. "The degradation of the herbicide bromoxynil and its impact on bacterial diversity in a top soil", J. Appl. Microbiol. Vol 104, pp. 1605–161, 2008.

[7] X. Li, J. Jiang, L. Gu L, W. Ali, and J. He. "Diversity of chlorpyrifos-degrading bacteria isolated from chlorpyrifos-contaminated samples", Int Biodeterior Biodegradation Vol 62, pp. 331-335, 2008.

[8] K.R. Aneja. "Experiments in Microbiology, Plant Pathology and Biotechnology", Fourth edition. New Age International Limited, 2013.

[9] Mbajiuka and S. Chinedu. "Isolation and identification of microorganisms associated with the use of Automated Teller Machine (ATM) in the Michael Okpara University of Agriculture, Umudike (MOUAU) and its environs", World Journal of Pharmaceutical Research Vol 4, pp. 85-99, 2015.

[10] I. Fawzy, E. A. H. Mahmoud, N. Osama, M. K. Ghanem, and M. I. Gomaa. "Biodegradation of Chlorpyrifos by Microbial Strains Isolated from Agricultural", Wastewater Journal of American Science, Vol 10, No 3, pp. 95-99, 2014.

[11] X. Yong, W. Raza, G. Yu, W. Ran, and Q. Shen. "Optimization of the production of poly-γ-glutamic acid by Bacillus amyloliquefaciens C1 in solid-state fermentation using dairy manure compost and monosodium glutamate production residues as basic substrates", Bioresour Technol Vol 102, pp. 7548-7554, 2011.

[12] C. Yang, N. Liu, X. Guo, and C. Qiao. "Cloning of mpd gene from a chlorpyrifos-degrading bacterium and use of this strain in

bioremediation of contaminated soil", FEMS Microbiol. Lett. Vol 265, pp. 118-125, 2006.

[13] M. Digrak, S. Ozcelik, and S. Celik. "Degradation of Ethion and methidation by some microorganisms", 35th IUPAC Congress. Istanbul. Vol 14, pp. 19-84, 1995.

[14] O. Ghisalba, M. K. Ramos, G. M. Tombo, and H. P. Schaer. "Organophosphorous microbial degradation and utilisation of selected organophosphorus compounds: Strategies and Applications", Chemia, Vol 41, pp. 206-210, 1987.