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Effect of Pesticides (Chlorpyrifos and Endosulfan) on Soil Microbial Diversity

Satyamvada Swayamprabha
Research Scholar,
Department of Biochemistry
Magadh University, Bodh Gaya – 824234, Bihar, India, satyam.swayam@gmail.com

ABSTRACT

The paper studied the effect of soil contamination on microbe population. The microbes mineralise and bio transforms 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. Use of pesticides like Chlorpyrifos in agricultural soil is the primary reason for the pollution of aquatic and terrestrial environments. Endosulfan is a broad-spectrum chlorinated cyclodiene insecticide widely employed as pesticide around the world and is reported to be extremely toxic to aquatic organisms and mammalian system. 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, Endosulfan, Xenobiotic Characteristics, Microbial Characterisation

Introduction

The 70% of total population of India is dependent on the agricultural primarily which is the maximum portion of the country's economy1. Pesticides are varied and large group of substances used for killing the harmful organisms like weeds, insects, rodents among others.

The extensive use of such pesticides results in the accumulation of pesticide in our atmosphere. Many of these pesticides can persist in the soil and they can also contaminate the surface and the ground-water.

The Organophosphate insecticides like Chlorpyrifos is also widely used and can lead to contamination of soil and water bodies. Because of its abundant usage and potential transport, endosulfan has been detected in the soil, sediments, atmosphere, surface and rain waters, and foods (Kumar, 2011). Though many countries have imposed ban on endosulfan production and/or usage, yet in India it is still one of the priority pollutants for pest control. The biodegradation of persistent compounds is an important mechanism for their dissemination in the environment. The microbial mineralisation of organic compounds and associated biotransformation 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 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. 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 (Singh 2009). The literature survey findings, in the present study was taken up with the following objective of examining the biological dissipation of pesticides in the Chlorpyriphos and Endosulfan contaminated soil and effect of pesticide on soil microflora and pesticide degradation by isolate/ consortium obtained from contaminated soil. Degradation of the pesticide depends upon the type of the soil, soil property, the moisture content of the soil and pH (Xu et al

2008). The amount of applied pesticides reaching the target organism is about 0.1% while the remaining bulk contaminates the soil environment (Carriger et al 2006 and Pimentel 1995). 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 have received more attention (Andrea et al 2003, Baxter et al 2008 and Li et al 2008).

2. Materials and Methods

2.1 Soil Sample Collection

The soil samples were collected from the organic garden; both without the contamination of any chemical pesticides and with the contamination of Pesticides (chlorpyrifos and endosulfan) 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 (Aneja 2013).

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 (Mbajiukaand Chinedu 2015). 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.

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.

Ten bacterial strains and three fungal strains (Fig 2 and 3) were isolated from the different region of organic garden soil.

Table 1: Total number of isolated bacterial pure strains

S.No.	Identification	Gram's Reaction
1	Coccus	Positive
2	Staphylococcus	Positive
3	Staphylococcus	Negative
4	Bacillus	Negative
5	Coccobacillus	Positive
6	Coccus	Negative
7	Bacillus	Positive
8	Bacillus	Positive
9	Pleomorphic	Positive
10	Streptobacillus	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

3.2 Microbial Characterisation

On the basis of the various biochemical tests performed the ten 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	-	-	+	+	+++	-	+++	-	-	-	-	-

+ve = positive result; -ve = negative result

Table 4: Result of another different biochemical test

Isolate No	Amylase Test	Cellulose Test	Casin Hydrolysis Test	H2S Production Test	Catalase Test
1	+	-	+	++	-
2	-	-	+	++	-
3	+	-	+	++	-
4	+	-	+	++	-
5	-	-	+	+	-
6	+	-	+	++	-
7	-	-	-	+	-
8	-	-	-	+	+
9	+	-	-	+	-
10	+	-	+	+	-

+ve = positive result ; -ve = negative result

3.3 Isolation from the Chlorpyriphos and Endosulfan Exposed Wheat Soil

Average total number of bacterial colonies was 3.8 X 103 CFU/ gm

Eight bacterial strains and two 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

Table 6: Total number of fungal isolates

Strain no.	Colony morphology	Microscopic characterstics	Identification on microscopic view
I	A black colony with dense cottony growth	Beak-like, alternate septation	Alternaria
II	Black colony	Conidiophores are smooth walled hyaline, conidial head are biseriate.	

3.4 Microbial Characterisation

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

Table 7: Result of biochemical test

Strain number	Fermentation test (glucose)		Amylase test	Cellulose test	Casein hydrolysis test	H2S production test	Catalase test
	Gas Production	Acid Production					
S1	+	-	-	-	-	-	-
S2	+	-	-	-	-	-	+
S3	+	-	-	-	+	+	+
S4	+	-	+	+	-	+	+
S5	+	-	+	-	-	+	-
S6	+	-	-	-	-	-	+
S7	+	-	-	-	-	-	+
S8	+	-	+	+	+	-	+

+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 (Yong et al 2011). 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(Yong et al 2006).Endosulfan also shows same activity. 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*.(Digrak et al 1995 and Ghisalba et al 1987).

4. Conclusion

Chlorpyrifos and Endosulfanhas 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 Chlorpyrifos designed for the control of diseases in crops and vegetables should be used carefully and according to the manufacturer's recommendations and Endosulfan is a persistent in nature not to be used. Uncontrolled doses distort the homeostasis of soil, which can have a strong impact on plant growth and yield.

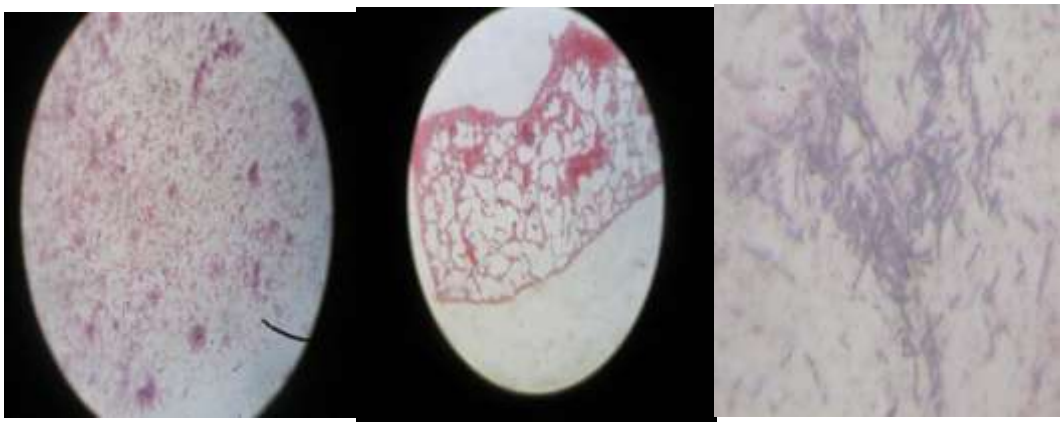

Fig 1a Fig 1b Fig 1c

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

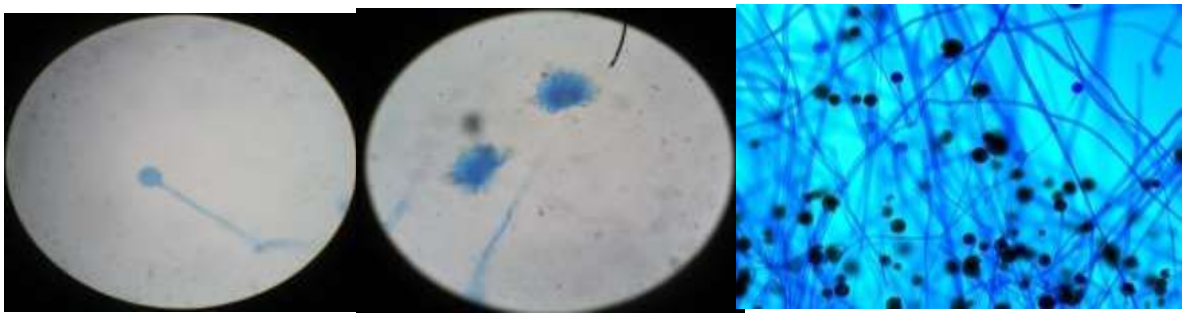

Fig 2a Fig 2b Fig 2c

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

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