EFFICIENCY OF BACTERIAL ISOLATES IN DEGRADATION OF QUINALPHOS INSECTICIDE

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ABSTRACT

The use of microbes for effective detoxify, degrades and removal of toxic compounds from contaminated soils has emerged as an efficient and cheap biotechnological approach to clean up polluted environments. In the present study the soil sample was collected from the paddy fields of Maloibari village of Dimoria Tribal Development Block, Kamrup, Assam, which is having a history of repeated pesticide applications. The isolation of pesticide degrading bacteria was carried out and the isolated bacterial isolates were identified as Staphylococcus species and *Bacillus licheniformis based on staining techniques and plating on selective media. The growth of these two pesticide degrading isolates viz., Staphylococcus species* and *Bacilluslicheniform was assessed in Minimal salt broth containing 50ppm of pesticides at different pH levels viz., pH 4, pH 5, pH 6, pH 7, pH 8 and different carbon sources viz., Dextrose, Fructose, Lactose, respectively. Among the two bacterial isolates, the bacteria Staphylococcus species utilized the pesticides effectively and showed maximum growth. The maximum growth rate of bacteria was recorded at pH 7. The growth of bacteria was maximum in the presence of Dextrose followed by Fructose and Lactose.*

Keywords: ***Bacteria, Biodegradation, Pesticides, Quinalphos.***

I. INTRODUCTION

The intensive nature of modern agricultural practices has led to the development and widespread utilization of synthetic pesticides in our environment. Pesticides, herbicides and polychlorobiphenyls are PCB's that are widely distributed in the environment. In India, alarming levels of pesticides have been reported in air, water, soil as well as in foods and biological materials. Some of these pesticides have also been reported to be toxic, mutagenic, carcinogenic and tumorogenic. The most important pollutants among the toxicants in India are organochlorine and organophosphorus pesticides. A hundreds of pesticides of different chemical moieties are widely used for agricultural purpose, soil receives large amounts of pesticides even from bulk handling, direct application at fields or accidental release which lead to occasional contamination of a wide range of water and terrestrial ecosystems, and accumulation of these compounds has many health hazards associated with it (Singh *et al*., 2004).

1.1 Environmental Impact of Pesticides

The major environmental concern of used pesticides is their capacity to leach down to subsoil and contaminate the ground water (Kookana et al., 1998) or if immobile, they would persist on the top soil where it could

accumulate to toxic level in the soil and become harmful to microorganisms, plants, animals and man (Amakiri, 1982). Pesticides have various characteristics that determine how they act once in soil. Excessive and persistent use of pesticides results in deterioration of the environment. The quality of soils, ground water, continental and coastal waters as well as the air, is compromised by pesticide contamination (Surekha et al., 2008). Globally, subsoil and groundwater pollution arethe major consequences/outcomes environmental effects of pesticides application.

1.2 Biodegradation of Pesticides

Pesticide degradation is the breaking down of toxic pesticides into a nontoxic compounds and, in some cases, down to the original elements from which they were derived. The most common type of degradation is carried out in the soil by microorganisms, especially the fungi and bacteria.Hence the degradation process of pesticides in the different ecosystems universally takes a large space of interest. Recently the use of microbes for effective detoxification, degradation and removal of toxic compounds from contaminated soils has emerged as an efficient and cheap biotechnological approach to clean up polluted environments (Strong and Burgess, 2008). From the great microbial population existing in soil, some bacteria strains show the capability to degrade some types of pesticides thru specific paths, such using it as a source of nutrients and the most common one to use it as carbon and energy sources due to the chemical nature (Aislabie and Lloyd-Jones, 1995).

1.3 Bacterial degradation of insecticides

Bacteria capable to uptake and degrade various insecticides are isolated from various sources (Singh et al., 2004; Saier, 2005). Some of the widely used insecticides like Carbofuran and DDT are degraded by bacteria like *Alcaligens, Flavobacterium, Pseudomonas* and *Rhodococcus* (Aislabie et. al., 1995). A widely available insecticides alpha-endosulfan, beta-endosulfan is degraded by single bacteria like *Klebsiellaoxytoca, Bacillus spp., Pandoraea sp., Micrococcus sp.* and by mixed bacterial co-culture (Bhalerao and Puranik, 2007). *Flavobacterium sp., Pseudomonas diminuta, Pseudomonas putida, Enterobacter Strain B-14* were isolated from chlorpyrifos contaminated sites and showed degradation capacity for chlorpyrifos (Singh et. al., 2004).

1.4 Objectives of the study

General agricultural use of pesticides carries with it potential hazards to man and directly by exposure to toxic residues in food and indirectly to the environment. In relation with the increasing pesticide and insecticides application and their effect on the environment, it is necessary to study and understand the effect of pesticide and insecticide on soil microflora and the pesticide utilizing capacity of microorganisms. Microorganisms are frequently the major and sometimes the only means by which the pesticides are eliminated from a variety of ecosystems. The native microflora degrades the toxic pesticides in the soil and reduces its toxicity. In this context, the present study was planned to isolate and estimate the pesticide degrading microorganisms from soil samples collected from selected paddy fields of Maloibari village of the Dimoria region of Kamrup, Assam.
The specific objectives of study are:

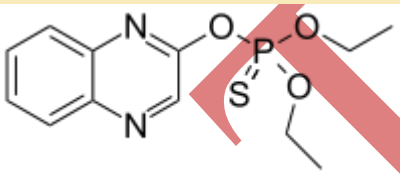
- To isolate the Quinalphos insecticide degrading microorganisms.
- To identify the pesticide (Quinalphos) degrading microorganisms in terms of its morphological characteristics like ***Colony morphology, Gram's staining, Motility test etc.***

- To study the effect of pH for the growth of pesticide degrading bacterial isolates.
- To study the effect of carbon sources for the growth of pesticide degrading bacterial isolates.

1.5 Pesticide Used For the Present Study

Quinalphos: Quinalphos is an organothiophosphate chemical chiefly used as a pesticide. It is a reddish brown liquid. The chemical formula is $C_{12}H_{15}N_2O_3PS$, and IUPAC name *O*, *O*-diethyl *O*-quinoxalin-2-yl phosphorothioate. Ranked 'moderately hazardous' in World Health Organization's (WHO) acute hazard ranking, use of quinalphos is either banned or restricted in most nations. Quinalphos, which is classified as a *yellow label* (highly toxic) pesticide in India, is widely used in the following crops: wheat, rice, coffee, sugarcane, and cotton. Quinalphos is a broad-spectrum organophosphorus insecticide and acaricide with good penetrative properties. Because of its broad spectrum application in tea plantation there has been growing concern among the regulation agencies, researcher and environmentalists to know its fate in tea and the surrounding environment. Quinalphos, being an inhibitor of acetylcholinesterase is a very effective, widely accepted broad-spectrum organophosphate pesticide applied as spray and used actively against the pests of cotton, vegetables, rice, groundnut and tea

Quinalphos



IUPAC name
O,*O*-Diethyl *O*-2-quinoxalinyl phosphorothioate

Other names

O,*O*-diethyl *O*-quinoxalin-2-yl phosphorothioate
Diethquinalphion;

Diethquinalphione

Figure: Structure of Quinalphos

II. METHODOLOGY

2.1. Sampling

2.1.1 Selection of Sampling Station

For the purpose of this study samples were collected from some selected paddy fields of Maloibari village of Dimoria region of Kamrup District, Assam. In all study areas the same method was used to collect soil sample. In total 5 stations were selected. Four stations each from **low land rice field** and one station from **organic paddy field (Control sample)** was selected. Three soil samples at 0-15 cm depth were collected randomly from each station to prepare a single composite sample of each station.

2.1.2 Sampling Procedure

In order to collect soil samples (0-15 cm depth) grasses, mosses, litter and other plant residues were removed from soil surface. Collection of soil samples was done by using an auger. In each case, a triangular block was cut with the help of the auger. Soils were collected in plastic bags, which were sealed and labelled properly. Three soil samples from a rooting depth of 15 cm were collected randomly from each sampling station and each sample composite was labelled as **S1**- Rice farm Composite sample 1; **S2**- rice farm Composite sample 2; **S3**-rice farm Composite sample 3; **S4**- rice farm Composite sample 4 and **S5**-Organic rice farm Composite sample 5

2.1.3 Soil Sample Preparation

Preparation of soil samples is based on the **ISO 11464 method** (Soil quality- pre-treatment of samples for physic- chemical and biological analysis). Collected samples were brought to the laboratory for analysis. Before analysis, the samples were spread out thinly on a piece of hard paper for drying in air in a shade. The big lumps were broken down, and visible plant roots, pebbles and other undesirable matters were removed. After the soil become completely dry, and after homogenization, a portion of each sample was passed through a 2-mm mesh screen and preserved in clean sealed polythene bagsand stored in sealed polythene boxes to avoid air contamination at 4°C before microbial and biochemical analysis. Another portion of the collected soils was stabilized at 25°C for seven days, after adjusting to 60% of its water holding capacity, before the determination of microbial and biochemical parameters.

2.2. MICROBIOLOGICAL PROCEDURES

2.3. Pesticides Used

Pesticides used in this present study is **Quinalphos**

2.2.2 Isolation Of Quinalphos Degrading Bacteria

The bacterial cultures capable of degrading Quinalphos were isolated from collected soil samples using enrichment technique, with some concentration of Quinalphos. Standard analytical grade solution of Quinalphos (25% E.C.) was purchased from the local market. 1gm of each soil sample was inoculated into 6 different 100ml Erlenmeyer flask containing 100ml of mineral salt medium(MSM) supplemented with 0.5ml concentration of Quinalphos in 300ml.

The composition of Mineral Salts Medium (MSM) is given below:

MS Medium composition	**Quantity(g)**
NaNO3	0.3
MgSO4	0.05
KCl	0.05
K2HPO4	0.1
KH2PO4	0.05
FeSO4	0.001
yeast extract	0.05
Glucose	1.0

The flasks were incubated on a rotary shaker at 150 cycles per minute for 7 days at room temperature (25-30◦C). At daily intervals, one loop full of enrichment culture from the flask was streaked on nutrient agar plates supplemented with Quinolphos (5g) and incubated at room temperature for 24-48hrs. Nutrient agar media was prepared by adding 7g of agar and 5g quinalphos in 250ml water. Individual colonies of bacteria which varied in shape and colour were picked up and were sub cultured onto nutrient agar plates containing same concentration of Quinalphos until pure culture was isolated. The isolated strain was maintained at 4◦C.

2.2.3. Identification of Bacterial Isolates

Identification of bacterial isolates were carried out by the routine bacteriological methods i.e., by the colony morphology, preliminary tests like Gram staining, Motility test etc. Gram staining reaction was performed to evaluate type of strain.

2.2.4 Microscopic Study of Bacterial Cultures

The bacterial isolates were studied for their various microscopic characters such as:

2.2.4.1. Colony Morphology

Study of colony morphology includes colour, size, margin, elevation etc.

2.2.4.2. Gram's Staining

Gram's staining of the cultures was performed by the method as described by ***Cappucino*.**

2.2.4.3. Motility Test

Motility test was performed by hanging drop method as described by **Cappucino.**

2.2.5 Effect of pH

In order to analyze the effect of pH variations on the growth of the organism, an experiment was conducted in a Erlenmeyer flask containing 50ppm of pesticides in 50ml MS Broth with pH **4.0, 5.0, 6.0, 7.0** and **9.0**.After sterilization by autoclaving the flasks were cooled and inoculated with the bacterial cultures after inoculation, flasks were kept at 37°C separately for 24 hrs. To analyze the effect of p^H the growth of pesticides degrading bacterial isolates were assessed by using **UV Spectrophotometer** at 560nm.

2.2.6 Effect of Carbon Sources

To evaluate the growth of pesticides degrading bacteria the bacterial isolates were cultured in 50ml of MS Broth with 50ppm of pesticides and **0.5gm** of various carbon sources and incubated at 37°C for 24 hrs. The growth of pesticides degrading Bacterial isolates were assessed by using UV spectrophotometer at 560nm. Carbon sources used are **lactose, dextrose, and fructose.**

III. RESULTS AND DISCUSSION

3.1. Isolation of Pesticides Degrading Bacterial Strain

A total of two different colonies were observed on nutrient agar medium enriched with QUINALPHOS from soil samples of Rice Farm Composites out of five samples collected for the present study and were designated as **QS1** and **QS2.**

QS1- Pesticides Degrading Bacterial Isolate from rice farm Composite sample 1.

QS2- Pesticides Degrading Bacterial Isolate from Rice Farm Composite sample 4.

3.2. Microscopic Study of the Bacterial Strains

3.2.1 Colony Morphology

The colony morphology of the bacterial isolates was recorded in the table 1.

Table 1: Morphology and characteristics of isolated bacterial strains

Sl. No.	Strain label	Colony colour	Size	Shape
1.	QS1	White to cream	1.5mm	Single round type
2.	QS2	Yellowish grey	0.5mm	Round, granular

3.2.2. Gram's Staining

The result of the gram's reactions was recorded in the table 2.

Table 2: Gram characters of the tested bacterial strains

Sl . No.	Strain label	Gram character
1.	QS1	Positive ,Coccus
2.	QS2	Positive , Rod

3.2.3. Motility Test

The result of the motility test is recorded in the table 3.

Table 3: Motility test of the tested bacterial strains

Sl. No.	Strain label	Motility
1.	QS1	Non-motile
2.	QS2	Motile

3.2.4 Identification of the Bacterial Isolates

Based on the morphological studies and preliminary tests like Gram staining, Motility test of the isolated colonies, the observed colonies may be identified as follows:

Table: 4 Identification of the bacterial isolates

Strain label	**Colony name**
QS1	*Staphylococcus species* (may be)
QS2	*Bacillus licheniformis* (may be)

The isolated bacterial isolates were identified as ***Staphylococcus species*** and ***Bacillus licheniformis*** based on staining techniques and plating on selective media.

3.3 Growth of Bacterial Isolates at Different Phand Carbon Sources

The growth of the pesticide degrading isolates *viz., Staphylococcus species, and Bacillus licheniformis* was assessed in Minimal salt broth containing 50ppm of pesticide at different pH levels *viz*., pH 4, pH 5, pH 6, pH 7 and pH 8 and in Minimal salt broth containing 50ppm of pesticides using different carbon sources viz., dextrose, lactose and fructose. The results of which is shown in Table 5 and 6.

Table 5: Spectrophotometer Reading Of Isolates Inminimal Salt Broth Containing Quinalphos.

Sample Label	**Readings (absorbance at 560 nm)**				
	pH				
	4	**5**	**6**	**7**	**8**
QS1	0.28	0.37	0.40	0.45	0.40
QS2	0.25	0.32	0.34	0.35	0.31

The maximum growth rate of bacteria was recorded at pH 7 followed by pH 6, pH 8 and pH 5 and pH 4.

Table 6. Spectrophotometer Reading of Isolates In Minimal Salt Broth Containing Quinalphos.

Sample Label	Readings (absorbance at 560 nm)		
	Carbon source reading		
	Lactose	Dextrose	Fructose
QS1	0.35	0.43	0.39
QS2	0.36	0.45	0.40

The growth of bacteria was maximum in the presence of dextrose followed by fructose and lactose.

IV. CONCLUSION

The increasing production and application of pesticides for agricultural and non-agricultural purposes has caused the pollution of air, soil, ground, and surface water which involves a serious risk to the environment and as well as human health due to either direct exposure or through residues in food and drinking water. In the world, alarming levels of pesticides have been reported in air, water, soil, as well as in foods and biological materials. Recently, bioremediation has been proven a suitable technique for reducing pesticide poisoning. The use of microorganisms for the degradation and detoxification of numerous toxic xenobiotics, especially pesticides, proved to be an efficient tool to decontaminate the polluted sites in the prevailing environment (Mervat, 2009). In this context, an attempt has been made to isolate microbial strains capable of degrading Quinalphos insecticides residues from the paddy field of Maloibari village of Dimoria Tribal Development Block, Kamrup, Assam, which is having a history of repeated pesticide applications with a view of bioremediation of contaminated sites. The study reveals that the bacterial isolates *Staphylococcus species* and *Bacillus licheniformis* have the capacity to utilize the pesticide and grow well in the medium supplemented with pesticides. Moreover, among the two bacterial isolates, the bacteria *Staphylococcus species* utilized the pesticides effectively and showed maximum growth. The maximum growth rate of bacteria was recorded at pH 7. The growth of bacteria was maximum in the presence of Dextrose followed by Fructose and Lactose. This research aims to elaborate the potential applications of micro biological agents in decontamination of agricultural soils, which have been polluted with continuous and higher doses of pesticides through process of biodegradation. Biodegradation is an eco friendly, cost effective, highly efficient approach and can be considered as a superior alternative to physical and chemical methods which are not only technically laborious and costly; also are not sufficient to completely degrade organic toxins.

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