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Research Article

BIODEGRADATION OF PESTICIDE DEGRADING MICROORGANISMS AND IT'S IMPACT

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ABSTRACT

The present investigation was focused on biodegradation of chlorinated pesticide monocrotophos. The enrichment culture technique was adopted in such a way that the high concentrations of 500ppm of pesticides were treated with Paddy field soil for a period of 15 days. Serial dilution technique was employed to isolate the dominant colony with the potential to degrade pesticide. The potential organisms were isolated and characterized morphologically and biochemically. The isolate 1 was presumed to be *Bacillus cereus* characterized based on morphologically and biochemically. The flask culture technique of biodegradation studies were performed with different concentration of monocrotophos pesticide and it was found out that in higher concentration the degradation was found to be increased. HPLC analysis, revealed a prominent result between control and experimental treated samples. The intermediate metabolites due to the action of enzymatic cleavage in the appearance of splitting isomer entities and breakdown of compounds were formed from *Bacillus cereus* (S1).

Keywords: Biodegradation, monocrotophos, pesticide, *Bacillus cereus*, HPLC

1. INTRODUCTION

Organophosphrous insecticides are compounds which act on the nervous system by inhibiting the action of several estersplitting enzymes, particularly acetylcholine esterase at the synapse. They have phosphorous at the active nucleus and are esters of alchohols with phosphoric acid or anhydrides of phosphoric acid with another acid. Organophosphorous insecticides form the largest and most diverse group of insecticides. The earliest compounds of this class were scharadan, dimefox and mipafox and had a very high mammalian toxicity and hence they are not in use at present the most commonly used are phosphamidon, chlorfenvinphos, parathion, methyl parathion, dimention, malathion, phorate, dimethoate etc [1].

Biodegradation of pesticides is determined by two groups of factors, the first related to microbial consortium and the optimum condition for their survival and activity while the second related to the chemical structure of the pesticides. Other factors related to microorganism including the presence and number of appropriate microorganism, the contact between microorganism and the substrate (pesticide),pH, temperature, salinity, nutrients, light quality and intensity, available water, oxygen tension and redox potential, surface binding, presence of alternative carbon substrates alternative electron acceptors [2].

Microbial degradation of pesticides applied to soil is the principle mechanism which prevents the accumulation of these chemicals in the environment. Yet, when pesticides degraded too rapidly, post control may be less effective. One factor that has been shown to increase the rate of microbial degradation of pesticides in soil is one or more provides applications of the same pesticide or another pesticide with a similar chemical structure. This phenomenon is known as accelerated or enhanced degradation [3].

Microorganisms are the primary soil decomposers driving key ecosystem processes such as organic matter decomposition, nutrient cycling and, thereby, plant productivity [4, 5]. Thus, agricultural practices affecting soil microorganisms are of particular interest. Modern agriculture worldwide uses a variety of pesticides including insecticides, nematicides, herbicides and fungicides to optimize crop production [6, 7].

The present endeavor was therefore initiated to evaluate the effect of pesticides and biopesticides on soil microbial biomass carbon in soil at controlled laboratory

conditions. To collect paddy field soil amended with pesticides (monocrotophos) were enrich soil with pesticide by enrichment culture technique and to isolate, dominant colony in pesticide degrading microorganisms was characterized by morphological and biochemically and to degradation study by flask culture method, and analyzed the metabolite pesticide degradation by HPLC (High performance liquid chromatography).

2. MATERIAL AND METHODS

2.1. Collection of Soil

The soil samples for the present study were collected from paddy field soil at selected pockets in and around Tiruvarur and brought to the laboratory for the experimental purpose.

2.2. Enrichment Culture Technique

Enrichment culture was carried out by taking 25 g of clay loam soil in a Petridis and calculated quantities of individual insecticide like monocrotophos was added so that find concentration is around 500ppm of soil. It was cultured for a period of 1-15 days, preferably in an incubator at 32°C.

2.3. Isolation of Pesticide Degrading Microorganism

Five (5)gms of enriched soil was taken and it was transferred to 100ml sterile water taken in 250ml conical flask stirred well and it was serially diluted (10^{-1} to 10^{-7}) through serial dilution method.

2.4. Morphological and Biochemical Characterization

The isolated microorganisms were characterized based on morphological and biochemical characterization were studied following tests, like gram staining, spore staining, indole production test, methyl red test, vogesproskauer test, citrate utilization test, triple sugar iron test, nitrate reduction test, gelatin hydrolysis and starch hydrolysis [8].

2.5. Determination of the growth of pesticide degrading bacterial isolates on minimal salt broth

The suspension of 24 hours old cultures of Bacillus species were used to prepare the bacterial inoculums. The growth of pesticide degrading bacterial isolates bacillus species was determined by using minimal salt broth. For this, 2ml of the bacterial inoculums was inoculated into 100ml of mineral salt broth containing 50ppm of pesticides. The flask was then incubated at 37°C for 7 days in a shaker. Five ml of culture was drawn and was disbanded and the supernatant was collected to evaluate the growth of pesticide degrading bacteria. The growth of the pesticide degrading bacterial isolates was assessed by using UV- spectrophotometer at 560nm.

2.6. HPLC Analysis of Monochrotophos

For HPLC analysis, extracted samples were centrifuged (at 7200 rpm) for about 10 min and supernatant was mixed with equal volume of dichloromethane (DCM). Organic layer was collected and DCM was evaporated (under nitrogen) at room temperature. Residues were filtered (0.45 µm diameter) after dissolving in acetonitrile. HPLC equipped with a ternary gradient pump, UV detector, electric sample valve, column oven and C18 reversedphase column was used for pesticide analysis using mobile phase of methanol, water (85:15,U:V) HPLC conditions were set as follows sample volume: 20µl, flow rate, 1ml min-1, retention time : 15min and wavelength,290nm.

3. RESULTS AND DISCUSSION

3.1. Isolation and Characterization of Biodegrading Bacteria from Soil

The results of enrichment of soil with pesticide monocrotophos was carried out for a period of 15days.Monocrotophos was selected and added to the soil in the concentration of 100ppm, 300ppm and 500ppm. The bacterial mixture was prepared by enrichment culture techniques [2a].

Five colonies were observed in a sample of 25gm soil which was treated with 100ppm of endosulfane. Colonies were observed in the soil which was treated with 300ppm.When the concentration of endosulfane was increased to 500ppm only one colony was seen. The results clearly show that with increasing concentration of monocrotophos there is decreasing number of colonies. By using gram staining techniques on subjecting them to various biochemical test mention in materials and methods and their growth in specific media, the isolated colonies were confirmed as *Bacillus* sp. The results were noted in Table1 and Plate 1-5.

3.2. Biodegradation of Pesticide by Flask Culture Method

During enrichment, the concentration of monocrotophos was increased from an initial concentration of 100, 300 to 500ppm. At high concentration of 500 ppm, only one bacterial strain survived. Which could degrade mono-

crotophos and used as a carbon source. The bacterial isolate were added in to 100 ml of minimal salt broth containing 100ppm, 300ppm and 500ppm of pesticides. It was kept in the shaker for 15 days. The results were shown in Plate 6. Simultaneously another isolate found to be *Bacillus cereus* was inoculated separately with 100, 300 and 500ppm respectively. After 15 days this samples from 100, 300 and 500ppm were taken and subject to analysis for pesticide degradation and its metabolites by HPLC (Fig. 1).

3.3. HPLC Analysis

HPLC or monitoring the formation or disappearance of peak obtained due to degradation of pesticides were recorded. The chromatogram of control treated pesticide exhibited to peaks the same retention time which appeared 3.040 and 3.534 min respectively. HPLC analysis on different concentration such as 300 and 500 ppm exposed to different isolates of *Bacillus cereus*. On analysis with an increase concentration of 500ppm inoculated with *Bacillus cereus* indicating a peak of 2.487 and 3.047 at 3 min of retention time revealing the degradation of parent molecular structure of the chlorinated compound monocrotophos (Fig. 2).

Table 1: Morphological and Biochemical Characterization of Pesticide Degrading Microorganism

Test	*Bacillus Cereus (S1)*
Gram Staining	Gram Positive Rod shape
Spore Staining	Spores Present
Indole	Negative
Methyl Red	Positive
Citrate utilization test	Positive
Triple sugar iron agar test	Alkaline Slant
Gelatin hydrolysis	Positive
Nitrate reduction test	Positive
Starch hydrolysis	Positive
Vp test	Negative
Carbohydrate test	
Dextrose	Positive
Fructose	Positive
Sucrose	Negative
Mannitol	Negative

PLATE:1. PURE CULTURE OF THE EFFICIENT DEGRADING MICROORGANISM

PLATE:2.

PLATE:3.

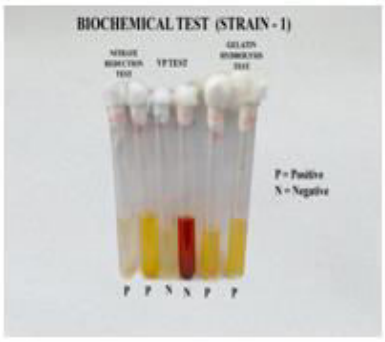
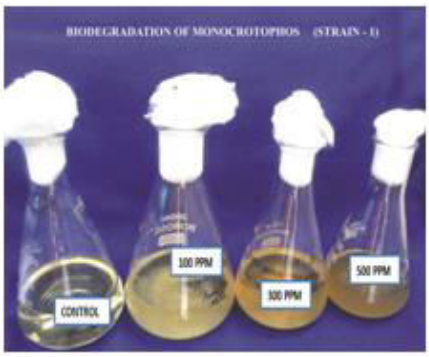


PLATE:4.

PLATE:5.

PLATE:6.



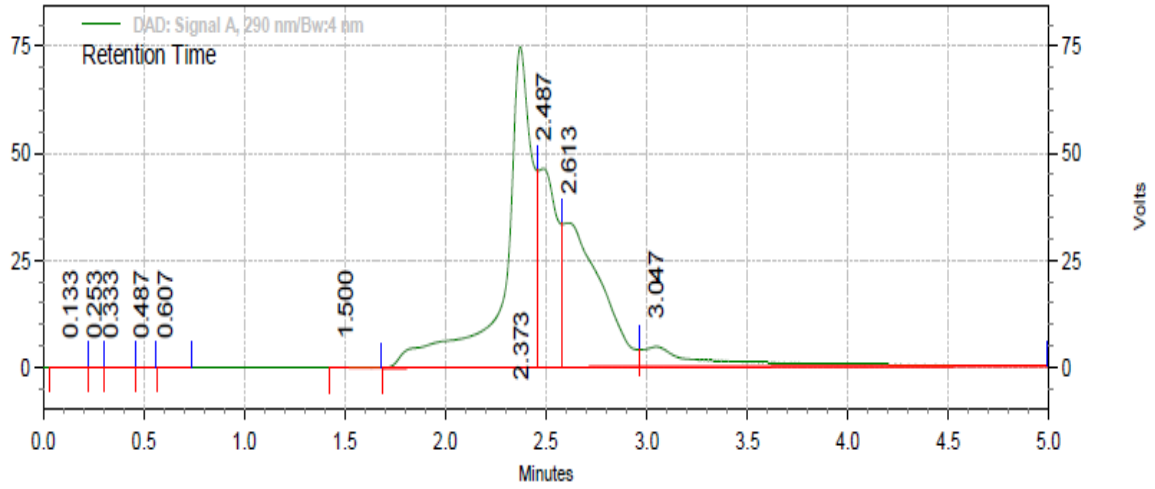


Fig. 1: HPLC analysis for biodegradation of pesticide degrading control treated sample

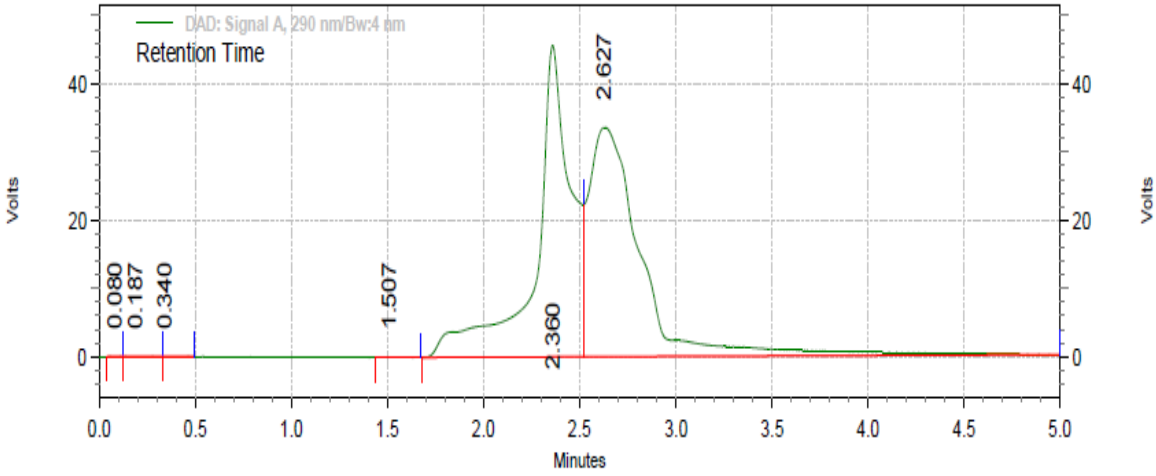


Fig. 2: HPLC analysis for biodegradation of pesticide degrading microorganism –s1 (*bacillus cereus*)

The role of microbes on biodegradation has been clearly investigated in the concept of enriching culture, it is very powerful and simple technique dating from 1980 in which the compound to be degraded is supplied as sole source and act as an essential nutrient in a culture medium, and only the organism with the necessary degradative ability will grow significantly under those condition and these organisms will outgrow in very large number of other organisms added at the start of enrichment.

In the present study, monocrotophos; an organochloride persistent compound, not easily biodegradable under sunlight (photometabolism) but can be cleared by the sporulating microorganism of *Bacillus* sp was easily degraded, it contain the xenobiotic pesticide molecules.

In the current research work the pesticide (mono-crotophos) degrading bacterial isolate strain 1 belongs to *Bacillus cereus* were isolated and identified by enrichment culture technique. It was interesting to note that the ability of bacterial strains isolated from pesticide enriched soils to tolerate and grow on higher concentration of pesticides at 500 ppm. Similar work was carried by Lu [8] with *Sphingomonas*. In tune with above discussion various contribution pertaining to degradation were performed by [9] wherein he emphasized bioconversion and biological growth kinetics of *Pseudomonas aeruginosa*, known to degrade HCH as sole carbon source.

In the present study, monocrotophos a widely used pesticide by agricultural farmers to ascertain role of pesticides in degradation using sporulating microbes to degrade the pesticides were monitored with flask culture experiment at laboratory.

The HPLC analysis revealed that strain 1 *Bacillus cereus* has good degradation efficiency as compared to control. The experimental work complete biodegradation yield by carbon and energy source of oxidation process utilized the microorganisms during the degradation of pesticides. The detailed pathway indicated that organochlorinated compound, monocrotophos was broken down into isomers and then dechlorinated into 2-pyridinol. The pyridine ring was then subjected to cleavage and then completes mineralization results.

Among the various factors that significantly affects monocrotophos degradation is the concentration of active material. Very high concentration leads to failure of biodegradation as microbes are not resistant against that, on the other hand a very low pesticide concentration leads to strong affinity with soil particles that make it not available to microbes.

Bacillus cereus is best adaptive at low concentration, the monocrotophos degradation decreases with increase in concentration. Monocrotophos degradative pattern of *Bacillus cereus* at different temperature which narrates that *Bacillus cereus* prefer 40°C as maximum degradation was observed at this temperature. Degradation rate at all the temperature ranges significantly. Similar findings were coincides by Ortiz-Hernande, etal. [10] with temperature as important feature in biodegradation experiments.

4. CONCLUSION

In conclusion, monocrotophos, an organochloride persistent compound, not easily biodegradable under sunlight (photometabolism) but can be cleared by enzymatic process leading to complete disappearance of parent molecular structure liberating carbondioxide and water. The sporulating microorganism *Bacillus* sp was employed to degrade the xenobiotic pesticide molecules.

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