Biodegradation of the Synthetic Pyrethroid, Deltamethrin by *Bacillus Subtilis* in Short Term Treatment

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Abstract

Background: Deltamethrin is a pyrethroid pesticide that kills pests on contact and through digestion. It works by paralyzing the insect's nervous system and therefore giving a quick knock-down effect. It is a highly persistent pyrethroid that is commercially used especially in soil containing a high proportion of organic matter. Deltamethrin is particularly highly toxic to mammals and aquatic organisms and also the breakdown is slow in the environment.

Materialsand Methods: Degradation of deltamethrin using *Bacillus subtilis* MTCC2423 obtained from IMTECH was performed in the present study. When cultured in minimal medium containing 50,100,150 and 200 ppm concentrations of deltamethrin, parameters such as pH, carbon dioxide, esterase activity and biomass were analysed for four days.

Results: Enhancement of carbon dioxide levels indicates the degradation of deltamethrin. Increase in pH, CO_2, esterase activity and biomass was observed.

Conclusion: Thus degradation of deltamethrin using *Bacillus subtilis* was efficient upto 100 ppm.

Key words: *Deltamethrin, degradation, Bacillus subtilis, pyrethroid.*

Introduction

Pesticides stand out as one of the major developments of the twentieth century. The use of pesticides is the most widespread method for pest control. Certainly pesticides have improved the longevity and the quality of life, chiefly in the area of public health. Insect control programs have saved millions of lives by combating diseases in plants. Their use constitutes an important aspect of modern agriculture tocontrol pests like insects, weeds, plant diseases, worms and rodents. Pesticides help in preventing economical loss to farmers. The use of pesticides becomes necessary as crop yields are reduced by 10% without them.[1] Along with the benefits of pesticide, there are risks to humans, livestock, wildlife, and the environment. Thewidespread use of these pesticides over the years has resulted in problems caused by their interaction with the biological systems in the environment. The use of pesticides decreases the general biodiversity of the soil, and use of pesticides can have uninvented effects on the environment. Over 95 - 98% of insecticides reach a destination other than their target species, including non-target species, air, water, soil and also other useful organisms.[2]

Pesticide degradation is the breaking down process of toxic substances into nontoxic compounds and in some cases, down to the original element from which they werederived. Pesticides are broken down by microbes, chemical reactions, and light or photodegradation. This process may take from days to years depending on environmentalconditions, and the chemical characteristics of the pesticides. The most common type ofdegradation is carried out in the soil

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by microorganisms, especially the fungi and bacteria.[3] Sunlight breaks down some pesticides. Generally chemical pathwaysresult in only partial deactivation of pesticides, whereas soil microorganisms can completely break down many pesticides to CO_2, water and other inorganic constituents.[4]Currently, among the various groups of pesticides that are used worldwide,synthetic pyrethroids form a major and most widely used group: Synthetic pyrethroids aretaken from pyrethrum, the oleoresin extract of dried chrysanthemum flowers. The insecticidal properties of pyrethrins are derived from ketoalcoholic esters ofchrysanthemic and pyrethroid acids.

Pyrethroids breakdown more slowly than the naturally occurring pyrethrins. Theypersist in the environment much longer.[5] They are often formulatedwith oils or petroleum distillates and packaged in combination with synergists, toincrease the potency of the pesticides: In the environment, pyrethroids are usuallydegraded by one or more biotic and abiotic processes, namely metabolic degradation by plants, animals, and microorganisms, and degradation by light. The rate of degradation bychemical and microbial action depends upon the pyrethroid, soil type, the species ofmicrobes present, and the size of their population.The studies so far carried out suggest that microorganisms endowed with theirproperty of degradation of toxic pollutants are a boon to mankind. Although synthetic pyrethroids are biodegradable in nature, their residues are present in the environment.[6]Considering their toxicity effective microorganisms that can rapidly degrade the pesticideare selected and used.In the present study,an attempt has been made to select a bacterialstrain, which degrades deltamethrin, and also to test its efficiency of degradation.

Materials and Methods

Pesticide

The selected pesticide, deltamethrin, was bought froman agrochemical company near Nelpettai in Madurai.

Reference strain

Bacillus subtilis MTCC2423 was obtained from MTCC, IMTECH, Chandigarh.

Degradation efficiency

The bacterial strain was inoculated on minimal broth containing different concentrations ofdeltamethrin (50, 100, 150 and 200 pm). The Erlenmeyer flasks were then kept in the room temperature for a period of 4 days and the samples were then analysed for theparameters pH, carbon dioxide, esterase activity and biomass of degradation products.

pH

The pH of the sample was measured for the different test concentrations using pH meter everyday for 4 days.

Carbon dioxide

Free CO_2 was determined by titrating the samples using a strong alkali (NaOH) atpH 8. Sodium hydroxide was prepared in CO_2 free distilled water (boiled) from which50ml was diluted in 1000ml of CO_2 free distilled water and titrated against 10ml of thesample. Phenolphthalein was used as the indicator and the endpoint is the appearance ofpink color.[7] The free CO_2, was estimated by the following formula

$$\text{Free } CO_2 \text{ (mg/l)} = \frac{(\text{Vol x N}) \text{ of NaOH x 1000 x 44}}{\text{Volume of the sample}}$$

Esterase activity

0.1 ml of the sample was mixed with 1.5ml of naphthyl acetate, 0.5ml of Na_2HPO_4 buffer (0.2 mM, pH 7.0) and 0.4ml of distilled water. The mixture was incubated at39^0 C for 10 minutes. 50ml of 10% lauryl sulphate solution containg 2.5mg of fast garnet GBC was added to the mixture. The mixture was incubated for 15 minutes at room temperature. Absorbance wasmeasured at 560nm and compared with the absorbance of a l-napthol curve (linear from0-0.08 mM).[8]

Biomass

Biomass of the sample was analysed by turbidometric method. The absorbance ofthe test sample was measured using a colorimeter at 600 nm for 4 days at an interval of24 hours.

Statistical analysis

Two way analysis of variance (ANOVA) for the parameters pH, CO_2, esteraseactivity and biomass was done using MS Excel package. Variations were considered significant only when the F value wasgreater than the tabulated value at 5% level.

Findings

Results

Figure 1 shows the changes in the medium during the degradation of deltamethrin by *Bacillus subtilis* in which the pH decreased in the short term treatment.

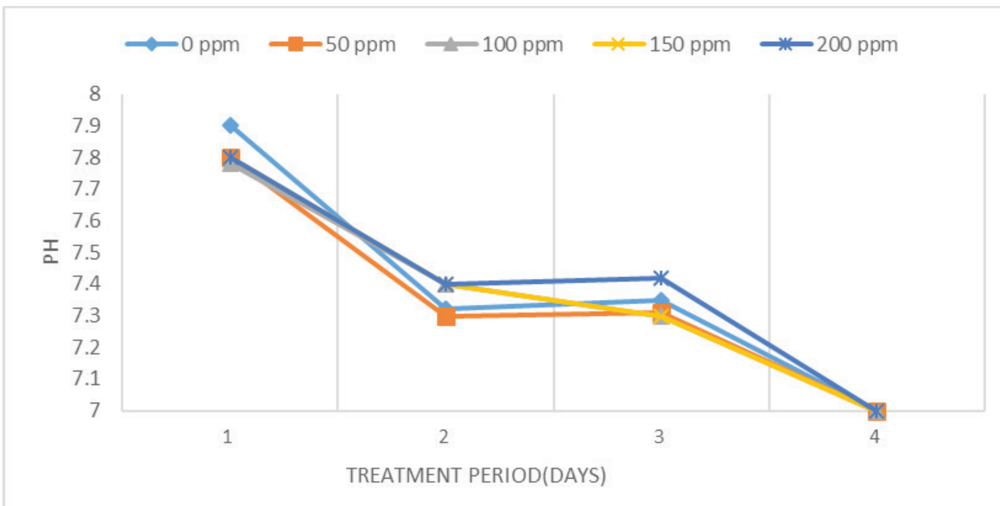


Fig 1.Changes in pH of the medium during the degradation of deltamethrin by *Bacillus subtilis* in the short term treatment

One of the end products of thedegradation of deltamethrin was CO_2 and it was analysed by titrimetry. The free CO_2 present in the medium increased during the 4 days.

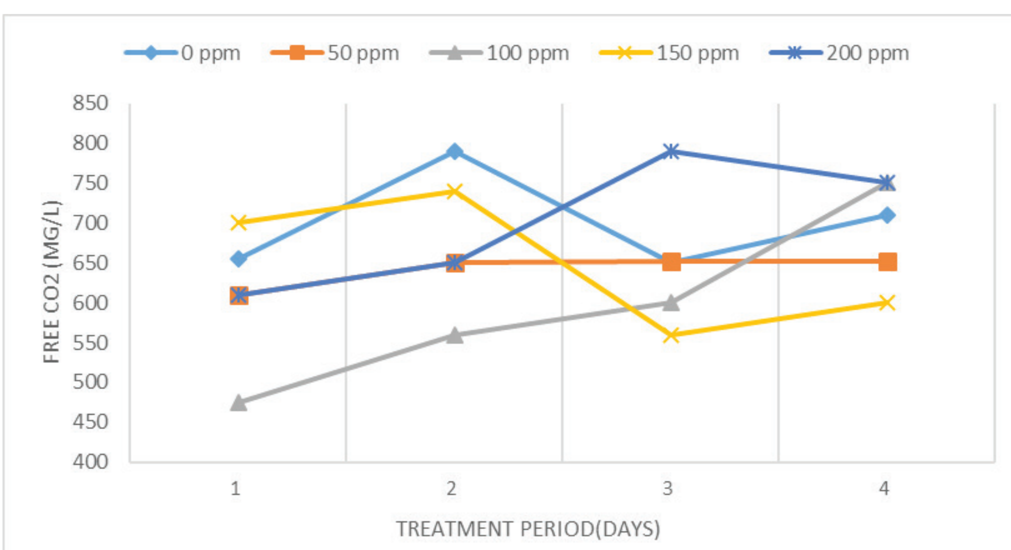
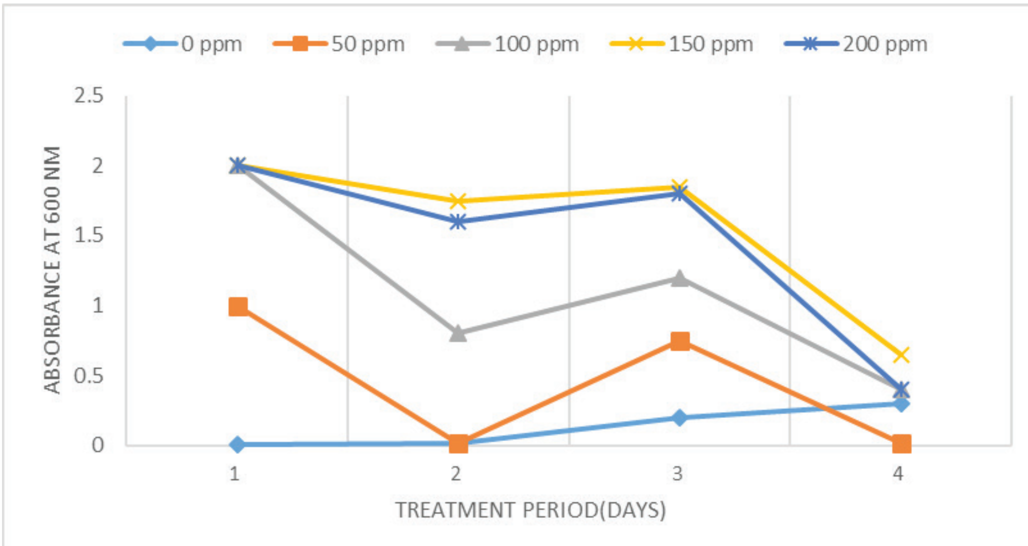


Fig 2.Carbondioxide released during the degradation of deltamethrin by *Bacillus subtilis* in the short term treatment

The maximum amount of carbon dioxide released was observed at 100 ppm concentration. Figure 2 shows the increase in free carbon dioxide in the medium in short term treatment

In the measurement of biomass,the bacterial strain showed increasing turbidity when measured by colorimeter. The increase in biomass results in increase in the turbidity of the medium. Figure 3 exhibitsincrease in the biomass up to three days of the short term treatment period.

Fig 3.Turbidity during the degradation of deltamethrin by *Bacillus subtilis* in the short term treatment



The enzyme esterase is responsible for the breakdown of deltamethrin to phenoxybenzoic acid and further to CO_2. Initially, the amount of napthyl acetate utilized by the enzyme increased upto 4 daysin short term treatment and Figure 4 shows the maximum enzyme activity for 100 ppm concentration.The degradation of deltamethrin by the strain is more efficient at 100 ppm and on increasing the concentration of the pesticide, there has been an increased lag phase in the growth of the organisms.

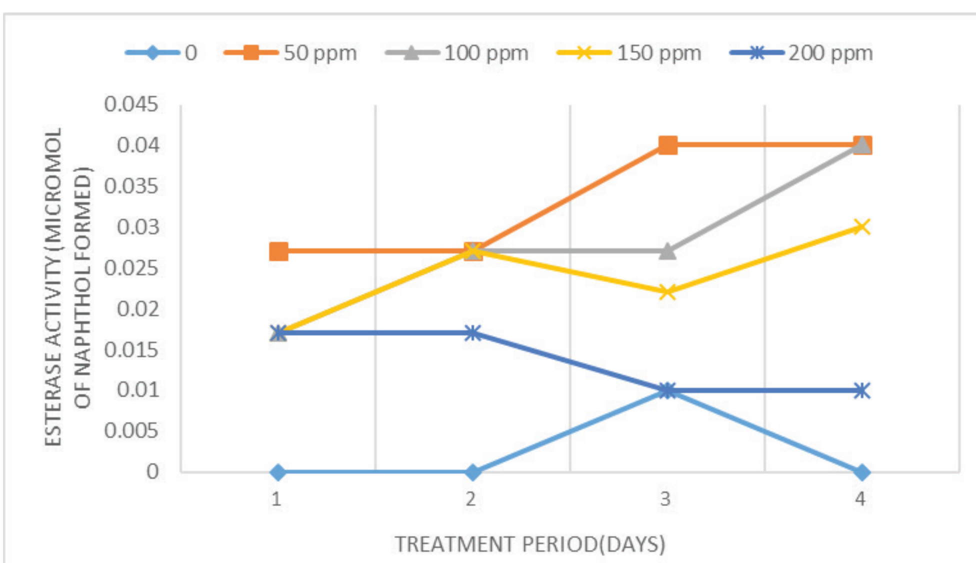


Fig 4.Esterase activity during the degradation of deltamethrin by *Bacillus subtilis* in the short term treatment

Table 1. Two way analysis of variance for the factors pH,CO_2, esterase and turbidity with the variables treatment period and Deltamethrin concentration for *Bacillus subtilis*(short term treatment)

Factor	Source of variation	SS	df	MS	F	Table value at 5% level	Level of significance
pH	Deltamethrin Concentration	0.00643	4	0.001607	1.288577	0.328429	Significant P<0.05
	Treatment period	1.518655	3	0.506218	405.7862	2.5	Significant P<0.05
CO_2	Deltamethrin Concentration	27684.8	4	6921.2	1.201681	0.359848	Significant P<0.05
	Treatment period	189772.8	3	6324.267	1.098039	0.387704	Significant P<0.05
Esterase	Deltamethrin Concentration	0.002505	4	0.000626	16.52507	8	Significant P<0.05
	Treatment period	0.000101	3	3.37E-05	0.890062	0.474143	Significant P<0.05
Turbidity	Deltamethrin Concentration	6.24985	4	1.562463	11.38634	0.000473	Significant P<0.05
	Treatment period	3.08228	3	1.027427	7.487305	0.004377	Significant P<0.05

Table 1 shows the two way analysis of variance for the factors pH, carbondioxide, esterase and turbidity with the variables, treatment period and deltamethrin concentration for *Bacillus subtilis* in the short term treatment. The variations due to treatment period and deltamethrin concentration were statistically significant (Table 1).

The *B.subtilis*MTCC 2423 was found to be efficient in degrading deltamethrin to a considerable extent.

Discussion

It has been suggested that bacteria with the ability to degrade specific compounds can be used for bioremediation of pesticide polluted sites.[9] Soil bacteria with the ability to degrade several pesticides have been isolated from soil showing enhanced biodegradation. Microorganisms need a possible acclimation time to induce the necessary degradative enzymes.[3]

From the changes in the pH, where there is a shift to acidity during the process of degradation, an acid was formed as a product or a transient material, and thus the decrease in the pH was noticed in the medium. The deltamethrin during the degradation initially gets converted into 3-phenoxybenzoic acid and this may be the cause for the decrease in the pH of the medium. During the degradation process of deltamethrin, after formation of phenoxybenzoic acid, in a particular amount, it will be converted into carbon dioxide.[10]Generally, chemical pathways result in only partial deactivation of pesticides, whereassoil microorganisms can completely break down many pesticides to CO_2 and water.[4]The turbidity of the medium indicates the growth of the organism in the medium.The increase in the turbidity of the medium indicates that the organism is growing in the medium having deltamethrin, and is also using it as a source of energy. Hence it is capable ofdegrading deltamethrin.

Bacillussubtilis is able to degrade five photosensitive pesticides including synthetic pyrethroid pesticides.[9]Bacteria of several different genera have been shown to degrade xenobiotics. Most of these xenobiotic degrading microorganisms harbour plasmids which code for thecatabolic genes. By understanding the biochemistry and genetics of plasmid that isresponsible for degradation, it is possible to characterize the appropriate genes andtransfer them to construct improved strains with enhanced ability for degradation ofseveral toxic compounds.[11]

Deltamethrin has a strong tendency to adsorb to soil sediments and degradesmore rapidly on sandy clay and sandy loam soil. It is partially soluble in water and isdegraded by sunlight, chemicals and microbes.During the process of degradation, the bacterium undergoes adaptation. In thepresence of high concentration of pesticide, the bacterium was greatly stressed and itsprocess of degradation and growth were slowed down. *Bacillus subtilis* was found to be effective to considerable extent and can be used as a bioremediation measure.

Conclusion

Biodegradation of Deltamethrin using *Bacillus subtilis* was effective upto 100 ppm concentration. In the presence of high concentration of pesticide, the bacterium was greatly stressed and its process of degradation and growth were slowed down.

Conflicts of Interest: Nil

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Ethical Clearance: No ethical issues involved

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