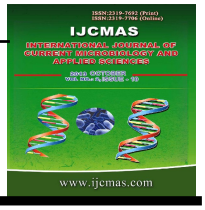
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 10 (2013) pp. 106-111**
http://www.ijcmas.com



Original Research Article

Degradation of delta methrin by organisms isolated from Koovam river water

S.Devisri and Priya R Iyer*

PG and Research Department of Biotechnology, Women`s Christian College, Chennai-600006.
****Corresponding author***

K e y w o r d s

Deltamethrin, *Bacillus* spp., *Klebsiella* spp., *Pseudomonas* spp. *Staphylococcus* spp.

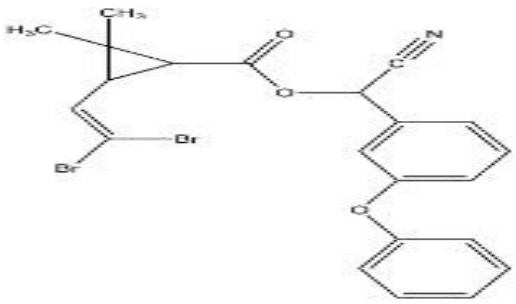
A B S T R A C T

Water samples collected from Koovam river water, Chetpet was used for isolation of organism degrading deltamethrin by inoculating the water samples in Berg's mineral salt medium. Four organisms were found to degrade deltamethrin - *Bacillus* spp., *Klebsiella* spp., *Pseudomonas* spp. *and Staphylococcus* spp.The growth of the isolated organisms were standardized by maintain them at various conditions like pH, temperature, concentration of deltamethrin, dextrose, yeast extract and potassium dihydrogen phosphate. The degradation was maximum at higher concentration of deltamthrin, pH 7-8 and temperature at 37ºC.The plasmid from all the four organisms were isolated and transformed into *E.coli DH5α* cells which indicate that the genes for degradation is present in the plasmid.

Introduction

Deltamethrin products are among some of the most popular and widely used insecticides in the world. This material is a member of one of the safest classes of pesticides: synthetic pyrethroids. While mammalian exposure to deltamethrin is classified as safe, this pesticide is highly toxic to aquatic life, particularly fish, and therefore must be used with extreme caution around water. [cyano-(3-phenoxyphenyl)-methyl] 3-(2,2-dibromoethenyl)- 2,2-dimethyl-cyclopropane-1-carboxylate. The available data indicates that the pesticide residues remain in surface soil, leading to toxicity in the soil water environment. The recent advances in bioremediation technology using microbial consortium has been found effective for treatment of pesticides in soil. A Surface Soil Treatment Unit has been designed wherein bioremediation of commonly used pesticides namely chlorpyrifos, cypermethrin, fenvalerate, and trichlopyr butoxyethyl ester at varying concentration viz. 25, 50 and 100 mg/kg have been carried out using cow-dung microbial consortia under simulated environmental conditions (Geetha *et al.*, 2008).

Deltamethrin



Materials and Methods

Isolation and identification of microorganisms degrading deltamethrin from Koovam river: Micobes were isolated from the river water in Berg`s mineral salt medium with various concentration of deltamethrin. The isolated organisms were identified using colony characteristics and biochemical tests.

Estimation of degradation of deltamethrin by colorimetric method using Brady`s reagent: Deltamethrin on degradation forms decamethrin which on hydrolysis with the methanolic potassium hydroxide forms phenoxybenzaldehyde. This aromatic aldehyde was used for the estimation of deltamethrin.

Standardization of Deltamethrin degradation: The degradation of deltamethrin was standardized by growing the organisms under various pH, temperature, carbon concentration, nitrogen concentration, phosphate concentration and deltamethrin concentration. The degradation of deltamethrin was analysed by estimating the concentration of decamethrin colorimetrically.

Isolation of the plasmid DNA from the isolated microorganisms: Bacterial cells were lysed using sodium hydroxide and sodium dodecyl sulphate which also denatures the protein. Sodium hydroxide denatures the chromosomal DNA and is separated out using centrifugation process. The plasmid is obtained in supernatant and is concentrated using ethanol.

Transformation: The three steps of bacterial transformation used were preparation of competent cells, introduction of foreign DNA in these cells and selection of transformants. The transformed cells were plated on LB agar plate containing deltamthrin.

Application of the isolated organism for bioremediation: The experiment was conducted to check the efficiency of the bioremediation by the isolated organisms in natural environment *viz*., soil.

Results and Discussion

Isolation and identification of microorganisms degrading deltamethrin from Koovam river

Four different colonies were observed on spread plating the Koovam water, the purpose of spread plating is to isolate individual bacterial cells that can degrade deltamethrin on a nutrient medium (Nwabueze, 2011). The isolated colonies were then sub cultured in nutrient agar and slants in order to obtain pure culture of all the 4 coloured colonies. The culture were subjected morphological identification and biochemical tests (Table 1).

From the above conducted tests the organisms isolated were found to be1. Green Colonies: *Pseudomonas* spp. 2.Yellow Colonies: *Staphylococcus* spp. 3.White Flat Colonies: *Bacillus* spp. 4.Mucoidal Colonies: *Klebsiella* spp.The identification and characterization of the isolates was performed using morphological, cultural and biochemical tests up to the stage of genus (Colins and Lyne 1985)

Estimation of degradation of deltamethrin by colorimetric method using Brady`s reagent:

After the addition of Brady's reagent, O.D. at 410 nm was taken. This was performed

Table.1 Identification of the isolated microorganisms

Biochemical tests	Green colonies	Yellow colonies	White flat colonies	Mucoidal colonies
Gram staining	Gram negative rods	Gram positive cocci	Gram positive rods	Gram negative rods
Indole	-	-	-	-
Methyl red	-	+	-	-
Voges-praeuker	-	+	+	+
Citrate	+	+	-	+
Mannitol Motility media	+	nonmotile ferments mannitol	+	+
Urease	-	+	-	+
TSI	Alk. /Alk.	acid/acid	alk/alk	acid/acid with gas
Catalase	+	+	+	+
Oxidase	+	-	-	-

Table.2 Degradation of deltamethrein by isolated organisms

Concentration of deltamethrin	***Bacillus* spp**	***Klebsiella* spp**	***Psuedomonas* spp**	**Staphylococcus spp**	**Mixed culture**
0.01%	0.08	0.06	0.08	0.04	0.11
0.1%	0.09	0.06	0.07	0.06	0.13
1%	0.10	0.09	0.11	0.08	0.14
5%	0.12	0.08	0.10	0.07	0.14

Table.3 Effect of pH on deltamethrin degradation

pH of the medium	*Bacillus* spp	*Klebsiella* spp	*Psuedomonas* spp	*Staphylococcus* spp
4	0.05	0.02	0.02	0.02
5	0.05	0.05	0.04	0.03
6	0.21	0.11	0.13	0.06
7	0.65	0.45	0.32	0.79
8	0.43	0.57	0.21	0.88

for each organism and also for mixture of all the four organisms . The following values were obtained (Table 2). Colorimetric analysis of phenoxybenzaldehyde using Brady's reagent was done to find out the concentration of deltamethrin at which maximum degradation can be observed. It was seen that the degradation increases with increase in concentration of deltamethrin. Thus it indicates that the esterase activity increases with increase in substrate concentration (Gonçalves *et al.*, 2010).

Table.4 Effect of Temperature on deltamethrin degradation

Temperature	*Bacillus* spp	*Klebsiella* spp	*Psuedomonas* spp	*Staphylococcus* spp
27ºC	0.45	0.40	0.11	0.12
37ºC	0.67	0.69	0.19	0.17
47ºC	0.12	0.10	0.02	0.04

Table.5 Effect of dextrose concentration on deltamethrin degradation

Conc. Of dextrose (gm/l)	*Bacillus* spp	*Klebsiella* spp	*Psuedomonas* spp	*Staphylococcus* spp
0.20	0.84	0.55	0.83	0.96
0.30	0.75	0.69	0.92	0.99
0.40	0.89	0.88	0.85	1.04
0.60	0.92	1.22	0.93	0.65
0.80	0.98	0.95	1.10	0.98

Table.6 Effect of yeast extract concentration deltamethrin degradation

Conc. Of yeast extract (gm/l)	*Bacillus* spp	*Klebsiella* spp	*Psuedomonas* spp	*Staphylococcus* spp
0.20	0.61	0.62	0.66	0.51
0.50	0.69	0.60	0.49	0.44
0.80	0.98	0.92	0.77	0.85
1.0	1.40	1.20	0.92	0.73

Table.7 Effect of Potassium dihydrogen phosphate concentration on deltamethrin degradation

Conc. Of KH_2PO_4 (gm/l)	*Bacillus* spp	*Klebsiella* spp	*Psuedomonas* spp	*Staphylococcus* spp
0.10	0.71	0.70	1.00	0.90
0.15	0.82	0.81	0.97	0.88
0.20	0.87	0.84	0.93	0.83
0.30	0.78	0.73	0.90	0.85

Table.8 The optimum conditions found for degradation

Characterization	*Bacillus* spp	*Klebsiella* spp	*Psuedomonas* spp	*Staphylococcus* spp
Optimum pH	7	8	7	8
Optimum temperature	37ºC	37ºC	37ºC	37ºC
Optimum dextrose concentration	0.80 gm/l	0.60 gm/l	0.80 gm/l	0.40 gm/l
Optimum yeast extract concentration	1.00 gm/l	1.00 gm/l	1.00 gm/l	0.80 gm/l
Optimum potassium dihydrogen phosphate concentration	0.20 gm/l	0.15 gm/l	0.10 gm/l	0.15 gm/l

Standardization of optimum growth conditions for degradation:

The optimum conditions for the degradation of deltamethrin using *Bacillus* spp, *Klebsiella* spp, *Psuedomonas* spp *and Staphylococcus* spp were standardized at different pH, temperature, carbon, nitrogen and phosphate source (Tables 3-8). The

standardization of various parameters is done in order to find out the optimal conditions at which the degradation is maximum for each isolated organisms. Thus the enzyme esterase which is involved in the degradation shows highest activity at temperature 37ºC and pH 7 to 8. Moreover from the result, it is evident that the *Klebsiella* spp. and *Pseudomonas* spp. share similar growth profile for the degradation of deltamethrin (Murugesan 2010).

Isolation of plasmid

The plasmids were isolated for all the four organisms and were run on 1% agarose gel. The plasmids were seen as sharp discrete bands under U.V. transilluminator. Plasmid is an extrachromosomal material which encodes for some properties like resistance against antibiotics, fertility, degradation of xenobiotics etc. Since all the four organism show plasmid, genes responsible for the degradation of deltamethrin may be present in the plasmid. (Kadam 2005).

Transformation

The isolated plasmids were transformed into *E.coli DH5 α* cells.

The transformed cells and competent cells were plated on LB agar plate. Growth was obtained on plate without deltamethrin where only competent cells were inoculated No growth was obtained on plate with deltamethrin where only competent cells were inoculated.Growth was obtained in the plate where transformed cells were inoculated (Rauf, 2001). Transformation was conducted to ensure that the degradation of deltamethrin is plasmid – mediated. Growth of transformed cells on plate with deltamethrin indicates that plasmid is successfully transformed. In order to check for the sensitivity of the competent cells, they were inoculated on plate with delatamethrin. Similarly, to check whether the cells are viable during transformation, competent cells were inoculated in the plate without deltamethrin (Kadam, 2005**).** Application of the organism for bioremediation: The soil sample were analysed for the presence of decamethrin colorimetrically which indicates degradation. The values obtained are as follows (Table 9). The microbial degradation was found to be highly efficient in *in vitro* conditions where the organisms are provided with appropriate nutrients for its growth. But in the natural environment viz., soil, degradation was found to be a very slow process. Thus *Bacillus* spp., *Klebsiella* spp., *Pseudomonas* spp. and *Staphylococcus* spp. can be used for the degradation of deltamethrin which is toxic to many animals and humans that poses serious threat to the environment.

Table.9 Degradation of deltamethrin in soil

S. No.	Organism	O. D. AT 410 nm
1.	*Bacillus* spp.	0.04
2.	*Klebsiella* spp.	0.02
3.	*Pseudomonas* spp.	0.04
4.	*Staphylococcus* spp.	0.01

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