Advanced Journal of Microbiology Research Vol. 2015
Available online at http://internationalscholarsjournals.org/journal/ajmr

Full Length Research Paper

Isolation and characterization of cypermethrin utilizing bacteria from Brinjal cultivated soil

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Accepted 5 November, 2014

The ability of five bacterial isolates (*Pseudomonas aeruginosa*, *Klebsiella* Sp., *Escherichia coli*, *Bacillus* Sp. and *Corynebacterium*) isolated from Brinjal cultivated field to degrade cypermethrin was studied. It was confirmed that these isolated organisms were able to utilize and degrade cypermethrin. In this five different bacterial colonies, *P. aeruginosa*, *Klebsiella* Sp., *E. coli* were found active in utilizing cypermethrin (1%) where as *Bacillus* Sp. and *Corynebacterium* were moderately active in utilizing cypermethrin (0.1%). The growth curve experiment was performed at 0.1 and 1% dose of cypermethrin to analyze the viable count of *P. aeruginosa*.

Key words: Cypermethrin, bacteria, utilization, growth kinetics, degradation, *Pseudomonas aeruginosa*.

INTRODUCTION

Pesticides have made a great impact on human health, production and preservation of foods, fibre and other cash crops by controlling disease vectors and by keeping in check many species of unwanted insects and plants. More than 55% of the land used for agricultural production in developing countries uses about 26% of the total pesticides produced in the world (Dollacker, 1991). However the rate of increase in the use of pesticides in developing countries is considerably higher than that of the developed countries. Pesticides are necessary to protect crops and losses that may amount to about 45% of total food production world wide (Tomlin, 1997). Even though several kinds of pesticides are present, pyrethroid is the most important pesticide because even at very low concentration it is more effective. They are very effective against flies, mosquitoes, stored grain insects, aphides. Pyrethroids have four major generations among this cypermethrin belong to the fourth generation (Casida, 1980). Cypermethrin is more effective against pests including moth pests of cotton, fruits and vegetable crops. Extensive and improper use of chemicals leads to greater health risk to plants, animals and human population which had been reviewed time to time by several workers. One of the major problems asides from toxicity and carcinogenicity of pesticides is their long persistence in nature that amplifies the toxicity and health risk problems in the area of contamination.

A variety of physical and chemical methods are available to treat the soils contaminated with hazardous materials but many of these physico chemical treatments do not actually destroy the hazardous compounds but are bound in a modified matrix or transferred from one phase to another, hence biological treatment is essential. The biological treatment of chemically contaminated soil involves the transformation of complex or simple chemical compounds into non-hazardous forms (Saraswat and Gaur, 1995). For biodegradation, ideally the target pesticide will be able to serve as the sole carbon source and energy for microorganisms, including the synthesis of appropriate enzymes if need able. The specificity of enzymes active against xenobiotic compounds differs from one microorganism to another and this non-specific metabolism provides as important mechanism xenobiotic degradation in the environment (Knackmoss, 1981; Shelton et al., 1996).

Cypermethrin has moderate persistence in soils. Under laboratory conditions, cypermethrin degrades more rapidly in soils (Saraswat and Gaur, 1995) and in aerobic conditions the half life of cypermethrin is 4 days to 8 weeks (Wauchope et al., 1992). Cypermethrin is co-

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Table 1. Incidence of total heterotrophic bacterial population and cypermethrin resistance at (0.01 and 0.1%) concentrations in Brinjal (*S. melangena*) cultivated field.

S/No	Dilutions	Total viable counts (CFU/g)	Cypermethrin resisting bacterial counts (CFU/g)	
			Cypermethrin concentration	
			0.01%	0.1%
1	10^{3}	5.80 ± 0.30 x 10^{5}	3.53 ± 0.40 x 10^{5}	1.10 ± 0.20x 10^{5}
2	10^{4}	4.36 ± 0.50 x10^{6}	2.53 ± 0.40 x10^{6}	0.80 ± 0.10x 10^{6}
3	10^{5}	3.96 ± 0.45 x 10^{7}	1.40 ± 0.30 x10^{7}	0.66 ± 0.57x10^{7}
4	10^{6}	3.43 ± 0.45x 10^{8}	1.30 ± 0.20 x 10^{8}	0.56 ± 0.57 x10^{8}
5	10^{7}	3.20 ± 0.40 x 10^{9}	0.63 ± 0.15 x10^{9}	0.46 ± 0.15x10^{9}

metabolized by bacteria in soil. *In vitro* studies have shown two soil-bacteria that are able to degrade this insecticide; they are members of the genera *Pseudomonas* and *Serratia* (Grant et al., 2002). A synthetic pyrethroid is often a mixture of different isomers. Bacteria preferentially degrade some enantiomers over others (Liu et al., 2005). The research aim was to identify the potential microbial strain able to utilize Cypermethrin from the soil. In this study, the pesticide degrading potential of a bacterial culture is examined with the hope of isolation and characterization of cypermethrin degrading potentials in the Brinjal cultivated soil. In addition, the optimum dose and the suitable conditions for Cypermethrin degradation using laboratory scale were also evaluated. The results of the present study suggest that the use of potential microorganisms in the treatment system can successfully overcome many of the disadvantages associated with the conventional method used for the degradation of inhibitory compound.

MATERIALS AND METHODS

Collection of soil samples

The soil samples were collected from the Brinjal (*Solanum melangena*) cultivated field. These fields had been already sprayed with cypermethrin for past few years. Soil samples were collected at different sites of the field by using sterile scalpel and these soil samples were transferred to sterile polythene bag and used for analysis.

Isolation and maintenance of bacterial colonies

The bacterial culture capable of degrading cypermethrin was isolated from agricultural soil using enrichment technique, with varying concentrations of cypermethrin in the medium. The soil sample (2 - 5 g) from an agricultural site was inoculated into 250 ml of nutrient medium in 500 ml Erlenmeyer flasks. The flasks were incubated in a shaking water bath operating at 240 cycles per minute for five days at room temperature (ranged from 20 - 28°C). At daily intervals one loop full of enrichment culture from the flasks was streaked on nutrient agar plates supplemented with cypermethrin (0.1 - 1%) and incubated at 35°C for 24 - 48 h. Individual colonies were sub cultured into nutrient agar plates containing same concentration of cypermethrin until pure culture was isolated. The isolated strain was maintained at 4°C and sub cultured after every three months.

Identification and characterization of resistant bacterial genera

The Cypermethrin impregnated plates showed morphologically dissimilar colonies and the purity of the colonies was isolated in a nutrient agar plates. Then the pure bacterial isolates were used for identification. The identification and characterization of the isolates was performed using morphological, cultural and biochemical tests as described by Colins and Lyne (1985) up to the stage of genus.

Enumeration of cypermethrin utilizing bacteria

In the enumeration of cypermethrin utilizing bacteria mineral salt medium is used in which cypermethrin as a carbon source. The microbial strains of cypermethrin resistant bacteria were streaked in triplicates on the mineral salt media containing cypermethrin at different concentration (0.01% to1%). After incubation the cypermethrin utilizing colonies were isolated.

Growth kinetic studies of cypermethrin resistant organisms

Growth of the isolates was determined by viable cell enumeration immediately after inoculation and at 2, 4, 6 and 24 h later. Sample of bacterial culture (1 ml) was drawn at regular intervals and serial dilutions (10^{-5} - 10^{-8}) of bacterial culture with and without addition of pesticide (control) was performed using 9 ml sterile saline blank (0.85% NaCl; pH = 7). Appropriate dilutions of bacterial samples were plated in triplicate on nutrient agar medium. After incubation the total viable colonies were counted using the method described by Collins and Lynes (1985).

RESULTS

This study was initiated to determine the total heterotrophic and insecticide resistant bacterial population. In the present investigation five different colonies were observed on nutrient agar medium enriched with Cypermethrin. One of the largest, most rapidly growing colonies of bacterial isolate was selected for growth kinetic study. The ability of isolated organism to utilize and degrade Cypermethrin was evaluated in this study. The total heterotrophic bacterial populations isolated from the soil contaminated with cypermethrin was represented in Table 1. Nearly 48 bacterial colonies were isolated and

Table 2. Cypermethrin resistance pattern of native bacterial isolates.

Bacterial Isolates	Cypermethrin Concentration										
	0.01%	0.1%	0.2%	0.3%	0.4%	0.5%	0.6%	0.7%	0.8%	0.9%	1.0%
P. aeruginosa	+	+	+	+	+	+	+	+	+	+	+
Klebsiella Sp	+	+	+	+	+	+	+	+	+	+	+
E. coli	+	+	+	+	+	+	+	+	+	+	+
Bacillus Sp.,	+	+	+	+	-	-	-	-	-	-	-
Corynebacterium Sp.,	+	+	+	+	-	-	-	-	-	-	-

+ Presence of growth.

- Absence of growth.

were isolated and identified. Among them 12.5% are gram positive rods and the rest 87.5% are gram negative rods. On the basis of morphological, cultural and biochemical characteristics, the bacterial isolates were identified as a member of the genus *Pseudomonas aeruginosa*, *Klebsiella* Sp, *Escherichia coli*, *Bacillus* Sp, and *Corynebacterium* Sp according to, Bergey's Manual of Determinative Bacteriology (1994). Among these *P. aeruginosa* was predominant (60.4%) followed by *E. coli* (18.8%) and *Klebsiella* Sp, (8.3%). The isolated native bacterial colonies exhibited remarkable resistance to the cypermethrin (Table 2). The generic composition of total viable bacterial strains at different concentrations of cypermethrin has been shown in Figure 1. This study revealed that except *Corynebacterium* Sp. and *Bacillus* Sp all other species like *P. aeruginosa*, *E. coli*, *Klebsiella* Sp., utilized cypermethrin as a carbon source at 1% concentrations. In the present investigation the growth curve experiment was performed with 0.1 and1% dose of cypermethrin to determine the viable count of *P. aeruginosa*. Results of the analysis are shown in Figure 2. On comparing the growth of *P. aeruginosa* in presence of Cypermethrin with that of control, it becomes clear that the bacteria grows faster and a higher number of cells was observed in 0.1 and 1% concentration of Cypermethrin. At 24 h the total viable count at control was 299 ± 10.53 x 10^5 CFU/g. The total viable count was 151.33 ± 5.03 x 10^5 CFU/g and 118.66 ± 6.5 x 10^5 CFU/g on using 0.1 and 1% concentration of cypermethrin at 24th h.

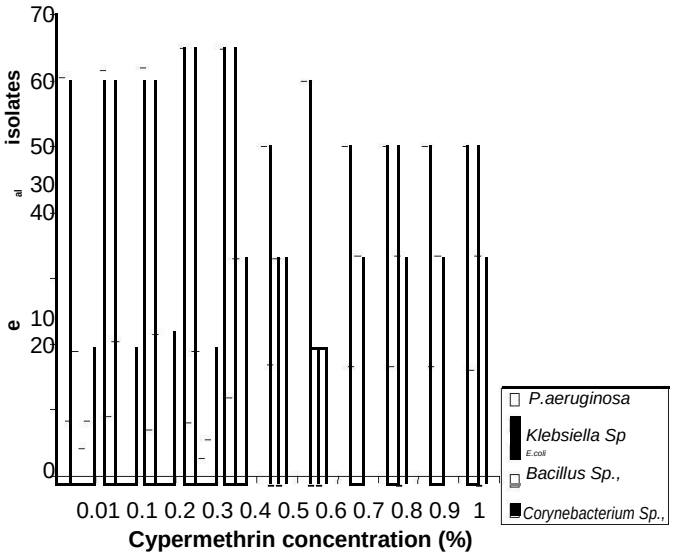

Figure 1. Generic composition of total viable bacterial strains isolated from Brinjal (*S. melangena*) cultivated field at 0.01 to 1.0% concentration of cypermethrin.

DISCUSSION

The potentials of microbial flora isolated from Brinjal (*S. melangena*) cultivated field to utilize cypermethrin was evaluated. Results of the present investigation revealed that five different bacterial genera showed different resistant capacities to various doses of the commercial insecticide, cypermethrin. The growth kinetics for the bacteria is high in cypermethrin concentration. Table 1 showed the maximum viable count 3.53 ± 0.40 x 10^5 CFU/g and 1.10 ± 0.20 x 10^5 CFU/g at 0.01 and 0.1% concentration of cypermethrin. Cypermethrin showed a higher number of counts at low concentration whereas at high concentration the number of organisms decreased or very slightly increased but no inhibition in the growth was observed when compared with the control tests (Jilani and Altaf Khan, 2006). Maria Kopytko et al. (2002) find out the growth kinetics for Gramoxone, it is clear that the bacteria grow faster and to a higher number of cells, when the herbicide concentration is the highest.

In the present study *Corynebacterium* and *Bacillus Sp*. were found to grow till 0.3% concentration of cypermethrin. When the concentration of cypermethrin was increased, the growth of *Corynebacterium Sp*. and *Bacillus Sp.*, was inhibited. But the growth of *P. aeruginosa*, *Klebsiella Sp*. and *E. coli* were seen up to 1% concentration of cypermethrin (Figure 1). Perclich and Lockwood (1978) observed that incidence of pesticide utilizing bacterial genera such as *Bacillus*, M*icrococcus*, *Pseudomonas* and *Vibrio* in the water and sediment samples of irrigational channel. Walker et al. (1993) investigated that, Pesticide is mainly degraded by *Pseudomonas* and *Bacillus* and this versatility might be

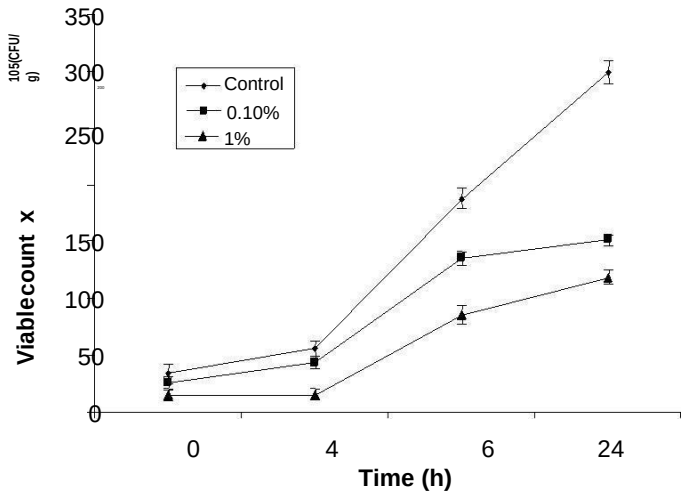

Figure 2. Growth of *P. aeruginosa* in the presence of 0.1 and 1% cypermethrin error bar represents ± S.D from the average of triplicates.

due to the presence of wide range of enzymes. Lal et al. (1995), Straube (1991), Safe (1984) have also isolated some naturally occurring soil bacteria capable of using certain organophosphate pesticides. Several researchers also reported similar results of lower degradation at high concentration of hazardous organic compounds (Smith and Adkins, 1995; Lee et al., 1998; Goudar and Strevett, 2000). Therefore the present study, reports that the bacteria isolated from soil confirm the degradation in natural habit.

The growth kinetics provides an evidence of mineralization potential of organism therefore such studies were carried out by several researchers (Karpouzas and Walker, 2000; Lee et al., 1998; Smith and Adkins, 1995; Haugland et al., 1990). In the present study, growth experiments were conducted by *P. aeruginosa* showed that it is also able to grow in the presence of Cypermethrin at 0.1 and 1%. It was noted that after incubation at 35ºC, plating on nutrient agar medium from the solution of nutrient broth inoculated with *P. aeruginosa* and Cypermethrin showed a higher number of viable count at low concentration whereas at high concentration the number of organisms decreased when compared with the control (Figure 2). Grant et al., (2002), reported that technical grade cypermethrin can be reduced from 60 to 6 mg/L by *Pseudomonas* sp. in 20 days. However, at increased concentration of cypermethrin, from 40 to 125 mg/L, a marked negative effect on the rate of degradation was observed (Jilani and Altaf Khan, 2006) . This may be due to mineral nutrients which are required for the growth of *Pseudomonas* and biodegradation of cypermethrin may become rate limiting in the wastewater sample after 48 h. (Lewis et al., 1986). This finding suggest that the utilization of cypermethrin by *P. aerugi*nosa may be feasible and this treatment option for the removal of pesticide from the soil and degradation observed only in the presence of microorganisms

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