

Original Research Article

Pyrethroid-Cypermethrin Degradation using Microorganisms Isolated from Rhizospheric Soil

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ABSTRACT

Keywords

Cypermethrin,
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Cypermethrin is a fourth generation pyrethroid insecticide. It is a Class – II pyrethroid which has a fast acting neurotoxic effect on insects. It is widely used in many household ant and cockroach killers due to this property. However, the wide use of cypermethrin insecticide has created certain problems such as accumulation in soils due to which it ultimately enters water bodies and has a severe detrimental effect on the aquatic ecosystems. The purpose of the present study was to isolate cypermethrin degrading micro-organisms from soil and to check their potential for the same.

Introduction

India is an agro based country. Large population is dependent on agriculture. But the various types of pests on the crops decrease the crop yield. So there is a need of pesticides.

These pesticides have great impact on production, human health and food preservation by controlling different pests and unwanted insects. However use of pesticides in developing countries is more than developed countries. Pesticides are necessary to protect crops and losses that may account for about 45% of total food production worldwide (Tomlin, 1997). Pyrethroids are effective even at very

low concentrations, so this group is important.

The synthetic pyrethroid insecticides are analogs of naturally occurring pyrethrins. Cypermethrin belong to fourth generation of pyrethroid group (Casida, 1980). Cypermethrin is more effective against pests of cotton, fruits and vegetable crops. It is unlikely to contaminate the ground water as it is tightly bound with the soil particles. The huge amount of pesticide application causes undesired side effects on population and activity of beneficial microorganisms in soil (Pandey and Singh, 2004). Therefore, soil

fertility is decreased and due to which crop production is also affected. In vitro studies have shown that many microorganisms have capacity of pesticide degradation mainly *Pseudomonas aeruginosa* is more effective than other organisms (Jilani and Khan, 2006). These microorganisms were isolated from soil having history of pesticide spread in the soil.

Cypermethrin has moderate persistence in soils. Under laboratory conditions, cypermethrin degrades rapidly in soil (Sarswat and Gaur, 1995) and in aerobic conditions the half life of cypermethrin is 4 days to 8 weeks (Wauchope *et al.*, 1992). Cypermethrin can be co metabolised by soil organisms.

Materials and Methods

Chemicals

Cypermethrin-25EC

Cypermethrin-25EC was purchased from local market, Agriculture shop, Hadapsar, Pune.

Composition

Cypermethrin-25EC technical: (70%Basis) W/W surfactants Ae1, Ae2, Ae3, (Calcium salt of Alkyl benzene sulphonate, Alkyl phenol Ethoxylate):10.00%w/w solvent-c-IX:54.00W/W, Total 100.00w/w.

Collection of soil sample

The soil samples were collected from the different sites of cotton, Brinjal and wheat cultivated field from Urulikanchan and Baramati, Dist. Pune, India. These fields were already spread with Cypermethrin-25EC, for past few years. The soil samples were collected in sterile polythene bags for further study.

Isolation of pesticide degrading microorganisms

The enrichment method was used to isolate pesticide degrading organisms. Enrichment of pesticide degraders was carried by using 150ml minimal mineral salt medium. Medium was sterilised by autoclaving at 121⁰c for 15 minutes. Cypermethrin was added after autoclaving as a sole carbon source.

The soil sample (2-5g) from an agriculture site was inoculated in above medium. Then second enrichment was carried out by transferring 1ml from first enriched flask culture into freshly prepared minimal medium. Incubation was carried out on shaker incubator at 240 cycles per minute for five days at room temperature.

Isolation was carried on minimal salt medium with 1ppm pesticide. After 48hrs incubation period, different types of colonies were observed. These colonies were picked up, checked for purity and maintained on slants for further study.

Secondary screening of the isolates and Maintenance of cultures

The isolated organisms were further screened for their growth in presence of different cypermethrin concentrations ranging from 0.25ppm to 3ppm. Isolates showing growth up to 3ppm pesticide concentration were used further.

All isolates were maintained as glycerol stocks at -20⁰c and on nutrient agar slants with 1ppm cypermethrin.

Morphological and biochemical characterization of isolated organisms

The organisms showing growth in presence of 3ppm cypermethrin on minimal medium

were morphologically characterized by Gram staining and motility. Identification of one isolate showing maximum growth was done by using API system of classification.

Medium optimization

Inoculum preparation

For inoculum preparation *Pseudomonas aeruginosa* culture was inoculated in minimal medium with 1ppm cypermethrin. Incubation was carried on rotary shaker at 240 rpm for 3-4 days. After incubation, cell pellet was obtained by centrifugation at 10000rpm for 10 minutes. Pellet was washed with sterile saline 3-4 times. Pellet was then resuspended in sterile saline and absorbance was taken at 600nm. Absorbance was set at 0.9 by taking saline as blank.

Optimization of Carbon source

Two carbon sources dextrose and maltose were used in different concentrations such as 0.1g%, 0.25g%, 0.5g%, 0.75g% and 1g%. These sugar concentrations were added in minimal medium broth with 3ppm cypermethrin. 5ml standardised culture suspension was inoculated in to the minimal medium broth. Incubation was carried on rotary shaker at 240 rpm for 24 hrs. Total viable count was determined on Davis Minimal agar medium plates supplemented with 3ppm cypermethrin.

Optimization of nitrogen source

Two nitrogen sources yeast extract and beef extract were used in different concentrations as 0.1g%, 0.25g%, 0.5g%, 0.75g% and 1g%. These concentrations were added in minimal medium broth with 3ppm cypermethrin.

5ml standardised culture suspension was inoculated in to the minimal medium broth. Incubation was carried on rotary shaker at

240 rpm for 24 hrs. Total viable count was determined on Davis Minimal agar medium plates supplemented with 3ppm cypermethrin.

Results and Discussion

Isolation of pesticide degrading microorganisms

Cypermethrin degrading isolates were isolated from the soil by enrichment technique. 100 isolates were isolated showing growth on the minimal agar plates plus cypermethrin.

Secondary screening of isolates

All the 100 isolates were screened for the growth on minimal medium with different cypermethrin concentrations.

9 Isolates were obtained showing growth on minimal medium supplemented with high cypermethrin as sole carbon source. Culture numbers FCM9, FCM44, FCM46, FCM65, FCM67, FCM93, showed growth in presence of 3ppm cypermethrin concentration. These cultures were used for further study.

Morphological and biochemical characterization of isolated organisms

The isolated organism was Gram negative motile rod. On the basis of morphological and biochemical characteristics BY API system of classification, the bacterial isolate was found to be *Pseudomonas aeruginosa* with 99% probability.

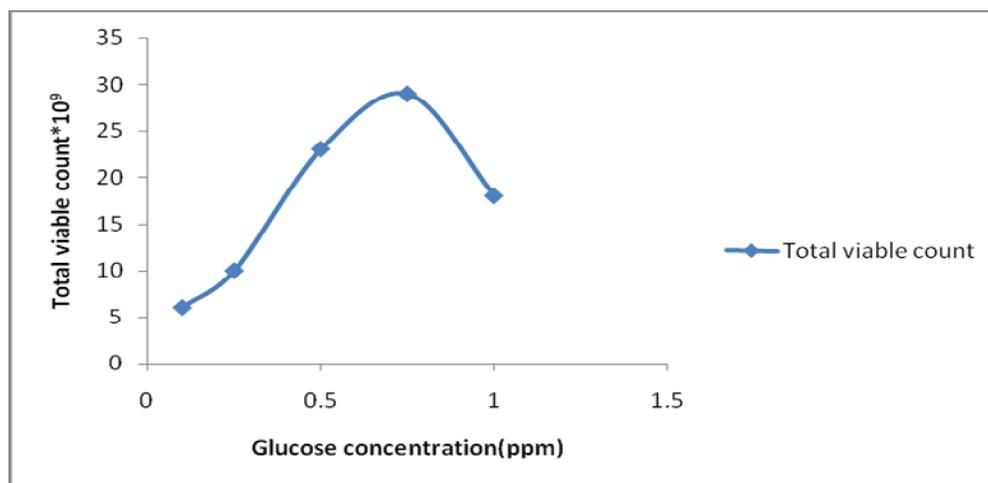
Maintenance of cultures

All isolates were maintained as glycerol stocks at -20⁰c and on nutrient agar slants with 1ppm cypermethrin.

Culture number	Cypermethrin concentration(PPM)								
	0.25	0.50	0.75	0.9	1.00	1.25	1.5	2.00	3.00
FCM44	+	+	+	+	+	+	-	-	+
FCM46	+	+	+	+	+	+	+	-	+
FCM65	+	+	+	+	+	+	+	-	-
FCM67	+	+	+	+	+	+	+	+	
FCM9	+	+	+	+	+	+		+	+
FCM93	+	+	+	+	+	+	+	+	+

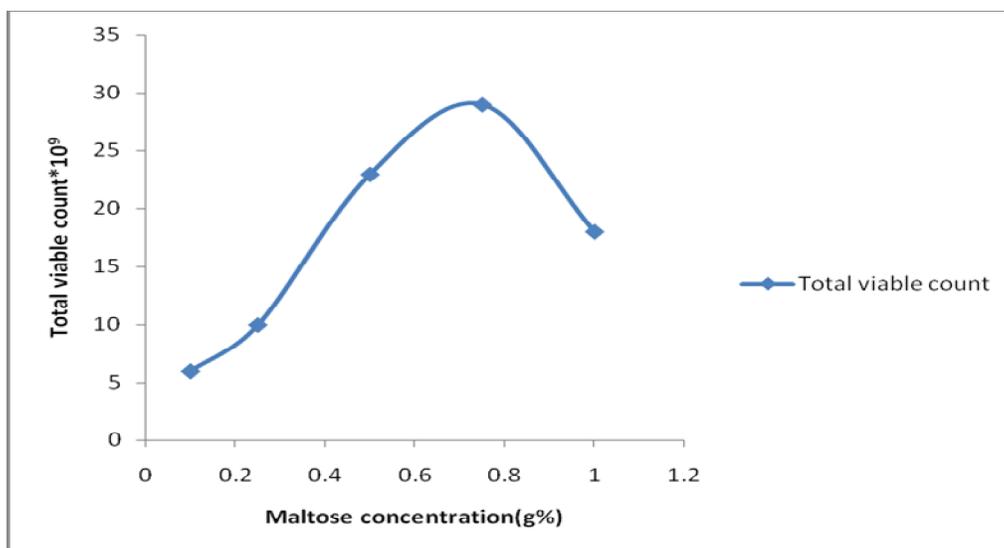
+ Presence of growth; - No growth

Graph.1 Effect of glucose concentration

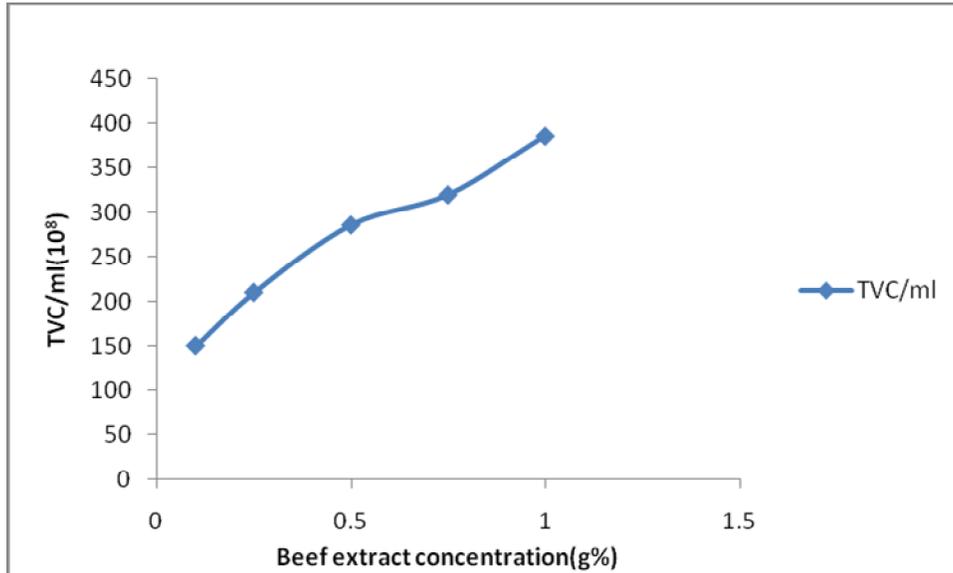


Graph1 shows maximum total viable count at 0.75g% glucose concentration.

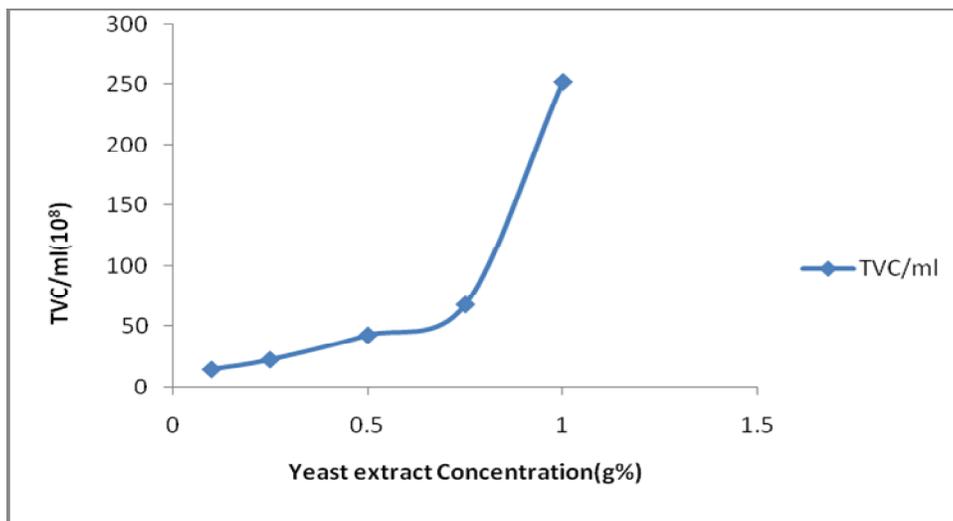
Graph.2 Effect of maltose concentration



Graph.3 Effect of beef extract concentration



Graph.4 Effect of yeast extract concentration



Inoculum preparation

Inoculum was prepared by using isolated culture and absorbance was set at 0.6-0.7 as per Mac Ferland standard.

Carbon source optimization

The selected isolate *Pseudomonas aeruginosa* was grown in presence of different concentrations of glucose and

maltose in minimal medium with 1ppm cypermethrin. The graph 1 clearly indicates that maximum total viable count was obtained at 1g% sugar concentration in presence of 1ppm cypermethrin concentration.

The selected isolate *Pseudomonas aeruginosa* was grown in presence of different concentrations of beef extract and yeast extract in minimal medium with 1ppm

cypermethrin. The graph clearly indicates that maximum total viable count was obtained at 1g% yeast extract and beef extract concentration in presence of 1ppm cypermethrin concentration.

The capacity of microbial flora capable of cypermethrin degradation was evaluated. Results show that two different isolates shows different resistant capacity to different doses of cypermethrin. In the present study, *Pseudomonas aeruginosa* showed maximum growth at 3ppm cypermethrin concentration. Several researchers have shown degradation of low levels of cypermethrin (Goudar and Strevett, 2000). This isolate showed growth on minimal medium supplemented with cypermethrin indicating degradation capacity.

The growth kinetics of *Pseudomonas aeruginosa* was checked in presence of different cypermethrin concentrations. It was noted that at concentration up to 3ppm, total viable count was increased but above this concentration, the number of organisms was decreased when compared with control. *Pseudomonas aeruginosa* showed maximum growth in presence of 0.75g% glucose and maltose. But glucose is found to be the carbon source utilized by organism more efficiently indicated by increased total viable count. *Pseudomonas aeruginosa* uses beef extract and yeast extract as complex nitrogen source and shows increased total viable count. This result indicates that *Pseudomonas aeruginosa* has the capacity of cypermethrin degradation and its use as source of carbon.

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