



Degradation of Cypermethrin (Pesticide) by Bacteria from Farmland Soil.

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ABSTRACT:

Cypermethrin is a pyrethroid pesticide, is use since 1980s in agricultural fields to check pest infestation in different crops for enhanced food production. Excessive use of cypermethrin results in soil contamination, affecting soil micro flora. Thus removal of cypermethrin contamination is important. The available method (physical & chemical) are time consuming and costly. Currently, bioremediation is one of the most ecofriendly, economical and efficient method for detoxification of the pesticide. A Cypermethrin-degrading E.coli, P.aeruginosa and B. subtilis are isolated from pesticide contaminated soil of a cauliflower field of district Samastipur, Bihar, India.

Keyword: Pesticide, E.coli, P.aeruginosa, B.subtilis, Biodegradation.

Introduction:

Chemical used to kill pests are called pesticides. Pesticide refers to wide range of compounds like insecticides, herbicides, fungicides etc. Cypermethrin [(+/-)- α -cyano-3-phenoxybenzyl (+/-)-cis, trans-3(2, 2dichlorovinyl)-2, 2-dimethylcyclopropane carboxylate] is a pyrethroid insecticide is use for insect control. Pyrethroid have four major generations, cypermethrin belongs to fourth generation of pyrethroids (Casida J.E.1980). No doubt cypermethrin enhanced yield in our agricultural product and also protect crop from the insects. It has been reported that extensive use of cypermethrin leads to undesired side effects on population and activity of useful soil micro-flora (Gurjar M.M &Hamde V.S., 2020). Cypermethrin persistence in environment varies from 14.6 to 76.2 days (half-life) depending on physicochemical properties of soil (Akbar S. et al., 2014).Cypermethrin affects the voltage-dependent sodium channel and ATPase system in neuronal membranes.it binds to nuclear DNA and leads to destabilization and unwinding of DNA(Patel et al.2006).

On the basis of the facts of toxicity and persistency of this pesticide, it is required to develop some methods to eliminate cypermethrin from the soil. Biodegradation process is one of the most eco-friendly, economical and cost effective method for detoxify or degrade the pesticide to less toxic forms. Till date ,several bacterial strain such as Pseudomonas aeruginosa(Zhang et al.2011), Streptomyces sp.(Lin et al.2011), Stenotrophomonas sp.(Chen et al.2011a)and Serratia marcescens(Cycon et al.2014a,b) have been reported to degrade pyrethroid pesticide. Many enzymes are involved in biodegradation of cypermethrin. In the present study, a coccus species able to degrade cypermethrin was recovered from the pesticide-contaminated soil of a cauliflower field of Samastipur district of Bihar (India).

Material and methods:

Soil

The Soil is used in this experiment was collected from cauliflower field of Samastipur district, Bihar with history of Cypermethrin and other pyrethroid application for more than 10 years. Sample was collected from 10-15 cm depth, transferred to sterile plastic bags, and stored at 4°C. Before use, the soil was dried.

Chemicals and media

Pesticide (cypermethrin) was obtained from local shop of samastipur district, Bihar and dissolved in acetonitrile to make a stock solution was filter sterilized and kept in refrigerator for use.

Bushnell haas agar medium (P^H 7.0) was used for the isolation and cultivation of cypermethrin degrading bacterial strains.

Composition of Bushnell haas agar medium-

MgSO₄- 0.2g/l, CaCl₂- 0.02g/l, KH₂PO₄- 1g/l, K₂HPO₄- 1g/l, (NH₄)₂SO₄- 1g/l and FeCl₃- 0.05g/l

Analysis of physico-chemical parameter

The physico-chemical properties of the soil samples namely p^H, temperature, and moisture content, Phosphate, magnesium and chloride were analyzed by standard methods. (M.Radhika &M. Kannahi)

Isolation of Cypermethrin degrading Bacteria

Isolation of bacteria from pesticide contaminated soil of Samastipur district, Bihar (India), by serial dilution method. Bacterial colonies identified by Gram's staining and biochemical tests.

Results and Discussion:

In the present study, bioremediation of cypermethrin in contaminated soil by the microbes E.coli, P. aeruginosa and B.subtilis.

Physico-chemical characteristics of soil sample analysis

The physico-chemical characteristics of soil sample the p^H (7.6), Temperature (28°C), moisture content (87%), Phosphate (30.6±0.0mg), Magnesium (13.0±0.6mg) and Chloride (10.5±0.5mg) (Table1).

Isolation of cypermethrin degrading bacteria

The bacterial species were isolated from pesticide contaminated soil of samastipur district (Bihar), by serial dilution method. Different bacterial colonies were observed in Bushnell Haas agar medium. This colonies were identified by Gram's staining and biochemical tests.

Identification and growth conditions of isolates

The identification characteristics of these isolated organisms growth were observed by measuring absorbance at 600nm in E.coli (0.55), P.aeruginosa (0.61) and B. subtilis (1.25) respectively (Table 2).

Cypermethrin utilizing bacteria

In this result of cypermethrin degradation of E.coli, P.aeruginosa and B.subtilis.

In E.coli, maximum zone of accumulate in 0.1%, 0.5%, 1.0% at 2.18±0.40, 1.94±0.3 and 0.77±0.20 respectively, followed by P.aeruginosa maximum zone of accumulate in 0.1%, 0.5% and 1.0% at 2.08±0.3, 2.13±0.8, 0.66±0.14 and B.subtilis maximum zone of accumulate in 0.1%, 0.5% and 1.0% at 2.27±0.50, 1.96±0.4, 0.88±0.10 (Table 3).

Table.1 soil analysis

S.No.	Soil analysis	Pesticide degrading soil
1	p ^H	7.6
2	Temperature °C	28°C
3	Moisture content	87%
4	Phosphate(mg)	30.6±0.0mg
5	Magnesium(mg)	13.0±0.6mg
6	Chloride(mg)	10.5±0.5mg

Values are Mean ±standard deviation

Table. 2 Analysis of cypermethrin degrading organisms

S.No.	Organisms	OD value at 600nm
1	E.coli	0.55±0.35
2	P.aeruginosa	0.61±0.30
3	B.subtilis	1.25± 0.5

Values are Mean±standard deviation

Table.3 Total bacterial population and degradation of Cypermethrin at concentrations of (0.01 and 0.1%) in contaminated soil.

S.No.	Organisms	Dilution	Total viable counts (CFU/g)	Cypermethrin resistance bacterial counts(CFU/g)		
				Cypermethrin concentration		
				0.1%	0.5%	1.0%

1	E.coli	10 ⁻⁴	5.60±0.30	3.55±0.40	2.54±0.3	1.00±0.20
		10 ⁻⁵	4.35±0.50	2.54±1.40	2.33±0.2	0.70±0.10
		10 ⁻⁶	3.86±0.45	1.41±0.30	1.34±0.6	0.65±0.55
		10 ⁻⁷	3.44±0.45	1.30±0.20	1.39±0.3	0.56±0.55
2	P.aeruginosa	10 ⁻⁴	5.50±0.40	3.32±0.30	3.52±0.30	1.07±0.10
		10 ⁻⁵	4.60±0.30	2.54±0.40	2.41±0.40	0.50±0.07
		10 ⁻⁶	3.95±0.50	1.30±0.30	1.20±0.20	0.50±0.05
		10 ⁻⁷	3.67±0.45	1.21±0.20	1.00±0.01	0.40±0.54
3	B.subtilis	10 ⁻⁴	5.60±0.50	3.65±0.50	2.62±0.4	1.07±0.10
		10 ⁻⁵	4.32±0.45	2.62±0.50	2.44±0.5	0.90±0.54
		10 ⁻⁶	3.94±0.55	1.50±0.40	1.40±0.4	0.80±0.47
		10 ⁻⁷	3.32±0.35	1.41±0.31	1.40±0.2	0.60±0.46

Values are Mean ±standard deviation

Table.4 Inhibiting the growth pattern of bacteria using cypermethrin.

Bacteria	Cypermethrin concentration		
	0.01%	0.5%	1%
E.coli	+	+	+
P.aeruginosa	+	-	-
B.subtilis	+	+	-

Conclusion:

E.coli, P.aeruginosa and B.subtilis were isolated from farmland soil sample collected from Samastipur district, Bihar (India).

It can utilize cypermethrin as sole source of carbon, nitrogen and energy. The optimal temperature and pH for biodegradation of cypermethrin by E.coli, P.aeruginosa and B.subtilis were 37°C and pH7.0, and the degradation rate reached 90.4% within one week under the optimal condition.

In conclusion, our results indicated that E.coli, P.aeruginosa and B.subtilis could be a good choice for the bioremediation of cypermethrin contaminated soil.

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