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Innovative exploration of crop beneficial bio-agents for reduction of insecticidal residues in vegetables grown soil of Surguja district of Chhattisgarh

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Abstract
An investigation was carried out to evaluate the crop beneficial bio-agents for reduction of insecticidal residues in vegetables grown soil of Surguja district of Chhattisgarh. One hundred and eighty three soil samples were collected from the fields of vegetable growing farmers of Surguja district where different insecticides were used over years for controlling of insects to reduce the losses due to their infestation. The soil samples were collected during *rabi* season 2017. From these soil samples two hundred and six crop beneficial insecticide tolerant microbial isolates were collected. These isolates were further characterized with respect to their cultural characteristics and screened for their potentiality of Chlorpyriphos+Cypermethrin degradation. Two hundred and six isolates of crop beneficial microorganism which belong to *Rhizobium,* PSB, *Azotobactor* and *Azospirillum* genus were taken in another study for their insecticide degradation potential under *in-vitro* conditions*.* All isolates had shown good growth in their respective culture media containing 3000 ppm Chlorpyriphos+Cypermethrin. With increasing concentration of Chlorpyriphos+Cypermethrin *Azospirillum* isolates did not show their survivility. However, five isolates of *Azotobacter,* 5 isolates of *Rhizobium* and 2 isolates of PSB shown their growth in presence of 15000, 16000 and 20000 ppm of tested insecticides, respectively.

Keywords: Vegetables, isolation, characterization, degradability

Introduction

Insecticides are widely used against a range of pests infesting agricultural crops. Modem agriculture largely relies on the extensive application of agrochemicals, which includes insecticides. Indiscriminate, long-term and over application of insecticides have severe effects on soil ecology that may lead to alterations in or the erosion of beneficial or plant probiotic soil microflora. Weathered soils lose their ability to sustain enhanced production of crops/grains on the same land. However, burgeoning concern about environmental pollution and the sustainable use of cropping land have emphasized inculcation of awareness and the wider application of tools, techniques and products that do not pollute the environment at all or have only meager ecological concerns (Kalia and Gosal, 2011) [4].

In Chhattisgarh, insecticides are tremendously used in different crop plants in general and in vegetable crops particularly. Districts like Surguja, Jashpur, Raigarh are the potential districts for vegetable production where the farming is leading by tribal community. With increment of agricultural productivity, the consumption of insecticides in these districts also increased manifolds. Microbial decomposition is one of the most important methods by which insecticides are decomposed in soil. Microorganisms consume the insecticide molecules and utilize them as a source of energy and nutrients for growth and reproduction. When a insecticide is applied to a soil, microorganisms may immediately attack it. The population of the particular microorganism that uses that insecticide for *a*n energy source will increase. After the insecticide is degraded, the microbial population may return to the original level, or it may stabilize at a level greater than before application. In agriculture many crop beneficial microorganisms are used as bio-fertilizer to increase the nutrients availability and enhance crop productivity. Many of them may degrade the insecticides at a faster rate. The biochemical basis of microbial degradation has received considerable attention. Several enzymes, which provide microorganisms with the ability to degrade organopesticides, have been identified and characterized. Thus, microorganisms provide a potential wealth in biodegradation.

Nitrogen-fixing micro-organisms proved to be a good bioagent for rapid degradation of pesticides (Gangawane and Francies, 1997) [2]. Kadam and Gangwane (2005) [3] isolated and tested *Azotobacter chroococcum* isolates having the highest degradation potential to phorate. So the present study aimed to identify such crop beneficial bio-agents which can conserve the environment from insecticidal pollution and make the vegetables insecticidal residue free by degrading them at a faster rate. The organisms simultaneously supplement nutrients to crop plants for their better productivity.

Materials and Methods

The experiment was carried out to evaluate the crop beneficial bio-agents for reduction of insecticidal residues in vegetables grown soil of Surguja district of Chhattisgarh. One hundred and eighty three soil samples were collected from the fields of vegetable growing farmers of Surguja district where different insecticides were used over years for controlling of insects to reduce the losses due to their infestation. The soil samples were collected during *rabi* season 2017. The isolation of insecticide tolerant crop beneficial microorganisms from the collected soil samples was done by dilution plate technique (Subba Rao, 1988) [6]. YEMA, Pikovskaya's media, Jensen's agar media, and Okon's agar media were used to isolate *Rhizobium*, phosphate solubilizing bacteria, *Azotobacter* and *Azospirillum* bacteria, respectively. From this isolation study 68 rhizobial, 39 phosphobacterial, 54 *Azotobactor* and 45 *Azospirillum* isolates were collected. The collected all isolates were tested for their insecticide tolerance behavior by growing them in their respective liquid medium supplemented with insecticide Chlorpyriphos + Cypermethrin at different concentrations *i.e* 1000 ppm, 200c ppm, 3000 ppm, 4000 ppm, 5000 ppm, 7000 ppm, 8000 ppm, 12000 ppm and 20000 ppm. After two weeks of incubation at 28±2 ^{0}C and at 150 rpm of shaking, each bacterial suspension (100 µl each) was then inoculated on plate containing desired agar medium. The plates were then incubated at 28±2 ^{0}C in inverted position. Counting of bacterial colonies was started after incubation of 24 hrs and the process of counting was continued up to 7 days of incubation. Plating of each sample was in duplicate and mean value was worked out for each sample. The population of bacteria was expressed per ml of broth.

Results & Discussion

In this experiment different crop beneficial microbial isolates were tested as individual to evaluate their degradation potential of Chlorpyriphos+Cypermethrin insecticide with respect to microbial properties of rhizosphere soil from the fields of vegetable growers. The results obtained from these studies are as follows:

1-Collection

One hundred and eighty three soil samples were collected from the fields of vegetable growing farmers of Surguja district where different insecticides were over years for controlling of insects to reduce the losses due to their infestation. The soil samples were collected during *rabi* season 2017. From these soil samples two hundred and six isolates of different crop beneficial microorganisms were collected which belong to *Rhizobium, Pseudomonas, Bacillus, Azotobactor* and *Azospirillum* genus (Table-1).

Table 1: Details of microbial isolates collected from fields received high dose of insecticides during last few years.

Soil sample no.	Village name	Block	District	Isolate No.			
				Rhizobium	PSB	*Azotobactor*	*Azospirillum*
1.	Mahavir-pur	Ambikapur	Surguja	Rh-In-1, 2, 3, 6, 8, 9, 10, 12, 13, 14, 15, 16, 17, 20, 21, 22, 24	PSB-In-1, 8, 9, 10, 13, 21, 24	Azo-In-1, 3, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 20, 21, 22, 24	Azos-In-1, 3, 6, 8, 9, 10, 12, 1, 14, 15, 17, 20, 21, 22, 24
2.	Sanjay Nagar	Ambikapur	Surguja	Rh-In-26, 27, 29, 31, 33	PSB-In-26, 27	Azo-In-26, 27, 29, 33	Azos-In-26, 27, 29, 33
3.	Bakalo	Ambikapur	Surguja	Rh-In-37, 40, 43	-	Azo-In-37, 43	Azos-In-37, 43
4.	Khala	Ambikapur	Surguja	Rh-In-47	PSB-In-47	Azo-In-47	-
5.	Amadarha	Ambikapur	Surguja	Rh-In-51, 54, 59, 62, 65, 69	PSB-In-51, 54, 59, 69	Azo-In-54, 59, 65, 69	Azos-In-65
6.	Kuberpur	Ambikapur	Surguja	Rh-In-73, 82, 86, 92, 96	PSB-In-73, 82, 86	Azo-In-73, 78, 82, 86, 92, 96	Azos-In-82, 86, 92, 96
7.	Udari	Lundra	Surguja	Rh-In-99, 103, 107, 111, 115, 118, 122, 126, 130, 132, 135, 140	PSB-In-99, 103, 107, 111, 118, 122, 126, 137, 140	Azo-In-103, 107, 111, 118, 122, 132, 140	Azos-In-99, 115, 118, 122, 132, 140
8.	Bulanga	Lundra	Surguja	Rh-In-142, 145, 148, 152, 154, 157, 162, 165, 171, 175, 178, 183	PSB-In-142, 145, 148, 152, 154, 157, 162, 165, 171, 175, 178, 183	Azo-In-142, 145, 148, 152, 162, 165, 175, 178, 183	Azos-In-142, 145, 148, 152, 154, 175, 183

2-Characterization

These isolates were further characterized with respect to their cultural characteristics and gram reaction. Confirmation of *Rhizobium,* PSB, *Azosprillum* and *Azotobactar* isolates was done by plant infection and phosphate solubilizing capacity, respectively. Other crop beneficial microorganisms were isolated by culture them on their respective media (Table-1).

Table 2: Characterization of top performing insecticide tolerance microbial isolates.

S. N.	Insecticide used	Isolated microorganisms	Color	Solubilization Zone diameter (mm)	Gram Reaction	Plant Infection	Characterization		
							Forms	Margins	Elevation
1.	Profenofos(40%)+Cypermethrin 4% EC	Rh-In- 41	White	-	-	+	Circular	Entire	Convex
2.	Profenofos(40%)+Cypermethrin 4% EC	Rh-In-25	White	-	-	+	Circular	Entire	Convex
3.	Chlorpyrifos +Cypermethrin (5%)	Rh-In-107	White	-	-	+	Circular	Entire	Convex
4.	Profenofos(40%)+Cypermethrin 4% EC	Rh-In-20	White	-	-	+	Circular	Entire	Convex
5.	Chlorpyrifos +Cypermethrin (5%)	Rh-In-24	White	-	-	+	Circular	Entire	Convex
6.	Chlorpyrifos +Cypermethrin (5%)	PSB-In-54	White	15	+	-	Irregular	Undulated	Raised
7.	Emamectin +Benzoate 5% SG	PSB-In-183	White	13	+	-	Irregular	Undulated	Raised
8.	Triazophos Deltramethrin	PSB-In-102	White	12	+	-	Irregular	Undulated	Raised
9.	Profenofos(40%)+Cypermethrin 4% EC	PSB-In-103	White	13.2	+	-	Irregular	Undulated	Raised
10.	Thiamethoxam 25% WG	PSB-In-19	White	14.5	+	-	Irregular	Undulated	Raised
11.	Chlorantraniliprole 18.5% WG	Azo-In-35	White	-	-	-	Circular	Entire	Convex
12.	Lambdacyhalothrin	Azo-In-167	White	-	-	-	Circular	Entire	Convex
13.	Thiamethoxam 25% WG	Azo-In-19	White	-	-	-	Circular	Entire	Convex
14.	Profenofos(40%)+Cypermethrin 4% EC	Azo-In-20	White	-	-	-	Circular	Entire	Convex
15.	Chlorpyrifos +Cypermethrin (5%)	Azo-In-24	White	-	-	-	Circular	Entire	Convex
16.	Lambdacyhalothrin	Azos-In-78	Blue	-	-	-	Circular	Entire	Convex
17.	Chlorpyrifos +Cypermethrin (5%)	Azos-In-103	Blue	-	-	-	Circular	Entire	Convex
18.	Thiamethoxam 25% WG	Azos-In-19	Blue	-	-	-	Circular	Entire	Convex
19	Chlorpyrifos +Cypermethrin (5%)	Azos-In-24	Blue	-	-	-	Circular	Entire	Convex
20.	Emamectin Benzoate 5% SG	Azos-In-1	Blue	-	-	-	Circular	Entire	Convex

3-Degradation potential of Chlorpyriphos+Cypermethrin insecticide

In the above isolation study two hundred and six crop beneficial insecticide tolerant microbial isolates were obtained. All isolates of *Rhizobium*, PSB, *Azotobactor* and *Azospirillum* were further tested for their ability to grow in a medium containing different concentration of Chlorpyriphos+Cypermethrin insecticide which commonly used in tomato to reduce the losses due to insect infestation. The isolates were grown in their respective nutrient liquid culture medium containing Chlorpyriphos+Cypermethrin at different concentrations (1000, 2000, 3000, 4000, 5000, 7000, 8000, 12000 and 20000 ppm). These isolates were subjected to continuous incubation at 28±2 ºC with 150 rpm for 15 days. After incubation the survibility of different isolates was tested by growing them on their respective media. All isolates had shown good growth in their respective culture media containing 3000 ppm Chlorpyriphos+Cypermethrin. With increasing concentration of Chlorpyriphos+Cypermethrin, *Azospirillum* isolates did not show their survibility. However, five isolates of *Azotobacter*, 5 isolates of *Rhizobium* and 2 isolates of PSB shown their growth in presence of 15000, 16000 and 20000 ppm of tested insecticides, respectively. (Table-3 & 4). The results obtained in this study were in agreement with earlier reports that indicated the involvement of different species of Enterobacteriaecae in the degradation of organophosphorous insecticides like chlorpyrifos (Singh *et al*., 2004). Similarly Pseudomonas auroginosa was more potential to degrade the toxic compound like Cypermethrin in soil and also beneficial for soil remediation for high yield production of crops (Bhosle and Nasreen., 2013).

Table 3- Growth behavior of isolated microbes under recommended dose of applied

S. N.	Growth ability							
	Insecticide: Chlorpyriphos + Cypermethrin							
	Concentration: 1000 ppm							
	Rhizobium		PSB		*Azotobacto*r		*Azospirillum*	
	Isolate	Growth*	Isolate	Growth*	Isolate	Growth*	Isolate	Growth*
1	Rh-In-38	-	PSB-In-38	+	Azo-In-35	++++++	Azos-In-44	+
2	Rh-In-41	++++++	PSB-In-39	+++	Azo-In-38	-	Azos-In-30	++
3	Rh-In-30	-	Rh-In-45	+	Azo-In-30	+++++	Azos-In-25	++
4	Rh-In-25	++++++	Rh-In-46	+++	Azo-In-25	+++++	Azos-In-78	+ +++++
5	Rh-In-45	+++	PSB-In-78	+	Azo-In-45	+	Azos-In-93	+
6	Rh-In-46	+++++	PSB-In-74	++++++	Azo-In-46	+	Azos-In-147	++++
7	Rh-In-78	+++++	PSB-In-52	+	Azo-In-78	-	Azos-In-163	-
8	Rh-In-93	+++++	PSB-In-57	+	Azo-In-93	+	Azos-In-167	+++
9	Rh-In-52	+++++	PSB-In-178	-	Azo-In-52	-	Azos-In-103	+ ++++
10	Rh-In-57	+++++	PSB-In-145	+	Azo-In-57	++	Azos-In-107	++
11	Rh-In-147	+	PSB-In1-46	-	Azo-In-178	-	Azos-In-138	+
12	Rh-In-163	-	PSB-In-183	++++++	Azo-In-145	-	Azos-In-10	-
13	Rh-In-167	++	PSB-In-102	++++++	Azo-In-167	++++++	Azos-In-9	-
14	Rh-In-99	+++++	PSB-In-103	++++++	Azo-In-106	+	Azos-In-19	+ +++
15	Rh-In-107	++++++	PSB-In-107	+	Azo-In-107	-	Azos-In-20	-
16	Rh-In-138	+++++	PSB-In-106	+	Azo-In-138	+	Azos-In-24	+ +++
17	Rh-In-20	++++++	PSB-In-9	+++	Azo-In-19	++++++	Azos-In-17	+++
18	Rh-In-24	++++++	PSB-In-19	++++++	Azo-In-15	-	Azos-In-1	++++++
19	Rh-In-9	+++++	PSB-In-23	+++	Azo-In-20	++++++	Azos-In-57	-

20	Rh-In-16	+++++	PSB-In-24	+	Azo-In-24	++++++	Azos-In-52	+++

Insecticides

Growth indication No. of colonies/50uLbroth

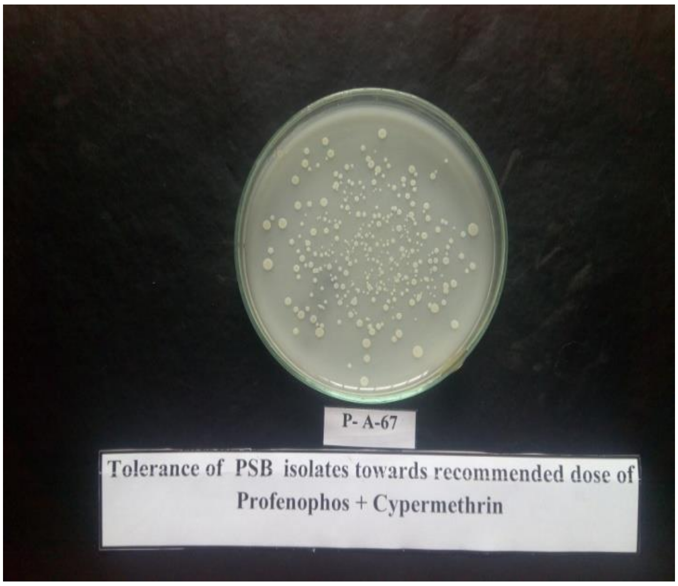
+ 1-100
++ 100-300
+++ 300-500
++++ 500-700
+++++ 700-1000
++++++ <1000

Table 4- Insecticide tolerance potential of top performing microbial isolates.

S. N.	Isolate	Growth ability							
		Insecticide:		Chlorpyriphos + Cypermethrin					
		2000 ppm	3000 ppm	4000 ppm	5000 ppm	7000 ppm	8000 ppm	12000 ppm	20000 ppm
1	Rh-In- 41	++++++	++++++	++++++	++++++	++++	+++	++	-
2	Rh-In--25	++++++	++++++	++++++	++++++	+++++	++++	+++	-
3	Rh-In-107	++++++	++++++	++++++	++++++	++++	+++	++	-
4	Rh-In-20	++++++	++++++	++++++	++++++	+++++	++++	+++	-
5	Rh-In-24	++++++	++++++	++++++	++++++	++++	+++	++	-
6	PSB-In-54	++++++	++++++	++++++	++++++	+++++	++++	+++	+
7	PSB-In-183	++++++	++++++	++++++	++++++	++++	+++	++	-
8	PSB-In-102	++++++	++++++	++++++	++++++	++++	+++	++	-
9	PSB-In-103	++++++	++++++	++++++	++++++	++++	+++	++	-
10	PSB-In-19	++++++	++++++	++++++	++++++	+++++	++++	+++	+
11	Azo-In-35	++++++	++++++	++++++	++++++	++++	+++	++	-
12	Azo-In-167	++++++	++++++	++++++	++++++	++++	+++	++	-
13	Azo-In-19	++++++	++++++	++++++	++++++	++++	+++	++	-
14	Azo-In-20	++++++	++++++	++++++	++++++	+++++	++++	+++	-
15	Azo-In-24	++++++	++++++	++++++	++++++	+++++	++++	+++	-
16	Azos-In-78	++	++	-	-	-	-	-	-
17	Azos-In-103	+++	++	-	-	-	-	-	-
18	Azos-In-19	+	+	-	-	-	-	-	-
19	Azos-In-24	+	+	-	-	-	-	-	-
20	Azos-In-1	+	+	-	-	-	-	-	-

***Growth indication No. of colonies/50uLbroth**

+ 1-100
++ 100-300
+++ 300-500
++++ 500-700
+++++ 700-1000
++++++ <1000



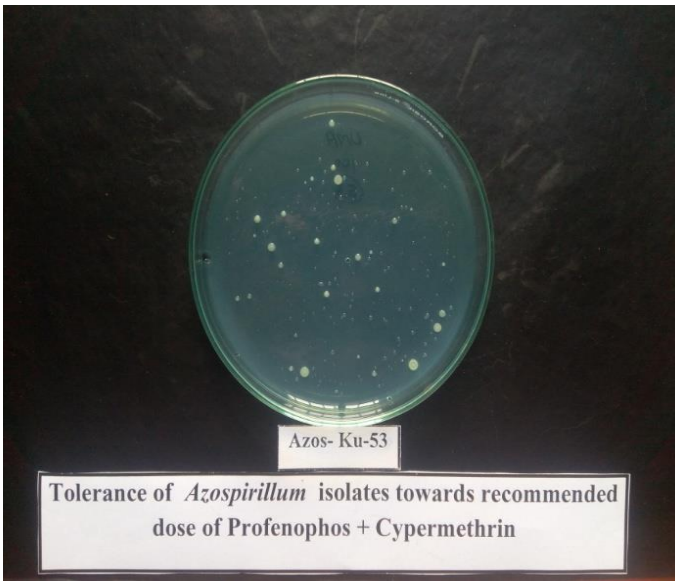


Fig 1: Insecticide tolerance micrroorganisms isolated from insecticides applied fields

Media Used: (i) For *Rhizobium*: YEMA broth
(ii) For PSB: Pikovskaya's broth
(iii) For *Azotobacter*: Jensen's broth
(iv) For *Azospirillum*: Okon's broth

Conclusion

One hundred and eighty three soil samples were collected from the fields of vegetable growing farmers of Surguja district where different insecticides were over years for controlling of insects to reduce the losses due to their infestation. From these soil samples two hundred and six crop beneficial insecticide tolerant microbial isolates were collected. These isolates were further characterized with respect to their cultural characteristics and behavior towards gram reaction. All isolates had shown good growth in their respective culture media containing 3000 ppm Chlorpyriphos+Cypermethrin. With increasing concentration of Chlorpyriphos+Cypermethrin, *Azospirillum* isolates did not show their survibility. However, five isolates of *Azotobacter*, 5 isolates of *Rhizobium* and 2 isolates of PSB shown their growth in presence of 15000, 16000 and 20000 ppm of tested insecticides, respectively.

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