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Identification of suitable crop beneficial bio-agents for rapid degradation of applied insecticide

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Abstract
The investigation was carried out to evaluate the identification of suitable crop beneficial bio-agents for rapid degradation of insecticide. Three soil samples were collected from the vegetable growing farms where different insecticides were over years for controlling of insects to reduce the losses due to insect infestation. From these soil samples 10 crop beneficial insecticide tolerant microbial isolates were collected. These isolates were further characterized with respect to their cultural characteristics and tested for their potentiality of Deltamethrin + Triazophos degradation. Three rhizobial two phosphobacterial, three *Azotobactor* and two *Azospirillum* isolates were collected. Isolates were taken in a study for their insecticide degradation potential under *in-vitro* conditions. All isolates had shown good growth in their respective culture media containing different concentration of Deltamethrin + Triazophos. Ten treatments were taken under this study. At 4000 ppm deltamethrine+trizophos concentration five isolates had shown poor growth while four did not show any growth. At 4000 and 5000 ppm concentrations all of the isolates had shown growth but at varying level. *Rhizobium*-1,2,3 *Azatobactar*-1,*2 Azospirillum*-1,2 PSB-1,*2* had shown highest population followed by PSB-*2 Rhizobium*-1,2, *Azatobactar*-1,*2 Azospirillum*-1 in 3000 ppm concentration and PSB-2 *Rhizobium*-2 *Azatobactar-2* in 4000 ppm concentration.

Keywords: Isolation, Characterization, Degradability

1. Introduction
Insecticides are tremendously used in different crop plants in general and in vegetable crops particularly. The increasing world population has therefore put a tremendous amount of pressure on the existing agricultural system so that food needs can be met from the same current resources like land, water etc. In the process of increasing crop production, herbicides, insecticides, fungicides, nematicides, fertilizers and soil amendments are now being used in higher quantities than in the past. Ideally, the applied pesticides should only be toxic to the target organisms, should be biodegradable and eco-friendly to some extent (Rosell *et al*., 2008) [6]. Unfortunately, this is rarely the case as most of the pesticides are non-specific and may kill the organisms that are harmless or useful to the ecosystem. In general, it has been estimated that only about 0.1% of the pesticides reach the target organisms and the remaining bulk contaminates the surrounding environment (Carriger *et al*., 2006) [3]. The repeated use of persistent and non-biodegradable pesticides has polluted various components of water, air and soil ecosystem. Pesticides have also entered into the food chain and have bioaccumulated in the higher tropic level. Several microorganisms have been isolated which are able to utilize pesticides as a source of energy. There are some examples of fungi including Trametes hirsutus, Phanerochaetechrysosporium, Phanerochaete sordia and Cyathus bulleri that are able to degrade lindane and other pesticides (Singh & Kuhad, 1999, 2000; Singh *et al*., 1999) [7]. However, most evidence suggests that soil bacteria are the principal components responsible for enhanced bio-degradation (Walker & Roberts, 1993). Several pure bacterial isolates with the ability to use specific pesticides as a sole source of carbon, nitrogen or phosphorus have been isolated (Singh *et al*., 1999, 2000) [7]. 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 an 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.

The increased population could cause more rapid insecticide degradation upon subsequent insecticide applications. Numerous microorganisms have the capacity to degrade the pesticides by the action of degradative genes in plasmids/transposons/chromosomes (Kumar *et al*, 1996) [4]. 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. Diverse symbiotic (*Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*) and non-symbiotic (*Pseudomonas*, *Bacillus*, *Klebsiella*, *Azotobacter*, *Azospirillum*, *Azomonas*), rhizo-bacteria are recognized and now being used worldwide as bioinoculants to promote plant growth and development under various stresses like heavy metals (Ma *et al*., 2011, and Wani and Khan 2010) [1], herbicides (Ahemad and Khan, 2011a and Ahemad and Khan, 2010) [1], insecticides (Ahemad and Khan, 2011b) [2], fungicides etc. So by exploiting them as microbial agents we can sustain the crop productivity and conserve environment simultaneously from the toxicity of insecticidal chemicals.

Methodology

The experiment was conducted at laboratory condition, Department of Agricultural Microbiology, IGKV, Raipur to evaluate the identification of suitable crop beneficial bio-agents for rapid degradation of applied insecticide. Three soil samples were collected from the vegetable growing farms where different insecticides were over years for controlling of insects to reduce the losses due to insect infestation. From these soil samples three rhizobial, two phosphobacterial, three *Azotobactor* and two *Azospirillum* isolates were collected. The experiment included 10 treatments *viz.*T1: *Rhizobium*-1, T2: *Rhizobium*-2, T3: *Rhizobium*-3, T4: PSB-1, T5: PSB-2, T6: *Azotobactor*-1, T7: *Azotobactor*-2, T8: *Azotobactor*-3, T9: *Azospirillum* -1, T10: *Azospirillum* -2. The history of application of insecticides in the above fields showed that in most of the fields the vegetable crops were sprayed with Deltamethrin + Triazophos, chlorantraniliprol and Profenofos + Cypermethrin insecticide on the field. The soil was Inceptisol (pH: 11, EC: 0.35 dSm^{-1}, available N: 232.47 kg ha^{-1}, available P: 7.86 kg ha^{-1}, and available K: 369.96 kg ha^{-1}, available Zn: 1.81 ppm, available Cu: 0.76 ppm, available Fe: 2.42 ppm, available Mn: 6.32 ppm).

Chemical composition of media-

1. **YEMA for *Rhizobium***

Mannital	10.0gm
K2HPO4	0.5gm
MgSO4. 7H2O	0.2gm
NaCl	0.1gm
Yeast extract	1.0gm
Agar	20.0gm
Distilled water	1000ml
Congo red soln (1%)	2.5ml
pH	7.0

2. **Pikovskaya's medium for PSB**

Mannital	10.0gm
K2HPO4	0.5gm
MgSO4. 7H2O	0.2gm
NaCl	0.1gm
Yeast extract	1.0gm
Agar	20.0gm
Distilled water	1000ml
Congo red soln (1%)	2.5ml
pH	7.0

3. Jensen's agar medium for *Azotobacter*

Sucrose	20.0gm
K_2HPO_4	1gm
$MgSO_4$	0.5gm
NaCl	0.5gm
$FeSo_4$	0.1gm
Na2MoO4	0.001gm
$CaCo_3$	2gm
Agar	15. 0gm
Distilled Water	1000ml
pH	7.0-7.2

4. Okon's medium for *Azospirillum*

Malic acid	5.0gm
KOH	4. 0gm
K_2HPO_4	0.5gm
$MgSO_4$	0.1gm
NaCl	0. 02gm
$FeSo_4$	0. 05gm
Na_2MoO_4	0.002gm
$CaCl_2$	0. 01gm
$MnSo_4$	0. 01gm
NH_4Cl	1.5gm
0.5%BTB	2.0gm
Agar	18. 0gm
Distilled Water	1000ml
pH	6.6-7. 0

Results & Discussion

In this experiment different microbial isolates were tested as individual to evaluate their degradation potential of Deltamethrin + Triazophos insecticide. The results obtained from these studies are as follows:

1. Collection

Three soil samples were collected from different plots of a long term insecticidal trail where different insecticides were over years for controlling of insects to reduce the losses due to insect infestation.

2. Isolation

From these soil samples ten isolates of different crop beneficial insecticide tolerant microorganisms were collected which belong to *Rhizobium*, *Pseudomonas*, *Bacillus*, *Azotobactor* and *Azospirillum* genus.

3. Characterization

These isolates were further characterized with respect to their cultural characteristics and gram reaction. Confirmation of *Rhizobium*, and PSB isolates was done by plant infection and phosphate solubilizing capacity, respectively. *Rhizobium* isolates were cultured in YEMA plates. The *Rhizobium* colonies were found white in colour, circular, entire and convex. Likewise *PSB* isolates were grown on pikovskaya's plates. The colonies of *PSB* were confirmed by the hollow zone formed around the colonies, light yellow in colour, circular, undulated and raised. The colonies of *Azotobacter* on Jensen's agar media was found small, white in colour and elevated. The margin of colonies was observed entire. The isolation of *Azospirillum* was done by using Okon's medium. Earlier the medium was green in colour but after growth of above bacterium the colour of the medium turned dark blue which was the indication of growth of above bacterium. The

colonies of *Azospirillum* were round in shape, elevated and their margin was found entire. Other crop beneficial microorganisms were isolated by culturing them on their respective media (Table-1).

Table 1: Characterization of isolated microorganisms collected from insecticide treated plots.

S.N.	Insecticide used	Isolated microorganisms		Characteristics				
			Colour	Forms	Gram reaction	Plant infection	Margins	Elevation
1.	Deltamethrin + Triazophos,	PSB-1*	White	Irregular	+	-	Undulate	Raised
		Rhizobium-1**	White	Circular	-	+	Entire	Convex
		Azotobactor-1***	White	Circular	-	-	Entire	Convex
		Azospirillum-1****	Dark blue	Circular	-	-	Entire	Convex
2.	Profenofos + Cypermethrin	*Rhizobium*-2**	White	Circular	-	+	Entire	Convex
		Azotobactor-2***	White	Circular	-	-	Entire	Convex
		Azospirillum-2****	Dark blue	Circular	-	-	Entire	Convex
3.	Chlorantraniliprole	*Rhizobium*-3**	White	Circular	-	+	Entire	Convex
		Azotobactor-3***	White	Circular	-	-	Entire	Convex
		PSB-3*	White	Irregular	+	-	Undulate	Raised

*Grown on Pikovskaya's medium *** Grown on Jensen's agar media **Grown on YEMA medium **** Grown on Okon's agar media

4. Degradation potential of Deltamethrin + Triazophos insecticide

Ten isolates which were collected from isolation study were further tested for their ability to grow in a medium containing different concentration of Deltamethrine + Trizophos insecticide which commonly used in brinjal to control insect. The isolates of *Rhizobium*, *PSB, Azosprillum* and *Azotobactar* were grown in bacterial nutrient liquid culture media containing Deltamethrine + Trizophos as a sole source of carbon and energy at varying concentrations viz. 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm. The isolates were subjected to continuous incubation at 28±20C with 150 rpm for 15 days after incubation period growth ability of the bacterial isolates were tested on their respective media. The growth data illustrated that after a period of 15 days of incubation all the isolates had shown no growth in 5000 ppm concentration. However, at 2000 ppm and 3000 ppm concentration all the isolates had shown good growth (Table 2). The results are in agreement with previous finding reported by Murugesan *et al*. (2010) who reported that different microbial isolated from Brinjal cultivated field produce enzyme have different degradation preferences.

Table 2: Growth ability of the bacterial isolates in liquid culture media containing Deltamethrin + Triazophos

S.N.	Isolates/ Treatment	Growth ability			
		Deltamethrin + Triazophos concentration			
		2000	3000	4000	5000
		ppm	Ppm	ppm	Ppm
1.	*Rhizobium*-1	++	+	-	-
2.	*Rhizobium*-2	+++	++	++	-
3.	*Rhizobium*-3	++	+	-	-
4.	PSB-1	++	-	-	-
5.	PSB-2	++	+	+	-
6.	*Azotobactar*-1	++	+	-	-
7.	*Azotobactar*-2	+++	++	+	-
8.	*Azotobactar*-3	+	-	-	-
9	*Azosprillum*-1	+	+	-	-
10	*Azosprillum*-2	+	-	-	-

+++High growth + poor growth
++ Medium growth - Nil

Conclusion

From these soil samples three rhizobial, two phosphobacterial, three *Azotobactor* and two *Azospirillum* isolates were collected. These isolates were further characterized with respect to their cultural characteristics and behavior towards gram reaction. The isolates of *Rhizobium*, PSB, *Azospirillum*, and *Azatobactar* were grown in their respective nutrient liquid culture medium containing deltamethrine+trizophos at different concentrations (2000, 3000, 4000 and 5000 ppm). These isolatets were subjected continuous incubation at 28±2o C with 150 rpm for 15 days. After incubation the survibility of different isolates was tested by growing them on their respective media. At 4000 ppm deltamethrine+trizophos concentration five isolates had shown poor growth while four did not show any growth. At 4000 and 5000 ppm concentrations all of the isolates had shown growth but at varying level. *Rhizobium*-1,2,3 *Azatobactar*-1,*2 Azospirillum*-1,2 PSB-1,*2* had shown highest population followed by PSB-*2 Rhizobium*-1,2, *Azatobactar*-1,*2 Azospirillum*-1 in 3000 ppm concentration and PSB-2 *Rhizobium*-2 *Azatobactar-2* in 4000 ppm concentration.

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