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Enhancement of Biodegradation of Chlorpyrifos and Carbaryl of Gill, Muscle, Liver Tissues of Cyprinus Carpio

Aurumugam Muniyasamy [a++*] and Victor Prabakaran [b#]

[a] Department of Biotechnology, Madurai Kamaraj University, Madurai, Tamil Nadu, India.
[b] Department of Zoology, Govt. Arts College, Melur, Madurai-625 106, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Pesticides are powerful agents that kill non- target animals and unwanted insects in a particular area. Chlorpyrifos bioaccumulate leading to increased contamination in soil and water. This study was focused with much attention to curb the menace of the pollution. Biodegradation is the process by which microorganism such as *Pseudomonas aeruginosa* advocate for biodegradation which is consider to be a clean-up technology. In our study 30% of degradation was recorded. The fish *Cyprinus carpio* was used as an indicator animal and its gill, muscle, liver tissue was analyzed for pesticide residue accumulation where in the biochemical changes such as protein were depleted due to pesticide toxicity. Subsequently an increase protein content was noted in the biodegradation

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[++] Research Scholar;
[#] Associate Professor;
**Corresponding author: Email: rrchola0@gmail.com;*

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experimental sets. Other pesticides carbaryl the active ingredient of sevin which cause drastic effect on non-target animals was degraded by the enzymatic machinery of the *Pseudomonas aeruginosa*.

Keywords: Bioaccumulation; biodegradation; Cyprinus carpio; aquaphyte; Ottelia.

1. INTRODUCTION

Chlorpyrifos, a synthetic pesticide is widely used for its low cost, effectiveness (Silva and Samayawardhena, 2002), [1,2,3,4]. Most of the insecticides used in crops are eventually expelled into nearby water bodies through canal, rains, and farm runoff. While pesticides serve a vital role in increasing land productivity and food quality for the world's rising population, particularly in developing nations, their presence in agricultural drainage poses a major threat to all aquatic ecosystem components [5], particularly fish [6]. These pollutants affect different parts of fish and food chain [7,8,9].

Carbaryl (n-naphthyl, 1-methyl carbamate), is a pesticide widely used because of its lower mammalian toxicity [10] especially in the aquatic environment [11,12,13]. Carbaryl is toxic to freshwater snails and other aquatic organisms [13,14].

In the present investigation, pesticides, of Chloropyrifos and carbaryl were commonly used by agriculture community. These pesticides indeed persistent that can be cleaved by the enzymatic pathway of microorganism. Pesticides once considered recalcitrant but now it can be cleaved into different metabolic and final end product, yielding carbon dioxide and water respectively. Similarly, carbaryl belonging to carbamate groups (commonly called sevin) which can be degraded by photo metabolism in spite carbaryl was chosen in our study in order to assess a comparative study between organophosphorus and carbamate group of pesticides. Bioaccumulation studies were carried out to assess the residues present in gills, muscle and liver tissue using in the indicator animal *Cyprinus carpio. In addition, biochemical evaluation of protein and exchange due to pesticides toxicity was also monitored.*

2. MATERIALS AND METHODS

2.1 Isolation and Characterization of Bacteria Collection of Soil

Soil samples collected from Madurai, Tamil Nadu. Soil samples were enriched with calculated amount of insecticide.

2.2 Isolation of Pesticide Degrading Microorganism

Enriched soil stirred well with sterile water and the supernatant was diluted by serial dilution method.

2.3 Preparation of Nutrient Agar Fortified with Pesticides

Nutrient blue agar medium mixed with 100 ppm, 300 ppm and 500 ppm of chlorpyrifos. The bacterial strain was identified by its uniform colony morphology on nutrient blue agar plates. Bacteria was mixed with nutrient broth. They were poured into the petri plates containing liquefied nutrient agar and agar 1.5 g in 100 ml of distilled water numbering 10-1, 10-2 and 10-3, respectively. The next day colonies on the Petri plates were seen. The number of colonies were counted by Quebec colony counter. Bacteria size was determined by cytometer.

2.4 Selection of Pesticides

The pesticides used in the study are

a) **Chlorpyrifos** (Dursban, Chemical name: 0,0-diethyl 0-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate)

b) **Carbaryl** (Sevin, Chemical name: 1. Naphthyl-N-Methyl, Carbamate)

2.5 Test Animal

The common carp *Cyprinus carpio* was used for the experiment, collected from Madurai District.

2.6 Preparation of Stock Solution

a) Chlorpyrifos

The stock solution was prepared by using technical grade Chlorpyrifos following way. Chlorpyrifos (0.1 g) was mixed well with 100 ml of DM water. From these various concentrations viz., 0.1, 0.01, 0.001 ppm were prepared by serial dilution method.

b) Preparation of sublethal concentration of pesticide Carbaryl

Stock solution was prepared by dissolving one hundred mg of carbaryl in 100 ml of DM water (1 ml contains 1 mg of pesticide). A safe concentration of 5.3 ppm of carbaryl is used in the present experiment, by dissolving 5.3 ml of stock solution to 1 lt. of water.

2.7 Test Aquarium

For the bio-assay experiments, plastic troughs 5 lit capacity were used. All the troughs were washed with small amount of acetone and kept in het sun for a day (II for bioassay experiments). The submerged plant Ottelia sp. (family: Hydrocharitaceae) were collected from a pond near TNACRI, Otthakadi, Madurai and maintained in cement tanks.

2.8 Experiment I

2.8.1 Assessment of biodegradation and bioaccumulation

2.8.1.1 Control I

To 1 Kg of enriched soil, 5 lit. Chlorpyrifos ,5.3 ppm carbaryl and 5 uniform sized *Cyprinus carpio* were added.

2.9 Experiment II With Plant of Uniform Size

To the above medium 100 gm of Ottelia plant was added.

2.10 Experiment III with Pseudomonas and with Plants

To 1 Kg of enriched soil, 5 lit. Chlorpyrifos , 5.3 ppm carbaryl and 5 *Cyprinus carpio*, 10 ml of nutrient broth containing *Pseudomonas* colonies, 100 gm of Ottelia plant were added.

2.11 Experiment IV (with plants only)

To one kg of sterilized soil 5 lit of chlorpyrifos and carbaryl treated water was added separately. 100 gm of Ottelia plant were added to this medium and residual levels of pesticides were analyzed.

3. RESULS AND DISCUSSION

3.1 Isolation and Characterization of Biodegrading Bacteria from Soil

The result shows that with increasing concentration of Chlorpyrifos there is a decreasing number of colonies.

3.2 Degradation of Chlorpyrifos and its Isomers by *Pseudomonas aeruginosa* Tested with gill, Muscle and Liver Tissue of *C.carpio*

3.2.1 Degradation of Chlorpyrifos by *Pseudomonas aeruginosa* tested with gill tissue of *C.carpio*

The results suggested that when concentration of chlorpyrifos increases form 100 to 500 ppm , decreases the number of colonies of *Pseudomonas aeruginosa* from five to one.

The degradation was gradually increased from 0.0358 to 0.0595 ppm as the duration time increased (Table 1).

3.2.2 Degradation of Chlorpyrifos by *Pseudomonas aeruginosa* tested with muscle tissue of *C. carpio*

From the Table 2, the prolonged exposure leads to the increase in the quantity of residues.

The degradation was generally increased as the days of incubation or exposure increased i.e. duration dependent (Table 3).

3.2.3 Degradation of Chlorpyrifos by *Pseudomonos aeruginosa* tested with liver tissue of *C. carpio*

The degradation was gradually increased as the days exposure increased (Table 3). The variation in the efficiency of degradation of Chlorpyrifos by gill, muscle and liver tissue of control fish and *Pseudomonas aeruginosa* treated fish were statistically significant

3.3 Degradation of Carbaryl and its Isomer by *Pseudomonas aeruginosa* Tested with Gill, Muscle And Liver Tissue Of C.Carpio

Table 4 showed that the degradation was gradually increased with the increasing days of incubation with *Pseudomonas aeruginosa* and duration of exposure.

Table 1. Degradation of chlorpyrifos by *Pseudomonas aeruginosa* during 10, 20, 30 days of treatment in the gill tissue of *Cyprinus carpio* (values are expressed in mg/gm of tissues)

Chlorpyrifos	Duration of exposure								
	10 Days			20 Days			30 Days		
Total	Control (Fish & pesticides)	Pesticide & Bacteria	Percentage reduction	Control (Fish & pesticides	Pesticide & Bacteria	Percentage reduction	Control (Fish & pesticides	Pesticide & Bacteria	Percentage reduction
	0.0509	0.0358	29.67	0.0753	0.0542	28.02	0.0800	0.0595	25.63

Table 2. Degradation of chlorpyrifos by *Pseudimonas aeruginosa* during 10, 20,30 days of treatment in the muscle tissue of *C.carpio* (values are expressed in mg/gm of tissues

Chlorpyrifos	Duration of exposure								
	10 Days			20 Days			30 Days		
Total	Control (Fish & pesticides)	Pesticide & Bacteria	Percentage reduction	Control (Fish & pesticides	Pesticide & Bacteria	Percentage reduction	Control (Fish & pesticides	Pesticide & Bacteria	Percentage reduction
	0.0833	0.0635	23.77	0.1184	0.0725	38.77	0.1214	0.0800	34.10

Table 3. Degradation of Chlorpyrifos by *Pseudomonas aeruginosa* during 10, 20, 30 days of treatment in the liver tissue of *C.carpio* (values are expressed in mg/gm of tissues)

Chlorpyrifos	Duration of exposure								
	10 Days			20 Days			30 Days		
Total	Control (Fish & pesticides)	Pesticide & Bacteria	Percentage reduction	Control (Fish & pesticides	Pesticide & Bacteria	Percentage reduction	Control (Fish & Pesticides	Pesticide & Bacteria	Percentage reduction
	0.0683	0.0501	26.65	0.0820	0.0614	25.18	0.0859	0.0631	26.54

Table 4 . Degradation of Carbaryl by *Pseudomonas* aeruginosa during 10, 20,30 days of treatment in the gill tissue of *C.carpio* (values are expressed in mg/gm of tissues)

Carbaryl	**Duration of exposure**								
	10 Days			**20 Days**			**30 Days**		
Carbaryl	Control (Fish & pesticides)	Pesticide & Bacteria	Percentage reduction	Control (Fish & pesticides	Pesticide & Bacteria	Percentage reduction	Control (Fish & pesticides	Pesticide & Bacteria	Percentage reduction
	2.374	1.8095	23.78	3.427	2.612	23.78	3.105	2.429	21.77
Total Carbaryl	3.854	2.479	35.68	4.9535	3.7303	24.69	5.293	3.5973	32.04

Table 5. Degradation of Carbaryl by *Pseudomonas aeruginosa* during 10, 20,30 days of treatment in the gill tissue of *C.carpio* (values are expressed in mg/gm of tissues)

Carbaryl	**Duration of exposure**								
	10 Days			**20 Days**			**30 Days**		
Carbaryl	Control (Fish & pesticides)	Pesticide & Bacteria	Percentage reduction	Control (Fish & pesticides	Pesticide & Bacteria	Percentage reduction	Control (Fish & pesticides	Pesticide & Bacteria	Percentage reduction
	2.486	1.9125	23.07	3.5765	2.7635	22.73	2.806	1.918	31.65
Total Carbaryl	3.878	2.5743	33.62	5.3175	3.971	25.32	4.983	3.2815	34.15

Table 6. Degradation of Carbaryl by *Pseudomonas aeruginosa* during 10, 20, 30 days of treatment in the gill tissue of *C.carpio* (values are expressed in mg/gm of tissues)

Carbaryl	**Duration of exposure**								
	10 Days			**20 Days**			**30 Days**		
Carbaryl	Control (Fish & pesticides)	Pesticide & Bacteria	Percentage reduction	Control (Fish & pesticides	Pesticide & Bacteria	Percentage reduction	Control (Fish & pesticides	Pesticide & Bacteria	Percentage reduction
	4.514	3.788	16.08	5.97	5.001	16.23	5.372	4.478	14.78
Total Carbaryl	5.933	4.789	19.28	8.2378	6.48	21.34	7.999	6.535	18.30

3.4 Degradation of Carbaryl by *Pseudomonas aeruginosa* tested with muscle tissue of *C. carpio*

The degradation gradually increased as the days of incubation of exposure increased which was positively related , shown in Table 5.

3.5 C. Degradation of Carbaryl by *Pseudomonas aeruginosa* tested with liver tissue of *C. carpio*

The degradation of carbaryl by the inoculation of *Pseudomonas aeruginosa*, during 10, 20 and 30 days of exposure was noticed and quantity of pesticide present was 4.789, 6.480 and 6.535 ppm respectively representing the total carbaryl (Table 6). The degradation was more predominant in the liver tissue than the other tissues in the order: liver>muscle>gill. The variation in the efficiency of the degradation of carbaryl by gill, muscle and liver tissue of control fish and *Pseudomonas aeruginosa* treated fish were statistically significant ($P<0.05$).

4. CONCLUSION

The results of the present study on biodegradation of chlorpyrifos with an interval 10, 20 and 30 days the fish tissuesuch as gill, muscle and liver were dissected out to analyse for biochemical changes such as, protein estimation. Among the different tissue such as tissue gill, muscle and there was notable indeed effective percentage of degradation of the chlorpyrifos in the liver tissue was found 23.77% on 10th day, 38.77 on 20th day and 34.10on 30th day respectively. Since liver is the sole site of accumulation and liver has numerous enzyme noted for detoxification mechanism. Similarly, degradation of carbaryl by P*seudomonas aeruginosa* tested with gill, muscle and liver tissue revealed 35.68 on 10th day, 24.69 on 20th day, and 32.04 on 30th day for gill, tissue and for muscle tissue the percentage of degradation of carbaryl residue was 33.62 on 10th day, 25.32 on 20th day, and 34.15on 30th day was recorded. But the degradation was predominant in the liver tissue of *Cyprinus carpio* which was 19.28 on 10th day, 21.34on 20th day and 18.30 on 30th day was significant.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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