Effects Of Carbaryl Exposure On The Protein Content Of *Cyprinus Carpio*

M. Arumugam[1*], Victor Prabakaran[2]

[1]Part-time Research Scholar, School of Biological Science, Madurai Kamaraj University, Madurai, Tamil Nadu, India
[2]Department of Zoology, Government Arts College, Melur, Madurai, Tamil Nadu, India

***Corresponding author:** M. Arumugam
*Email: aru9865@gmail.com

Abstract
The effect of carbaryl on the protein content of gill, muscle and liver tissues of *C. carpio* was studied. The fish *Cyprinus carpio* was employed as an indicator animal and its gill, muscle, liver tissue was analyzed for pesticide residue accumulation where in the biochemical changes such as protein was found to be depleted due to toxicity of pesticides. Subsequently an increase protein content was noted in the biodegradation experimental sets.

Keywords: Bioaccumulation, Carbaryl, *Cyprinus carpio,* protein degradation, toxicity

INTRODUCTION

Carbaryl, a globally renowned carbamate insecticide known for its exceptional pest control efficacy across various crop types [1]. This pesticide plays an essential role in both increasing agricultural productivity and ensuring food security [2]. However, the extensive use of pesticides carries significant downsides, as they can potentially harm local estuarine and coastal aquaculture through the discharge of agricultural wastewater [3,4]. The liver plays a vital role in several key metabolic functions [5] and is the primary organ for the accumulation, biotransformation, and excretion of pollutants in fish, including the degradation and bioactivation of pesticides [6,7]. In experimental studies, assessing the biochemical and histological changes in fish livers has become an important tool for monitoring environmental exposure to pollutants [8]. Boran et al. (2010) identified focal necrosis in the liver, head kidney, and spleen of rainbow trout (*Oncorhynchus mykiss*) during static tests involving exposure to carbaryl [9]. Furthermore, carbaryl can impair the normal neurological functions of trout by inhibiting acetylcholinesterase, leading to a loss of normal neural behavior and reduced predation efficiency [10]. Watershed research has consistently shown that carbaryl poses significant risks due to its acute toxicity to various small marine fish species as well as to aquatic and benthic invertebrates in estuarine environments [11]. Numerous studies have documented these effects, illustrating how carbaryl exposure can disrupt local ecosystems and affect the survival of marine life [12]. The immune system in fish serves as the principal defence against environmental pathogens and toxicants [13]. Pesticides interact with intracellular receptors and signalling molecules in fish, thereby modulating gene expression regulatory mechanisms. This interaction precipitates notable alterations in the expression of genes associated with immune, metabolic, and neurological functions, consequently disrupting normal physiological and immune responses in these organisms [14]. Evidence shows that exposure to the pesticide carbaryl induces an upregulation in immune gene expression in hybrid catfish [15]. The alterations in immune gene expression not only provide insights into the immune status of the fish but also elucidate their molecular response to chemical pollutants [16]. Due to limited data on carbaryl's impact on fish, this study employed *Cyprinus carpio* as a model organism. The effect of carbaryl on the protein content of gill, muscle and liver tissues of *C. carpio* after treating with bacteria, plants and both with bacteria along with plants was studied.

MATERIALS AND METHODS

Preparation of stock solution : One hundred mg of carbaryl was dissolved in 100 ml of distilled water and kept as stock solution (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 one litre of water.

Test aquarium: For the bio-assay experiments, plastic troughs 5 litre capacity were used. All the troughs were washed with small amount of acetone and kept in het sun for a day (ll for bioassay experiments). The submerged plant *Ottelia sp.* (family: Alismoides) were collected from a pond near Tamil Nadu Agricultural College and Research Institute, Otthakadi, Madurai (Tamilnadu. India) and maintained in cement tanks.
The common carp *Cyprinus carpio* (15 cm in length + 1cm) was collected from the Fish Farmers Development Association, Vaigai Dam at Fisheries Farm, Madurai District, (Tamil Nadu, India) and transported to the laboratory in polythene bags containing oxygenated water. Nearly one hundred *Cyprinus carpio* were stored in large aquaria capacity and care was taken to reduce the hyper activity and physical injuries to the fish. All

the individuals were given salt treatment (dip. In 3.5% NaCl solution for 3 minutes) a precaution against any parasitic infections. Fish were observed daily and water was changed once in a week. Ground nut cake was offered as food to test fish. In industrialized nations species like the common carp (*Cyprinus carpio*) have been recommended and employed as standard test fish,

The following combinations of the experimental made along with the control set.

Control set : *C. carpio*
Expt. Set I : *C. carpio* + carbaryl
Expt. Set II: *C. carpio* + carbaryl + *Pseudomonas aeruginosa*
Expt. Set III: *C. carpio* + carbaryl + Ottelia sp.
Expt. Set IV: *C. carpio* + carbaryl + *Pseudomonas aeruginosa* + Otellia sp.

(i) **Carbaryl on the protein content in the gill tissue of *C. carpio* treated with bacteria, plant and both bacteria and plant**

The control fish showed 42.1, 41.2 and 41.9 mg/100mg dry weight of tissue during 10, 20 and 30 days of exposure respectively (**Table 1)**. Fish were subjected to carbaryl showed 40.0, 38.2 and 37.7 mg of protein per 100mg of dry weight of tissue during 10, 20 and 30 days of exposure respectively. This clearly shows a reduction in the protein content in the gill tissue due to exposure of the pesticide. When compared to control set, the percentage of reduction of protein content in the experimental set I was 4.9, 7.2 and 10.0 during 10, 20 and 30 days of exposure respectively.

Table 1: Content of protein in gills of *C.carpio* exposed to carbaryl and bacteria, carbaryl and plants ,carbaryl plants and bacteria treated simultaneously. (Values are expressed in mg/100 mg of tissue)

Treatment group	10 Days		20 Days		30 Days	
		% Reduction		% Reduction		% Reduction
Control Set	42.1+ 0.48.		41.2 + 0.48.		41.9 + 0.96	
Expt.set I	40.0 + 0.96	4.9	38.2 + 0.65	7.2	37.7 + 0.5	`10.0
Expt.set II	41.1 + 0.96	2.4	40.0 + 0.63	2.9	40.2 + 1.1	4.0
Expt.set III	40.8 + 0.48	3.1	40.2 + 0.48	2.4	40.1 + 0.85	4.0
Expt.set IV	41.5 + 0.65	1.4	41.1 + 0.48	0.2	41.2 + 2.5	1.6

Table 2: The percentage of change in gills level in muscles *C.carpio* exposed to carbaryl, carbaryl and bacteria, carbaryl and plant, bacteria treated simultaneously (Values are expressed in mg/gm of tissue)

S.No.	Treatment	DURATION OF EXPOSURE		
		10 Days	20 Days	30 Days
		% Increase	% Increase	% Increase
1.	Fish + Carbaryl + *P aeruginosa*	2.5	4.3	6
2.	Fish + Carbaryl + *Ottelia sp.*	1.8	4.8	6
3.	Fish + Carbaryl + *P aeruginosa* + *Ottelia sp.*	3.5	7.0	8.4

When compared to the control set, the percentage of reduction was 2.4, 2.9 and 4.0 during 10, 20 and 30 days of exposure respectively. When the fish were exposed to carbaryl and bacteria, the concentrations of protein in gill tissue were 41.1, 40.0 and 40.2 mg per 100 mg of tissue during 10, 20, and 30 days of exposure respectively. When compared to the control set the percentage of reduction was 2.4, 2.9 and 4.0 during 10,20 and 30 days of exposure respectively. When the fish were exposed to the carbaryl and the aquaphyte, the concentrations of protein in gill tissue were 40.8, 40.2 and 40.1 mg / 100 mg of tissue for 10, 20 and 30 days of exposure respectively. The percentage of reduction was 3.1, 2.4 and 4.0 during 10, 20 and 30 days of exposure respectively. When the fish were exposed to the pesticide and bacteria along with plant, the concentrations of proteins in gill tissue were 41.5, 41.1 and 41.2 mg/100mg of tissue for 10, 20 and 30 days of exposure respectively. When compared to the control set, the percentage of reduction in this set was 1.4, 0.2 and 1.6 during 10, 20 and 30 days of exposure respectively. It is clear that the percentage of increase in the protein content in the experimental set II due to bacterial treatment was 2.5, 4.3 and 6.0 during 10, 20 and 30 days of exposure respectively **(Table 2)**. When compared to experimental set 1, the percentage increase in protein content in the experimental set III due to the bio-accumulation by the plant were 1.8, 4.8 and 6.0

during 10, 20 and 30 days of exposure respectively. Similarly the percentage increase in protein due to bio-degradation by bacteria and bio- accumulation by plants was 3.50, 7.0 and 8.4 during 10, 20 and 30 days of exposure respectively in the experimental set IV.

(ii) Carbaryl on the protein content in the muscle tissue of *C. carpio* treated with bacteria, plants and both bacteria and plants:

The effect of carbaryl on the protein content of the muscle tissue of *C. carpio* after treating with bacteria, plants and both with bacteria and plant was studied. The results were shown in (**Table 3)**. The control fish which were kept in tap water showed 46, 45.2 and 45.0 mg of protein per 100 mg of dry weight of tissue during 10, 20 and 30 days of exposure. The fish which were subjected to carbaryl, showed 42.0. 41.0 and 38.2 during 10, 20 and 30 days of exposure. When compared to control set, the percentage of reduction in the experimental set I was 8.6, 9.2 and 15.1 during 10, 20 and 30 days of exposure t to the pesticide respectively. When the fish were exposed to pesticide and bacteria, the protein concentration in muscle tissue were 43.3, 43.1 and 43.0 mg/100mg of tissue during 10, 20 and 30 days exposure respectively.

Table 3: Content of protein in muscles of *C.carpio* exposed to carbaryl and bacteria, carbaryl and plants, carbaryl plants and bacteria treated simultaneously. (Values are expressed in mg/100 mg of tissue)

Treatment group	10 Days		20 Days		30 Days	
		% Reduction		% Reduction		% Reduction
Control Set	46.0+ 2.16		45.2 + 1.47		45.0 + 0.48	15.1
Expt.set I	42.0 + 1.22	8.6	41.0 + 0.58	9.2	38.2 + 0.85	4.4
Expt.set II	43.3 + 0.75	5.9	43.1 + 0.50	4.6	43.0 + 0.85	6.4
Expt.set III	42.9 + 0.82	6.7	42.3 + 0.96	6.4	42.1 + 0.58	2.8
Expt.set IV	44.2 + 0.41	3.9	41.8 + 0.48	7.5	43.7 + 0.82	

Table 4: The percentage of change in gills level in muscles *C.carpio* exposed to carbaryl, carbaryl and bacteria, carbaryl and plant, bacteria treated simultaneously (Values are expressed in mg/gm of tissue)

S.No.	Treatment	DURATION OF EXPOSURE		
		10 Days	20 Days	30 Days
		% Increase	% Increase	% Increase
1.	Fish + Carbaryl + P aeruginosa	2.7	4.6	10.7
2.	Fish + Carbaryl + Ottelia sp.	1.9	2.8	8.7
3.	Fish + Carbaryl + P aeruginosa + Ottelia sp.	4.7	1.7	12.3

Compared to the control set, the percentage of reduction was 5.9, 4.6 and 4.4 during 10, 20 and 30 days of exposure respectively. When the fish exposed to carbaryl and the aquaphyte, the concentrations of protein in the muscle tissue were 42.9, 42.3 and 42.1during 10, 20 and 30 days of exposure period respectively. The percentage of reduction was 6.7, 6.4 and 6.4 for 10, 20 and 30 days of exposure period respectively. When the fish exposed to carbaryl and bacteria along with plant, the concentration of protein in muscle tissue were 44.2, 41.8 and 43.7 mg/100mg of dry weight of tissue during 10, 20 and 30 days of exposure respectively. When compared to control set, the percentage of reduction was 3.9, 7.5 and 2.8 during 10, 20 and 30 days of exposure period respectively. It is clear from the **(Table 4**) that, the percentage of increase in protein content in the experimental set II due to the degradation of the pesticide by bacteria was 2.7, 4.6 and 10.7 during 10, 20 and 30 days respectively. When compared to experimental set, I the percentage of increase in protein content in the experimental set III due to the bio accumulation by plant was 1.,2.8 and 8.7 during 10, 20 and 30 days of exposure period respectively. Similarly, the percentage of increase in protein due to biodegradation by bacteria and bioaccumulation by plants was 4.7,1.7 and 12.3 during 10, 20 and 30 days of exposure respectively in the experimental set IV.

Table 5: Content of protein in muscles of *C.carpio* exposed to carbaryl and bacteria, carbaryl and plants, carbaryl plants and bacteria treated simultaneously. (Values are expressed in mg/100 mg of tissue)

Treatment group	10 Days		20 Days		30 Days	
		% Reduction		% Reduction		% Reduction

Control Set	44.2+ 0.48		43.2 + 0.48		43.2 + 0.96	
Expt.set	41.6 + 0.96	6.7	39.1 + 0.65	9.5	39.1 + 0.5	9.4
Expt.set	42.3 + 0.96	4.3	42.3 + 0.63	2.1	42.0 + 1.1	2.7
Expt.set	42.7 + 0.48	3.4	42.0 + 0.48	2.7	41.2 + 0.85	4.6
Expt.set	42.0 + 0.65	1.8	42.1 + 0.48	2.5	42.8 + 2.5	0.2

Table 6: The percentage of change in protein level in muscles *C.carpio* exposed to carbaryl, carbaryl and bacteria, carbaryl and plant, bacteria treated simultaneously (Values are expressed in mg/gm of tissue)

S.No.	Treatment	DURATION OF EXPOSURE		
		10 Days	20 Days	30 Days
		% Increase	% Increase	% Increase
1.	Fish + Carbaryl + P aeruginosa	2.4	7.4	6.7
2.	Fish + Carbaryl + Ottelia sp.	3.3	6.5	4.8
3.	Fish + Carbaryl + P aeruginosa + Ottelia sp.	1.8	7.0	0.2

The effect of carbaryl on the protein content of the liver tissue of *C. carpio* after treating with bacteria, plants and both with bacteria and plant was studied. The results were shown in (**Table 5**). The control fish which were kept in tap water showed 44.2, 43.2 and 43.2 mg of protein per 100 mg of dry weight of tissue during 10, 20 and 30 days of exposure. The fish which were subjected to carbaryl, showed 41.2, 39.1 and 39.1 during 10, 20 and 30 days of exposure. When compared to control set, the percentage of reduction in the experimental set I was 6.7, 9.5 and 6.7 during 10, 20 and 30 days of exposure to the pesticide respectively. When the fish exposed to pesticide and bacteria the protein contents in liver tissue were 42.3, 42.3 and 41.2 mg/100mg of tissue during 10, 20 and 30days exposure respectively when compared to the control set the percentage of reduction was 4.3, 2.1 and 2.7 during 10, 20 and 30 days of exposure respectively. When the concentrations fish of protein were exposed to carbaryl and aquaphyte, the in the liver tissue were 42.7, 42.0 and 41.2 during 10, 20 and 30 days of exposure period respectively. The percentage of reduction was 3.3, 2.7 and 4.8 for 10. 20 and 30 days of exposure respectively. When the fish were exposed to carbaryl and bacteria along with plant, the concentration of protein in liver tissue were 42.0, 42.1 and 42.8 during 10, 20 and 30 days of exposure respectively. When compared to control set, the percentage of reduction was 4.9, 2.5 and 9.2 during 10, 20 and 30 days of exposure respectively. It is clear from the (**Table 6)** that, the percentage of increase in protein content in the experimental set II due to bacterial degradation was 2.4, 7.4 and 6.7 during 10, 20 and 30 days respectively. When compared to experimental set I, the percentage of increase in protein content in the experimental set III due to the bioaccumulation of by plant was 3.3, 6.0 and 4.8 respectively. Similarly the percentage of increase in protein due to biodegradation by bacteria and bioaccumulation by plants was 1.8, 7.0 and 0.2 during 10, 20 and 30 days of exposure respectively in the experimental set IV.

CONCLUSION

In summary, this study investigated the effects of carbaryl exposure *C. carpio*. Carbaryl was capable of reducing the protein content in gill, liver and muscle tissue of *C.carpio*. However, when they are treated along with bacteria and aquaphyte the reduction was minimized by the degrading capacity of the bacteria and bioaccumulation by the aqua phyte in the muscle tissue, gill and liver tissue of the fish *C.carpio*. Reduction in protein contents of carbaryl treated *C. carpio* indicates fall in nutritional value or quality of food, underlining the need for further research on protective strategies against pesticide impacts in aquaculture. This research not only provides fundamental data for evaluating the biochemical and immunological impacts of carbaryl toxicity on fish but also lays the groundwork for further studies on protective strategies against pesticides in aquaculture.

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