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Effects of a Fungicide, Carbendazim on the Biochemical Changes in the Fresh Water Fish, *Cyprinus carpio*

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ABSTRACT

The biochemical components like Glucose, Glycogen and Protein were estimated quantitatively in the tissues of liver, muscle and kidney of control and fungicide treated fishes. The fishes were treated with the 0.1%, 0.25% and 0.5% concentration of carbendazim for 24, 48 and 72 hours. The Glycogen and Protein of liver, muscle and kidney of the control fish were very high when compared with the treated ones. In treating fish, the glucose content (0.1%) was high in liver, muscle and kidney when compared to the control. Maximum reduction was observed at 72 hours exposures.

INTRODUCTION

Fungicides are chemical compounds used to prevent the spread of fungi or plants in gardens and crops, which can cause serious damage resulting in loss of yield and thus profit. Fungicides are also used to fight fungal infections. Fungicides can either be contact or systemic. A contact fungicide kills fungi when sprayed on its surface; a systemic fungicide has to be absorbed by the plant.

Carbendazim is a fungicide of major concern due to the suspected hormonal effects. Carbendazim is a systemic benzimidazole fungicide that plays an important role in plant disease control. Pesticide pollution is known to alter the freshwater ecosystems to a greater extent, thereby affecting the different components of the ecosystem. The chief non-target species that are very much affected by these pesticides are fishes which are economically important and contributing much to our nation's protein value.

Biochemical changes induced by fungicide strain lead to disturb the metabolism, inhibition of an important enzyme, retardation of growth and reduction in longevity of the organs. The present study was designed to understand the impact of fungicide (carbendazim) on Protein, Glucose and Glycogen in freshwater fish, *Cyprinus carpio* with an exposure period of 24, 48 and 72 hours.

MATERIALS AND METHODS

The freshwater fish, *Cyprinus carpio* (body length 5-7 cms, body weight 5-6 gm) were collected from Aliyar Dam and acclimatized to laboratory condition for 2 weeks in a large cement tanks (6'×4×3') at (24±3ºC). The fishes were fed regularly with conventional diet. Rice bran and oil cake 1:1 ratio. Feeding was stopped one day prior to the start of the experiment. Fishes about the same size irrespective of sexes were selected for the experiment. Technical grade of carbendazim fungicide was used in the investigation. Batches of 10 healthy fishes were exposed to different concentration of the fungicide. LC_{50} value for 72 hrs was calculated by using probit analysis (Finney, 1971).

The fishes were exposed to different concentration of the fungicide, carbendazim for 24, 48 and 72 hours respectively. Another group was maintained as control. At the end of each exposure period, fishes were sacrificed and tissues such as liver, muscle and kidney were dissected and

removed. The tissues were homogenized with 80% methanol, Centrifuged at 3500 rpm for 15 minutes and the clear supernatant was used for analysis of different parameters. The Protein was estimated following method of Lowery *et al*. (1951). Glycogen and Glucose were estimated by Kemp and Kits (1954). The results were expressed as mg/g wet weight of the tissues.

RESULTS AND DISCUSSION

Protein levels

The estimated protein content of control liver tissue *Cyprinus carpio* was 5.48 ± 0.18 mg/gm. The protein level shows lesser changes from the control. It either increases or decreases (4.53 mg/gm, 5.24 mg/gm, and 5.26 mg/gm) from the control insignificantly at 24 hrs, 48 hrs and 72 hrs duration of 0.1% concentration. This was the same condition in muscle and kidney tissues at 0.25% and 0.5% concentration of fungicide treated fishes, for the experimental durations. Among the three types of tissues, the kidney tissues were the most affected tissues and it loses its protein content throughout the period of toxication and never regains whereas muscle and liver tissues lose this protein content at the starting period of toxication (24 hrs) as 4.53 mg/gm and 2.31 mg/gm at 0.25% concentration and later at 48 hrs and 72 hrs of period it regains. The liver protein level increases (5.24 mg/gm & 5.26 mg/gm) at 48 hrs and 72 hrs towards the control (5.48 mg/gm) after depletion at 24 hrs (4.53 mg/gm). The same is the condition in the muscle protein, which regains (48 hrs and 72 hrs), after depletion at 24 hrs. (Table 1).

In the present investigation, the pesticide depletes the protein content of the tissue at 24 hrs of exposure period in 0.1%/ 0.25% and 0.5% concentration levels (4.53 mg/gm, 4.91 mg/gm, 4.98 mg/gm) than the control (5.48 mg/gm). The depletion was higher as the period of exposures prolonged in all concentrations. The decrease may be due to the rapid utilization of body protein or poor intake of dietary protein by the fish during stress conditions. If the dietary protein has been taken in a considerable amount, it would not have been stored in the body tissue. Instead, that would have been utilized to get extra energy.

Glucose levels

The liver glucose level of control *Cyprinus carpio* was estimated to be 4.16 ± 0.19 mg/gm and when exposed to 0.1% carbendazim, the glucose level fell down (1.87 mg/gm) at 24 hrs period.

During 48 hrs duration, the liver glucose increased slightly (3.58 mg/gm and at 72 hrs it further increased to 4.61 mg/gm. Even at 0.25% and at 0.5% carbendazim glucose get decreased first (1.90 mg/gm (at 24 hrs) and steadily increased further (48 hrs & 72 hrs), (4.05 and 5.23 mg/gm) towards the control (4.16 mg/gm). This phenomenon is carried even at 0.5% concentration of carbendazim.

The muscle glucose content at 0.1%, 0.25%, and 0.5% fungicidal effects were prominent at 0.1% concentration of fungicide; the fish loses its glucose almost completely at 24 hrs. (1.21 mg/gm). Then at 48 hrs and 72 hrs of duration, the fish attains steadiness in increasing the muscle glucose (3.41, 3.47 mg/gm). As the concentration increases, the fish tend to lose more of glucose at 24 hrs of treatment (1.91 mg/gm to 1.48 mg/gm). As the duration of treatment as well as the percentage of concentration increase, the animal regains its muscle glucose level (Table 2).

The liver glucose level of control *Cyprinus carpio* was 4.16 ± 0.19 mg/gm. After the treatment of pesticides, the fish loses its glucose level in all the tissues. The loss of glucose content in the liver tissues for 24 hrs exposure is directly proportional to the percentage of pesticide. The same result was noticed in the freshwater teleost *Labeo rohita*. When the duration was prolonged to 48 hours and 72 hours, the level of glucose increased to reach the control level slowly (3.58 mg/gm to 4.61 mg/gm, 4.05 to 5.23 mg/gm). Increased glucose levels in blood and other tissues, as a response to pesticide stress, reported in a number of fishes has been suggested as a result of glycogenolysis (Mukhopadhyey and Dehedrai, 1980). The same would be the reason for the depletion and like in the glucose levels of muscle and kidney tissues.

Glycogen levels

The liver glycogen level of control *Cyprinus carpio* was estimated to be 17.13 ± 0.33 mg/gm and when exposed to 0.1% carbendazim, the fish showed an overall insignificant change in liver glycogen level during 24 hrs, 48 hrs and 72 hrs exposure while an insignificant increase was observed during 24 hrs and 0.05 level significant decrease was noticed at 48 hrs and 72 hrs. The 0.25% pesticide-exposed fishes showed decreased glycogen levels of 15.7 mg/gm, 14.1 mg/gm, 14.2 mg/gm at 24 hrs, 48 hrs and 72 hrs respectively. These levels are significant at 0.01 and 0.05 freedom levels. There is an overall decreased of glycogen level at 0.5% concentration (15.1

mg/gm, 12.47 mg/gm and 11.03 mg/gm) for 24 hrs, 48 hrs and 72 hrs exposure periods than the control (17.13 mg/gm).

The muscle glycogen level shows a steady increase at 0.1% concentration over the control at various exposures. But at 0.25% concentration the muscle glycogen gets disturbed and shows a significant decrease (6.56 mg/gm, 5.81 mg/gm and 6.3 mg/gm) from the control (17.13 mg/gm). Likewise, at 0.5% concentration there is significant at 0.01 and 0.05 level in the muscle glycogen as it decreased (5.53 mg/gm, 5.51 mg/gm and 4.92 mg/gm) towards the prolongivity (48 hrs, and 72 hrs).

24 hrs and 48 hrs exposures of fishes at 0.1% carbendazim produced insignificant decreased of glycogen level (0.42 mg/gm/ 0.47 mg/gm) than the control (0.50 mg/gm). But prolonged exposure (72 hrs) showed an increased level of glycogen (0.58 mg/gm). Fishes treated with 0.25% concentration lost the kidney glycogen (0.35 mg/gm, 0.23 mg/gm and 0.32 mg/gm) at higher significant level than the control (0.58 mg/gm). Higher concentration of pesticide (0.5%) exposures, highly decreased the kidney glycogen (0.27 mg/gm, 0.23 mg/gm, and 0.23 mg/gm) at (24 hrs, 48 hrs and 72 hrs) (Table 3).

The glycogen content of the liver, muscle and kidney tissues get reduced after 24 hrs of exposure to 0.25% and 0.5% concentration of pesticide, even though it showed a slight increase at 0.1% conc. (17.31 mg/gm) than the control (17.13 mg/gm). Decrease in glycogen has been reported by many authors in various fishes at different pesticide treatment. Srivastava and Singh (1981) have reported that utilization of stored glycogen in different tissues (like liver, muscle, brain etc.,) results in severe glycogenolysis in fishes under pesticide stress.

In the present study, the glycogen level has increased than the control after the prolonged exposure of 48 hrs and 72 hrs at 0.1%, 0.25% and 0.5% concentration of the fungicide used. This is due to glycogenesis through 'Cori cycle''. There are supportive evidences to show the increased level of glycogen. Bakthavathsalam (1980) has reasoned the reduction in lactic acid levels in the muscle tissue of lindane exposed *Anabas testudineus* as an indication of adaptive glycogenesis. From the present study it was concluded that, the toxicity of the commonly used fungicide carbendazim was used to analyze the effect on the protein, glycogen and glucose levels of the tissues of liver, muscle and kidney. Based on the results, the carbendazim reduces the protein and

carbohydrate levels at short period of exposure. For a prolonged exposure, *Cyprinus carpio* tends to acclimatize to the environment by evolving by adaptations to overcome the stress. Anything beyond the levels of tolerance may have ill effect over the survival ability of the fish. That expresses the toxicity of the fresh water forms non edible for human beings and to the aquatic animals. Thus the *Cyprinus carpio* can be a bio-indicator of carbendazim toxicity.

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Table 1. Changes in the level of protein (mg/g) in different tissues of the fish, *Cyprinus carpio* exposed to different durations and concentrations of the fungicide carbendazim.

Tissues	Control	24 hours			48 hours			72 hours		
		0.1%	0.25%	0.5%	0.1%	0.25 %	0.5%	0.1%	0.25 %	0.5%
Liver	5.48 ± 0.18	4.53 ± 0.26**	4.91 ± 0.05	4.98 ± 0.14	5.24 ± 0.16	3.95 ± 0.13	4.82 ± 0.19	5.26 ± 0.03	3.43 ± 0.12	3.66 ± 0.24
Muscle	2.87 ± 0.13	2.31 ± 0.21	2.30 ± 0.09	2.49 ± 0.20	2.01 ± 0.08**	2.15 ± 0.15	1.90 ± 0.29	2.9 ± 0.33	1.59 ± 0.08	1.50 ± 5.78
Kidney	2.0 ± 0.01	1.87 ± 0.16	1.6 ± 0.17	2.00 ± 0.11	1.55 ± 0.17	0.29 ± 0.16	1.67 ± 6.41	0.90 ± 0.08**	0.18 ± 0.08	1.66 ± 0.25

Each value mean is the ± SE of 6 individual observations.

**- Significant at 0.05 level; NS- others are not significant.

Table 2. Changes in the level of glucose (mg/g) in different tissues of the fish, *Cyprinus carpio* exposed to different durations and concentrations of the fungicide carbendazim.

Tissues	Control	24 hours			48 hours			72 hours		
		0.1%	0.25%	0.5%	0.1%	0.25%	0.5%	0.1%	0.25%	0.5%
Liver	4.16 ± 0.19	1.87 ± 0.04**	1.90 ± 0.21**	1.4 ± 0.17**	3.58 ± 0.11**	4.05 ± 0.07**	3.76 ± 0.14	4.61 ±0.10**	5.23 ± 0.15**	5.17 ± 0.20**
Muscle	2.36 ± 1.21	1.21 ± 0.10**	1.91 ± 0.18	1.48 ± 0.13**	3.41 ± 0.13**	3.84 ± 0.09	3.30 ±0.21**	3.47 ±0.07**	4.55 ± 0.12**	4.39 ± 0.19**
Kidney	0.41 ± 003	0.22 ± 0.01**	0.2 ± 0.02**	0.16 ± 0.01	0.41 ± 0.33**	0.40 ± 0.03	0.46 ±0.02	0.48 ±0.02**	0.63 ± 0.02**	0.7 ± 0.02

Each value mean is the ± SE of 6 individual observations.

**- Significant at 0.05 level; NS- others are not significant.

Table 3. Changes in the level of glycogen (mg/g) in different tissues of the fish, *Cyprinus carpio* exposed to different durations and concentrations of the fungicide carbendazim.

Tissues	Control	24 hours			48 hours			72 hours		
		0.1%	0.25%	0.5%	0.1%	0.25%	0.5%	0.1%	0.25%	0.5%
Liver	17.13 ± 0.33	17.31 ± 0.25	15.7 ± 0.16**	15.10 ±0.36**	15.81 ± 0.27**	14.1 ± 0.32**	12.47 ± 0.29**	17.03 ± 0.30	14.2 ± 0.17**	11.03 ± 0.37**
Muscle	6.56 ± 0.33	6.85 ± 0.23	6.57 ± 0.03	5.53 ±0.19**	7.07 ± 0.46	5.81 ± 0.06**	5.51 ± 0.15**	7.3 ± 0.38	6.3 ± 0.06**	4.92 ± 0.36**
Kidney	0.50 ± 0.03	0.42 ± 0.3	0.35 ± 0.01**	0.27 ±0.04**	0.47 ± 0.31	0.23 ± 0.01**	0.23 ± 0.02**	0.58 ± 0.25	0.32 ± 0.02**	0.23 ± 0.02

Each value mean is the ± SE of 6 individual observations.

**- Significant at 0.05 level; NS- others are not significant.