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Impact of Carbendazim Fungicide on Acid Phosphatase Activity in Freshwater Fish *Rasbora daniconius*

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A B S T R A C T

Carbendazim is a systemic fungicide that is extensively used to control fungal diseases in agriculture, forestry, and veterinary care. These fungicides infiltrate water bodies through runoff and soil erosion, harming non-target animals including fish. The current study investigated at the toxicological effects of carbendazim at a lethal concentration on Acid Phosphatase (ACP) activity in *Rasbora daniconius* of liver and muscle tissue. For seven days, the experimental fish were acclimatised in glass aquaria in laboratory settings. The treatment (LC_0 and LC_{50} concentration) group had significantly reduced Acid Phosphatase activity in the liver and muscle than the control group. Carbendazim, a fungicide, suppresses enzymatic activity in the freshwater fish *Rasbora daniconius*, according to the findings. It has been discovered that a higher concentration of carbendazim affects the natural activities of aquatic species, particularly fish.

***Key words*:** Acid phosphatase, Carbendazim, *Rasbora daniconius*, Acute toxicity, LC_0 and LC_{50}

Water pollution has become a serious issue all over the world as a result of the addition of numerous pollutants to water bodies in a variety of ways, which alters the inherent properties of water [1]. Pesticides that impact fish development, reproduction, and nutritional value are generally known in the case of inland water contamination. The widespread and indiscriminate use of harmful chemicals to pollute the aquatic environments through drift, runoff, drainage, and leaching has become one of the world's most serious concerns [2]. Carbendazim is a 2-metheoxy carbamoyl benzimidazole systemic fungicide, that is effective against a variety of plant diseases. It's commonly used to protect fruits, vegetables, and decorative plants from illness [3]. It's also used to treat and manage a wide range of diseases before and after harvest [4]. They can easily penetrate plants' roots and leaves, and they can drain directly into natural water. After application, the majority of these substances linger in the environment for months, if not years. Carbendazim is a plant disease control agent that works by preventing mitosis and cell division. It has been found to result in maternal and developmental toxicity in mammals [5-6].

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Enzymes play a major role in metabolic processes. They perform very well and are very specific with regard to the nature of the catalyzed reaction and the substrate used. Therefore, changes in enzymatic concentrations are one of the basic steps in evaluating the effects of toxic substances. Enzymes are biochemical macromolecules that regulate the metabolic processes of animals, therefore, minor changes in enzyme activity can have an impact on the organism [7]. Phosphatases are excellent markers of stress in biological systems [8]. Acid phosphatase has been found as a lysosome detecting enzyme in cell fractions [9]. The metabolism of the fish can be affected by changes in acid phosphatase activity. Changes in phosphatase activity have been used as indicators of fish growth, sickness, and spawning in fisheries science [10-11]. However, very scanty information is available on the alterations in enzyme activities due to Carbendazim in the *Rasbora daniconius*. The effect of carbendazim on acid phosphatase activity in the liver and muscle of *Rasbora daniconius* were investigated in this study.

MATERIALS AND METHODS

Test organism and chemicals

The healthy freshwater fish *Rasbora daniconius*, length 7-9 cm and weight 8-10 gm, were collected from Urmodi river near Satara. The fishes were acclimatized in laboratory condition for 10 days in rectangular glass aquarium and abundant dechlorinated water is used and proper aeration. The fishes were fed twice a day and change the water once a day during acclimatization.

The Carbendazim (97%) fungicide were collected from Sigma-Aldrich chemical Pvt. Ltd., India.

Estimation of acid phosphatase activity

After acute exposure period, the fishes were sacrificed and separated fresh tissue viz. liver and muscle. The estimation of Acid Phosphatase activity was carried by Linhardt and Walter [12] method using as a substrate P-nitrophenyl phosphate. The optical density was measured at 400 nm by UV spectrophotometer using blank. The ACP activity was calculated in term of µ mols of P-nitrophenol/mg protein.

The formula of ACP activity = Optical density × 2.76 ×1000/ amount of protein/ mg tissue.

Statistical analysis

In the present study, the data obtained from each group was expressed as an arithmetic mean ± S.D. The statistically analyzed values were calculated by using student 't' test.

RESULTS AND DISCUSSION

The (Table 1) exhibit the effect of carbendazim fungicide on the acid phosphatase activity in liver and muscle of the fish *Rasbora daniconius* in the control group, LC_0 and LC_{50} concentration group after acute exposure (96 hours) and graphically represented in (Fig 1). The ACP activities were higher in Liver than the Muscle. In control group fishes, 5.0341 µ mole PNP/ mg protein/hr of the liver, however, in LC_0 concentration group fish liver exhibited 4.89004 µ mole PNP/ mg protein/hr and in the LC_{50} concentration group fish showed 4.53658 µ mole PNP/ mg protein/hr of liver. The muscle of control group fish exhibited 2.9962 µ mole PNP/ mg protein/hr. While, in the LC_0 concentration group fish showed 2.6138 µ mole PNP/ mg protein/hr of muscle and in the LC_{50} concentration group was 2.47132 µ mole PNP/ mg protein/hr of muscle. There was a significant depletion in ACP activity of the liver and muscle tissues of LC_0 and LC_{50} concentration group as compared to control group after acute exposure of Carbendazim fungicide.

Table 1 Effect of carbendazim on acid phosphatase activity in liver and muscle tissue of the fish *Rasbora daniconius* after acute exposure (µ mole PNP/ mg protein /hr)

Tissue	Liver (Mean ± SD)	Muscle (Mean ± SD)
Control	5.0341 ±0.18692	2.9962 ±0.38192
LC_0	4.89004 ±0.24333	2.6138 ±0.33049
(p-value)	0.1548	0.1111
	NS	NS
LC_{50}	4.53658 ±0.35143	2.47132 ±0.33477
(p-value)	0.01587	0.02778
	*	*

(Values expressed is an average of five animals ± SD) NS = Not Significant; * = P-value < 0.05; ** = P-value < 0.01; *** = P-value < 0.001

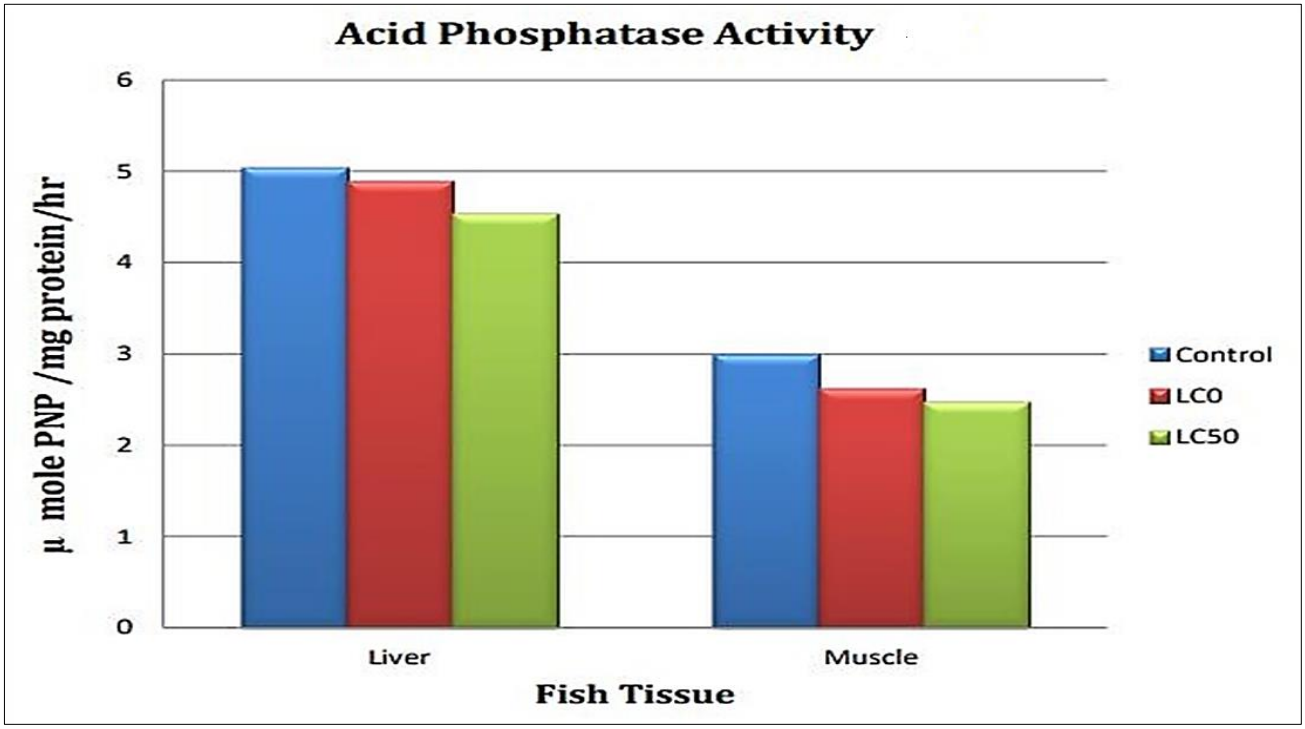

Fig 1 Shows carbendazim induced changes in acid phosphatase activity in liver and muscle tissue of the fish *Rasbora daniconius* after acute exposure

Acid phosphatase and alkaline phosphatase are enzymes found in the plasma membrane that aid in cytolysis and differentiation [13]. ACP is a marker enzyme for detecting lysosomes in cell fractions, and its activity can be influenced by xenobiotics [9]. Because of the potential toxicity and genotoxicity caused by contaminants, acid phosphatases are required biological biomarkers [14]. Phosphatases are often considered to be excellent markers of stress in the biological system [15]. Acid phosphatases are hydrolytic lysosomal enzymes that are released by lysosomes for the hydrolysis of foreign material, and hence they play a role in the detoxification of some poisons. According to Sherekar and Kulkurni [16] the

substantial rise in protease activity could be due to disruption to the lysosomal membrane, allow the lysosomal enzyme to leak into the cytosol. Uncoupling of oxidative phosphorylation is also thought to be the main cause of acid phosphatase inhibition, according to Dalela *et al*. [17]. Decrease or increase in the enzyme activity represents the stress in any organism that results in metabolic burden.

In the present investigation, the acute exposure (96 hours) of Carbendazim at LC_0 and LC_{50} concentration showed a significant decrease in the ACP activity of liver and muscle tissue of freshwater fish *Rasbora daniconius* as shown in (Table 1). The ACP activity significantly decreased at LC_0 & LC_{50} concentration group as compared to the control group. The resultant decrease is attributed due to damage of the lysosomal membrane after exposure to Carbendazim. Similar results were also observed by Mir *et al*. [18] observed decrease in ACP activity in the liver & kidney after exposing *Labeo rohita* to heavy metal concentration. Magar and Shaikh [19] reported decrease in ACP activity in the liver and muscle after exposure *Channa punctatus* to sub-lethal concentrations of Malathion for 4 days. Humtsoe *et al*. [20] studied the effect of Arsenic in the phosphatase activity of *Labeo rohita* and concluded that the ACP activity decreased in both experimental groups compared to control group.

Observations of the present work are correlation with Palanisamy *et al*. [21] noticed significant decrease ACP activity was observed in gill and air bladder tissue of catfish *Mystus cavasius* exposed to electroplating industrial effluent Chromium. Binukumari and Manimegalai [22] studied the exposure of Quinalphos leads to significantly decreased ACP activity upto 20 days in various tissue of fresh water fish *Labeo rohita*. Verma *et al*. [8] Observed inhibition in ACP activity in different tissue of fish *Notopterus notopterus* on exposure both separately chemicals Phenol and Dinitrophenol. Similar observations were reported in Zebra fish (*Danio rerio*) in chronic exposure to Azoxystrobin has altered ACP activity in various tissue by Hazira *et al*. [23]. The significant decline in ACP activity in various tissue of fish *Catla catla* on exposure to sublethal concentrations of heavy metal chromium by Suresh *et al*. [24]. On the contrary, rising levels of ACP the muscle, liver and intestine of *Cirrhinus mrigala* exposed to Quinalphos by Nair and Radha [25]. The decrease in ACP activity was observed in intestine, liver and muscle of fish *Cirrhinus mrigala* exposed to organic solvent methanol by Desai *et al*. [26]. Deshpande *et al*. [27] studied the activity of acid phosphatises inhibited significantly due to sublethal concentration of synthetic pyrethroid Fenvalerate and Cypermethrine in liver and intestine of fresh water fish *Labeo rohita*. Changes in enzyme activity cause a severe metabolic burden in fish [28].

CONCLUSION

In present investigation it has been concluded that after acute exposure the ACP activities decrease in liver and muscle tissue of the fish *Rasbora daniconius* exposed to Carbendazim acts on lysosomal membrane. The percent depletion was highest in the liver than muscle.

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