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Research Article

EFFECT OF COPPER AND MORINGA DIET ON SURVIVAL AND BEHAVIOURS IN *CYPRINUS CARPIO*

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ABSTRACT

The effect of copper toxicity and *Moringa* diets on survival and behavioural responses in fresh water fish *Cyprinus carpio*, was studied for 96 hr LC_{50} as a function of body size groups. The 96 hr LC_{50} value of *C. carpio* fed with control diet was low as compared to fish pre-fed with *Moringa* diets (25 and 50%). LC_{50} value was low in small size group as compared to large size group. However, the LC_{50} value in relation to unit weight was high in small size group (0.99 ppm) in relation to large size (0.35 ppm). It showed that, copper elicited the three times more toxicity in small size groups than in large size group fed with control diet; however it was reduced to two times in *Moringa* fed diets groups. The 96 hr LC_{50} value increased in Cu exposed fish pre-fed with *Moringa* diets; however, it responded well in large size group as compared to small size group of *C. carpio.* Hence the present study, *Moringa* diet reduced the copper toxicity and thereby increase the 96 hr LC_{50} value in copper exposed *C. carpio* pre-fed with *Moringa* diets.

Keywords: *Cyprinus carpio*, *Moringa*, Copper toxicity, LC_{50}, Size groups.

INTRODUCTION

Fishes are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic system (Farkas *et al.,* 2002). Copper occurs in the environment and are toxic to fish (James *et al.,* 1992; James, 2011). The reduction of toxic elements in aquatic systems by acceptable methods is needed urgently (Boyd, 1990; James *et al.,* 2004). Researchers have reported the therapeutic effects of *Moringa* leaf as a growth promoter, and booster of immune system in animals including fishes (Fahey *et al.,* 2005). So far *Moringa* leaf is known for its nutritive value only and its role in alleviating metal toxicity in fishes and other cultivate organisms are completely unexplored. Hence the present study has been undertaken to study the impact of *Moringa* leaf diets on the reduction of copper toxicity on behavioural responses and enhancement of survival in common carp, *Cyprinus carpio* as a function of body size groups.

MATERIALS AND METHODS

Experimental fish *C. carpio* were collected from Rajan Fish Farm, Vellanguli, Tirunelveli district, Tamil Nadu. They were acclimatized to the ambient laboratory conditions for 3 weeks. The water was changed daily and fish were fed *ad libitum* with pelletized diet containing 35% protein. The water used was clean and unchlorinated. Static renewable bio-assay method (Sprague, 1973) was adopted to determine the LC_{50} value of copper for 96 hrs. Stock solution of copper was prepared by dissolving 3.93 g of Analar grade $CuSO_4$ (MERCK) in 1 *l* of distilled water and then diluted with fresh water to obtain the desired concentrations.

Seven hundred and twenty individuals of small (0.90 ± 0.14 g) and large (4.15 ± 0.34 g) sized groups of *C. carpio* were separately selected from the stock tanks. Each size group is further divided into three groups and separately fed *ad libitum* with control diet, 25 and 50% *Moringa* leaf diets respectively for 30 days. Thereafter, each sub-groups

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were separately exposed to toxic concentrations of copper (small size: 0, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1 and 1.2 ppm; large size: 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.4 ppm) separately for 96 hrs. Duplicates were maintained for each sub-group. One day prior to the experiment and throughout the bio-assay test, the fish were starved. The experiment was conducted in plastic trough containing 10 *l* of freshwater with various toxic concentrations of copper. The medium was changed daily to maintain constant toxicant (Sprague, 1971). Control group was also maintained in freshwater. During the bio-assay test, the behavioural changes of fish were observed and dead fish were removed immediately from the test media. Profit analysis was followed for the calculation of 96 hr LC_{50} value, its 95% confidence limits and slope function(s) (Litchfield and Wilcoxon, 1949).

RESULTS

The chosen size groups of C. carpio behaved abruptedly when they were exposed to various toxic concentrations of copper. When test animals were exposed to copper, C. carpio showed an avoidance response even at low concentrations. At lethal concentrations, they tried to avoid the toxicant by irregular erratic swimming, jerky movements, rapid opercular movements, restlessness, frequent surfacing (4 and 3 times / min in small and large groups) and upside-down surface movement (3 times / min in both groups). Finally, they lost their equilibrium and settled at the bottom before death. The dead animals showed blood clots on the gill surface and widely opened mouth and gills. The observation revealed that, mortality of *C. carpio* dependent on the toxic concentrations of copper and duration of the exposure. It was more pronounced in small size group as compared to large size group; while it was completely absent in the control fish.

The 96 hr LC_{50} value of copper for small and large size groups of *C. carpio* fed with control diet was 0.89 and 1.46 ppm respectively (Figure 1-2). The slope function calculated for small and large size groups of *C. carpio* was 1.25 and 2.29 ppm respectively. A positive correlation co-efficient was obtained for the relationship between the copper concentrations and per-cent mortality and it was statistically significant for both size groups ($P < 0.01$) of *C. carpio* exposed to toxic levels of copper (Figure 1-2). The relationship between the toxic concentrations of copper and per-cent mortality of small and large size groups of *C. carpio* expressed by the regression lines ($Y = a+bx$) were $Y = -91.68 + 154.79\ x$ and $Y = -3.30 + 36.25\ x$ respectively. The 'b' value obtained for small size group was 155 and it significantly declined to 36 in large size group of *C. carpio* exposed to copper levels (Figure 1-2). It showed that 'b' value obtained for small size group was five times high as compared to large size group.

The 96 hr LC_{50} value obtained for fish pre-fed with *Moringa* diets (25 and 50%) was similar to those of control fish. However, 96 hr LC_{50} value and other related values were improved in *Moringa* pre-fed *C. carpio* as compared to 96 hr LC_{50} value of fish fed with control diet. For instance, 96 hr LC_{50} value in small and large size groups of fish pre-fed with 25% *Moringa* diet was 0.96 and 1.94 ppm respectively. Similarly, it was 1.01 and 2.27 ppm in copper exposed fish pre-fed with 50% *Moringa* diet (Table 1).

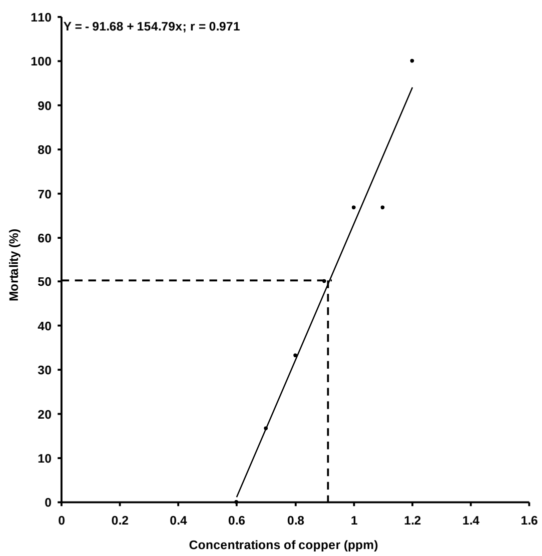

Figure 1. Effect of copper concentrations on per-cent mortality in small size group of *Cyprinus carpio* exposed for 96 hr.

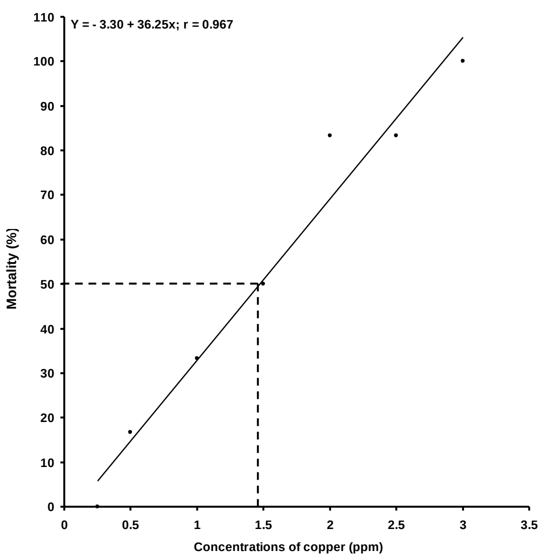

Figure 2. Effect of copper concentrations on per-cent mortality in large size group of *Cyprinus carpio* exposed for 96 hr.

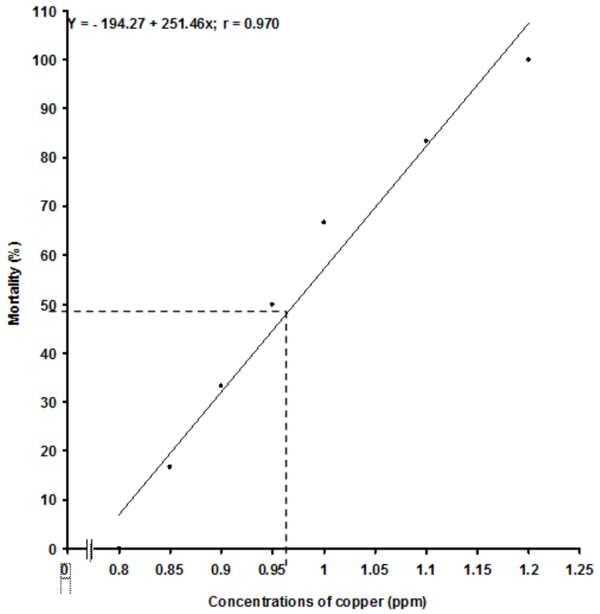

Figure 3. Effect of copper concentrations on per-cent mortality in 25% Moringa diet treated small size group of *Cyprinus carpio* exposed for 96 hr.

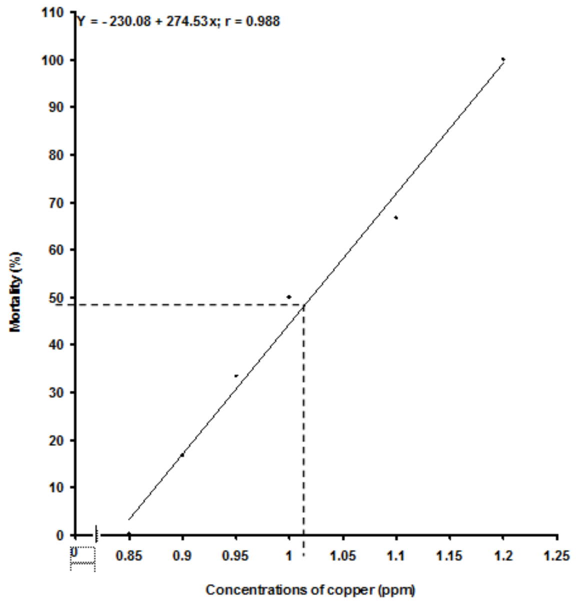

Figure 4. Effect of copper concentrations on per-cent mortality in 50% Moringa diet treated small size group of *Cyprinus carpio* exposed for 96 hr.

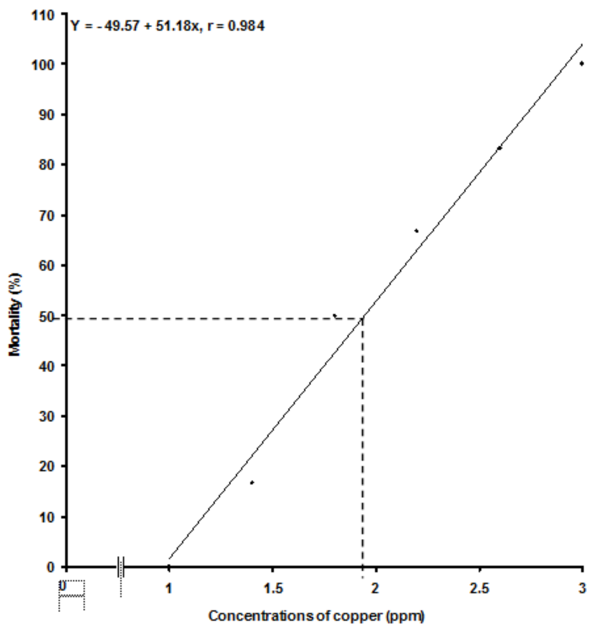

Figure 5. Effect of copper concentrations on per-cent mortality in 25% Moringa diet treated large size group of *Cyprinus carpio* exposed for 96 hr.

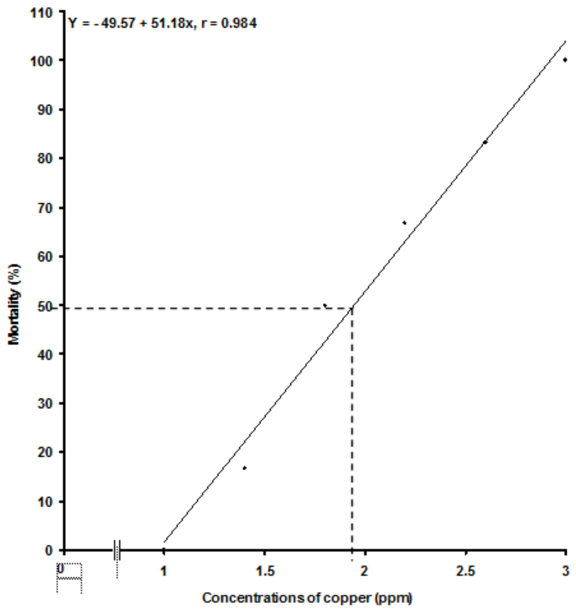

Figure 6. Effect of copper concentrations on per-cent mortality in 50% Moringa diet treated large size group of *Cyprinus carpio* exposed for 96 hr.

Table 1. Effect of copper toxicity on per-cent median lethal levels (LC_{50}) and slope function in *Cyprinus carpio* as a function of body size and pre-fed *Moringa* diets.

Groups	Lethal concentration (ppm)			LC_{50} in relation to unit weight	Slope value
	16%	50%	84%		
Small size group					
Control diet	0.70	0.89	1.08	0.99	1.25
Pre-fed with 25% of *Moringa* diet	0.85	0.96	1.07	1.07	1.12
Pre-fed with 50% of *Moringa* diet	0.91	1.01	1.12	1.12	1.11
Large size group					
Control diet	0.50	1.46	2.42	0.35	2.29
Pre-fed with 25% of *Moringa* diet	1.40	1.94	2.48	0.47	1.33
Pre-fed with 50% of *Moringa* diet	1.64	2.27	2.90	0.55	1.32

DISCUSSION

The behavioural studies revealed that, mortality of *C. carpio* related to the concentrations of copper and duration of exposure. Heavy metals in general cause inhibition of mitochondrial enzymes (Hingorani *et al.,* 1973) and ATP ase (Farmanfarmaian *et al.,* 1980) resulting the decline of cellular respiration and death of animals (Agarwal, 1991). *C. carpio* showed rapid movement initially when exposed to tested concentrations and a subsequent reduction in these activities while increasing the concentrations of copper or the duration of exposure. The hyperactivity in the initial period indicates an avoidance behavior of the fish due to metal stress (James, 1990). Moreover, a concentration and duration based reduction in these activities was a sign of physiological adjustment (Verma *et al.,* 1979).

The present study reveals that, the secretion of mucous was regarded as a defense and excretory response (Bennett and Dooley, 1982), which might help in protecting gills and skin from heavy metal toxicity. Irregular, erratic and jerky movements were observed in the fish exposed to copper and these abnormal behaviors are certainly a sign of metal toxicity. The erratic swimming and upside-down movement of copper exposed fish indicates that, the region in the brain which is associated with maintenance of equilibrium should have been affected under the metal toxicity.

The peculiar behavior of surfacing frequency was more frequent in *C. carpio* exposed to copper at high concentrations as compared to less toxic concentrations tested. The abnormal behaviours of the fish which is greater in small size group as compared to large size group. Also, the above mentioned abnormal behaviours were less in copper exposed *C. carpio* pre-fed with *Moringa* diets (25 and 50%) as compared to *C. carpio* fed with control diet. Sharma and Sharma (2005) reported that *Spirulina* added feed improved the tolerance of *Poecilia reticulate* when exposed to an azo dye methyl red by considerable reduction in cytotoxic effects on RBC's count at higher concentrations of dye. The dietary ascorbic acid reduced the toxicity of water borne copper in trout, *Salmo gairdneri* and carp by preventing copper accumulation in tissues. It is likely that, pre-fed dietary *Moringa* may also reduce the metal toxicity in tissues and protect *C. carpio* from copper toxicity and there by occurrence of abnormal behaviours were minimal as compared to copper exposed fish received control diet.

The results of the present study also showed that, small and large size groups of *C. carpio* fed with control diet (without *Moringa* diet) was less tolerant or more sensitive to copper toxicity than those pre-fed with 25 and 50% *Moringa* diet. It was confirmed with the 96 hr LC_{50} value arrived in small and large size groups of *C. carpio* fed without *Moringa* and with *Moringa* diets (See Table 1). It was evidently showed that, *Moringa* diet reduced the copper toxicity which in turn enhanced the 96 hr LC_{50} value and it was more pronounced in fish fed with 50% *Moringa* diet as compared to 25% diet. Vinodini *et al.* (2014) reported that pre-treatment with *M. oleifera* leaf extract in cadmium exposed rats showed a significant decrease in the levels of liver enzymes alanine aminotransferase (ALT) and asparate aminotransferase (AST) as compared with the cadmium alone treated rats. Therefore, they suggested that, pretreatment with *M. oleifera* leaf extract alters the levels of the liver enzymes and hence *Moringa* diet can improve the liver functions in cadmium chloride-induced rats. This study supports the present investigations. The *Moringa* leaves are also used as nutritional supplement and growth parameters due to the significant presence of protein, P, Ca, selenium, β-carotene and α-tocopherol (Sanchez *et al.,* 2009). β-carotene of

Spirulina maintains the mucous membrane firmly (Henrikson, 1994) and thereby prevents the entry of toxic element into the body. Chlorophyll of *Spirulina* acts as a cleansing and detoxifying factor against the toxic substances (Henrikson, 1994). James (2011) found that dietary supplementation of 75 and 225 mg selenium per kg diet required to reduce the copper and cadmium toxicity when the metals existed individually in the environment. Also, they suggested that, the protective role of selenium against metals toxicity in carp, *Cirrhinus mrigala*. It is likely that, presence of selenium and β-carotene in the *Moringa* diet reduced the copper toxicity and thereby increase the 96 hr LC_{50} value in copper exposed *C. carpio* pre-fed with *Moringa* diet.

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