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TOXIC EFFECTS OF A SYNTHETIC FUNGICIDE TEBUCONAZOLE ON SURVIVAL AND BEHAVIOUR OF A FRESH WATER FISH, *CYPRINUS CARPIO*

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Abstract: Tebuconazole is a systemic fungicide used in agriculture to control rust and mildew on fruits, vegetables and seeds. Fungicides affect a number of physiological potential of non-target organisms. The present investigation were conducted to determine toxic effects of tebuconazole with reference to the survival and behaviour on the fresh water fish, *Cyprinus carpio*. At a concentration of 47 ppm tebuconazole caused 100% mortality within 24 h. The 24 h LC50 for the pesticide exposure was 45.6 ppm. At a concentration of 32 ppm and below tebuconazole caused no mortality (sub-lethal concentration). The sub-lethal concentrations of the fungicide increases the total glucose (52.34%) and decreases the total protein (13%) in the muscles of *C. carpio*. The sublethal concentrations of the fungicides decreases the opercular beats, while a gradual increase were noticed at lethal concentrations. The result of the present study indicates that tebuconazole exerts toxic effect on fish and show quick and lethal response to this toxicant. Thus the use of tebuconazole should be properly and strictly controlled and regulated by appropriate legislation in order to prevent bioaccumulation in the environment, aquatic animals and ultimately to the human beings.

Key words: *Cyprinus carpio*, Tebuconazole, Sub-lethal concentration.

INTRODUCTION

Fisheries and aquatic resources are exceptionally valuable natural assets enjoyed by millions of people. The vigorous development of industries especially the chemical industry and the widespread use of chemicals in agriculture, forestry has inevitably resulted in the pollution of water bodies (Louis *et al*., 2009). Insect pest control, an essential component of crop protection and public health, has evolved over a recorded history of three millennia (Casida and Quistad, 1998). They have a profound effect on aquatic life and water quality. Most of these pesticides are discharged into the soil and aquatic system and found to be highly toxic to many organisms in the ecosystem (Frid *et al*., 2003). The main target for these chemical compounds is the insect nervous system, at a very limited number of primary points: inhibition of alpha-lanosterol demethylase which decreases ergosterol biosynthesis for fungicides (Graf, 1993). The pesticide pollution is also known to affect a number of physiological, biochemical and reproductive potential of non-target organisms. Literature review reveals that little work has been carreid out on the toxicity of this fungicide (Crofton, 1996; Narotsky and Kavlock, 1995; Toni *et al*., 2011). The present investigation is an attempt to study the effects of a fungicide, Tebuconazole on *Cyprinus carpio*.

MATERIALS AND METHODS

Experimental organism

Healthy juveniles (3.11 ± 0.87 cm) of *C. carpio* were collected from hatchery and acclimated to laboratory conditions. They were fed with artificial feed once in a day and faecal matters were removed daily without disturbing the fishes. Desired concentrations of tebuconazole (30, 34, 38, 42, 46 and 50 ppm) were prepared by dissolving in 1 liter of water. The mortality was noted for each concentrations at 24 hour after pesticide treatment (n=5) and the mean value was taken for probit analysis using SPSS software (version 10). The effects of lethal (32 ppm), median lethal (45.6 ppm) and lethal (47 ppm) concentrations of tebuconazole in the opercular beat, swimming behavior of *C. carpio* were studied during first, second and third half hour of exposure. The estimation of muscle

glucose (Nelson, 1944; Somogyi, 1952), and total muscle protein (Lowry, 1951) was carried out at 24 hr after the application of sub-lethal of concentration of tebuconazole.

RESULTS AND DISCUSSION

The percentage of mortality of the fish when exposed to various concentrations of tebuconazole as a function of time was shown in Table 1. At concentration of 47.0 ppm tebuconazole caused 100% mortality, within 24 hours of exposure (acute toxicity). The exposure of tebuconazole on *C. carpio* showed some deleterious toxic effect on its survival. The LC_{50}, the median lethal concentration which kills 50% of the test individuals during 24 hours of exposure, was 45.6 ppm. At concentration 32 ppm and below no mortality was observed (sub lethal concentration). The reported LOELs (lowest observable effect levels) ranged from 50 to 60

Table 1. Effect of different concentrations of tebuconazole on the mortality of *C. carpio* as a function of 24 hours

Concentration (ppm)	Percentage of mortality (%)
32.0	0
41.5	25
45.6	50
46.5	75
47.0	100

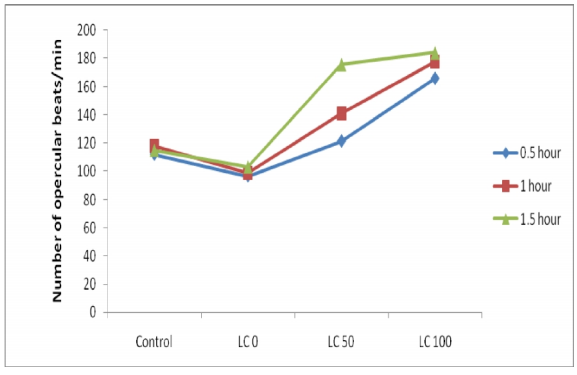

Fig 1. Effect of different concentrations of tebuconazole on the rate of opercular beats of the fish, *C. carpio*. Each value represents the average performance of an individual over the range of 1.5 hours

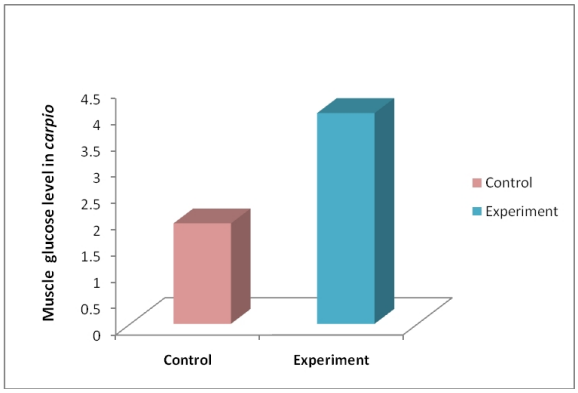

Fig 2. Effect of different concentrations of tebuconazole on the mortality of *C. carpio* as a function of 24 hours

mg/kg/day (USEPA, 2000). In the present investigation the lethal concentrations (LD_{50} and LD_{100}) are 45.6 and 46 ppm respectively. The reported LD_{50} in rats is quite high (4 g/kg), but systemic toxicity, teratogenicity, and developmental toxicity has been reported at doses as low as 50 mg/kg/day (USEPA, 2000). Thus, the present data agree with the effective dose range.

When the *C. carpio* was transferred from fresh water to different concentration of fungicides, showed marked variation in opercular beat (Fig. 1). The opercular beat increased at lethal concentrations. The opercular beat was decreased (96.6 ± 3.40) within half hour and showed marked increase (103.0 ± 3.08) after sub lethal exposure. The opercular beat showed marked increase (175.8 ± 7.79 and 184.2 ± 4.30) at median lethal concentration and acute lethal concentration respectively whereas the average opercular beats in control fishes were 115.4 ± 2.19/ min. within 1.5 hours. A survey of literature on fish and pesticide pollution clearly indicates that some pesticides increase respiratory metabolism (Holden, 1973).

At lethal concentrations they showed less active movement for half an hour, and then become hyperactive. The breath rate becomes very high. As soon as the fishes were transferred from fresh water to different concentrations of tebuconazole, a marked increase in swimming activity was observed. At sublethal concentrations they showed only a slight increase in swimming activity compared to

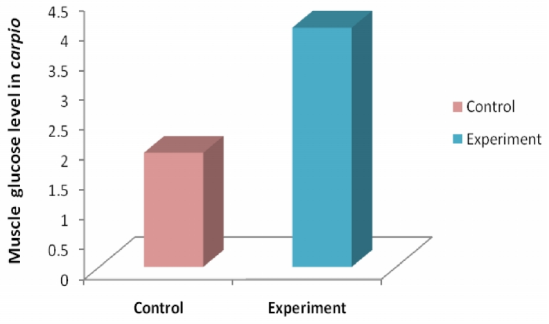

Fig. 3. Effect of sublethal concentrations of tebuconazole in the levels of muscle protein (mg/gm) of the fish, C. *carpio*

control. At higher concentrations of tebucona zole the fishes showed erratic vertical swimming, resting at bottom, rapid eye movement and sometimes lost the sense of direction and balance compared to the normal. The effect has been also reported on operculum beat (Chindah *et al.*, 2004) was similar in tebuconazole exposed fishes. As the fish came in contact with pesticide restlessness was observed and supplemented by sudden rapid movement in circles (Patnaik and Patra, 2006) was also observed in the present study. The behavioral changes observed in fish during this study may be due to the effects of tebuconazole on cholinesterase activity in various parts of nervous system. When *C. carpio* were exposed to acute toxicity of dimethoate, the test fish exhibited erratic swimming, increased surfacing, decreased rate of opercular movement, copious mucous secretion, reduced agility and inability to maintain normal posture and balance with increasing exposure time (Singh and Narain, 1982).

The total glucose in the muscles of treated and control fishes are presented in Fig. 2. The result indicates a 52.34% increase in total glucose in the muscles of fish at 24 hour after tebuconazole application. The changes in the amount of glucose observed in the fish when exposed to sublethal concentration of tebuconazole demonstrate a mechanism similar to that of stress. Similar results were found in zebra fish (Sancho *et al.,* 2008), produced hyperglycemia. The protein in the muscle of treated and control fishes are presented in table 3. The result indicate a 13% reduction in total protein in the muscle of fish at 24 hour after tebuconazole application. A decrease in protein content was also reported in *Mystus vittatus* after pesticide exposure (Palanichamy *et al.*, 1989). Rath and Mishra (1980) have also reported a reduction in protein content in *Tlapia mossambica* exposed to pesticide. Moreover reduction in the free aminoacids after pesticide treatment has been reported (Binitha *et al.*, 2009), which may results an imbalance in the uptake and utilization of protein within 24 hour after treatment. The decreased opercular movement after application of sublethal concentration of tebuconazole indicates that the fish adaptively decrease opercular movement to decrease its oxygen uptake to meet the pesticide stress. On the other hand, higher concentration of this pesticide caused excitatory effect on opercular movement even from the half hour of exposure. The result of the present study indicates that tebuconazole exerts toxic effect on fish and show quick and lethal response to this toxicant. Thus the use of tebuconazole should be properly and strictly controlled and regulated by appropriate legislation in order to prevent its bioaccumulation in the environment, aquatic animals and ultimately to the human being.

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