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The curative role of vitamin c on alteration in behavioral and morphological characteristics of cyprinus carpio fish after exposed to Malathion

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Abstract
This study aims to determine of malathion effects in behavior, Feeding and morphological deformation of *Cyprinus carpio* and protective activity of vitamin C in reduction toxicity**.** Common carp *C. carpio* (total n= 75) were exposed to different concentrations (0, 1, 3, 6 and 10 mg/L) of malathion (EC 57) for 28 days. The median lethal concentration (LC50) for 96 hours was computed by the probit method, and was found to be 2.04 mg/L. The fish were exposed to 0.5 mg/L (approximately 1/4 of the 96-h LC_{50}) malathion. A total number of 108 were allocated into six treatment groups (with three replicates): group 1 as control; group2 malathion (0.5 mg/L), group3 malathion (0.5 mg/L) plus vitamin C (300 mg/kg in diet), group4 malathion (0.5 mg/L) plus vitamin C (1000 mg/kg in diet), group5 vitamin C (300 mg/kg in diet) and group6 vitamin C (1000 mg/kg in diet). The experiment was carried on for 28 days. The behavioral changes and morphological deformation were recorded. Fish in malathion groups showed irregular swimming, loss of equilibrium. The main morphological changes were caudal bending. However, this toxic effect was neutralized by the administration of ascorbic acid. Thus, the present results suggest that simultaneous treatment with ascorbic acid may alleviate malathion-induced oxidative stress.

Keywords: Malathion, organophosphate pesticides, cyprinus carpio oxidative stress, reactive oxygen species, vitamin c

Introduction
Malathion is an organophosphate insecticide, it has been used in agriculture to improve food production via eliminating unwanted pests and controlling disease vectors. (Srivastava *et al*., 2012) [26].
Organophosphorus insecticides exert their biological effects through electrophilic attack on the cellular constituents of hepatic and brain tissues (Samanta and Chainy, 1995) [25]
The production of ROS is to be caused by a mechanism in which pollution may produce oxidative stress and induce various tissue damage i.e., liver, kidney and brain (Dwivedi *et al*., 1998; Oncu *et al*., 2002; Yu *et al*., 2008) [8, 19, 29]. pesticide toxicity in fish may be related to increased reactive oxygen species (ROS) production (Yonar and Sakin, 2011) [28]. ROS production can induce oxidative damage in aquatic organisms.
Oxidative stress occurs when the production of ROS overrides the antioxidant capacity in the target cell, resulting in the damage of macromolecules such as nucleic acids, lipids and proteins causing alterations in the target cell function and leading to cell death (Bachowski *et al*., 1997) [2].
Contaminant induced neurotoxicity can produce a variety of effects encompassing the altered behaviour. Changes in behaviour are among commonly used biomarkers having potential to link biochemical effects to ecological outcomes of environmental pollution (Dogan, 2017) [7].
The activity of acetylcholinesterase (AChE) which is involved in the termination of impulse transmission by rapid hydrolysis of the neurotransmitter is one of the most widely used indicator of altered neural function. Pesticide evoked inhibition, mainly by organophosphates and carbamates, leads to changes in behaviour including reduced swimming performance, hyper excitability andaltered social behaviour (Colovic *et al*., 2013) [5].
Vitamin C (ascorbic acid) is one of the non-enzymatic antioxidant factors both in extracellular (interstitial and intercellular fluids) and intracellular fluids (cytosol), being able to neutralize many oxyradicals (Bigard, 2001) [4]. Vitamin C prevents the lipid peroxidation process through inhibiting reactive oxygen species in aqueous phase (Frei *et al*., 1988; Jialal *et al*., 1990) [10, 14].

Materials and methods
The fish used in the present study common carp (Cyprinus carpio), were obtained from local fish farm, juvenile fish, 131±2 gm in weight and 20±2 cm tall. Samples were transported to laboratory by using plastic tanks. The healthy fish were selected, while the weak and sick fish were rejected. The fish were being acclimated to the laboratory circumstances for 14 days in a dechlorinated and aerated freshwater that changed every 48 hours and were fed commercial fish food twice daily at 800 and 1600 hours. Before the initiation of the experiments. The water used was analyzed for temperature, dissolved oxygen, EC and pH.

Determination of the LC_{50}
Healthy and active specimens of C. carp (total n= 75). Malathion (57 EC, Topsin, China) was purchased from local retail pesticide shop. Different concentrations (0, 1, 3, 6 and 10 mg/L) of malathion were prepared by adding required volume from the stock solution prepared by diluting the original formulation. The entire experiment was independently repeated three times, and each replicate of each group contained six fish. Fish feeding was stopped during the experiment to minimize the effects of metabolic products. Malathion was added directly to the aquarium water two hours before the fish were placed for good spread

of the pesticide. Every 24 h the mortality count was monitored and dead fish were removed to avoid possible deterioration of the water quality (Gooley *et al*., 2000) [11] The lethal concentration (LC_{50}) for 96 h was computed by the probit method (Finney, 1971) [9].

Chronic toxicity

Healthy and active specimens of C. carp (total n= 108) were procured from a local fish farm. The length and weight of fish was 20±2 cm and 131±2 g, respectively. The fish were exposed to 0.5 mg/L (approximately 1/4 of the 96-h LC_{50}) malathion for 28 days. The entire experiment was three replicate, and each replicate of each group contained six fish. The fish were divided into six groups as follows:

Treatment 1: the control group, was maintained in tap water and received a commercial basal diet that did not contain ascorbic acid.
Treatment 2: were exposed to 0.5 mg/L malathion and received a commercial basal diet feed for 28 days.
Treatment 3: were exposed to 0.5 mg/L malathion and received a commercial basal diet enriched with 300 mg vitamin C per 1 kg diet for 28days.
Treatment 4: were exposed to 0.5 mg/L malathion and received a commercial basal diet enriched with 1000 mg vitamin C per 1 kg diet for 28 days.
Treatment 5: the specimens were fed with a diet enriched with 300 mg ascorbic acid per 1 kg diet for 28 days.
Treatment 6: the specimens were fed with a diet enriched with 1000 mg ascorbic acid per 1 kg diet for 28 days.
Replaced concentrations were doing every 48 hours.

Results

Median Lethal Concentration (LC_{50})

The experiment of calculating median lethal concentration (LC_{50}) of *C. Carpio* was carried out in 24, 48, 72 and 96 hours, and used different concentrations of malathion (0, 1, 3, 6 and 10 mg/L). The median lethal concentration (LC_{50}) for 96 h was computed by the probit method (Finney, 1971) [9], the log of concentration and probit unit shown in table (1).

Table 1: Median lethal concentration (LC_{50}) of malathion for *C. Carpio*

Concentration ppm	Log of Concentration	mortality rates%	mortality Probit
0	-	0	-
1	0	28	4.42
3	0.477	61	5.28
6	0.778	83	5.95
10	1	100	-

The lc_{50} was determined by Graph plotted for log of concentration versus % of mortality of *C. carpio* exposed to malathion then calculate the lc_{50} from the equation of straight-line figure (1).

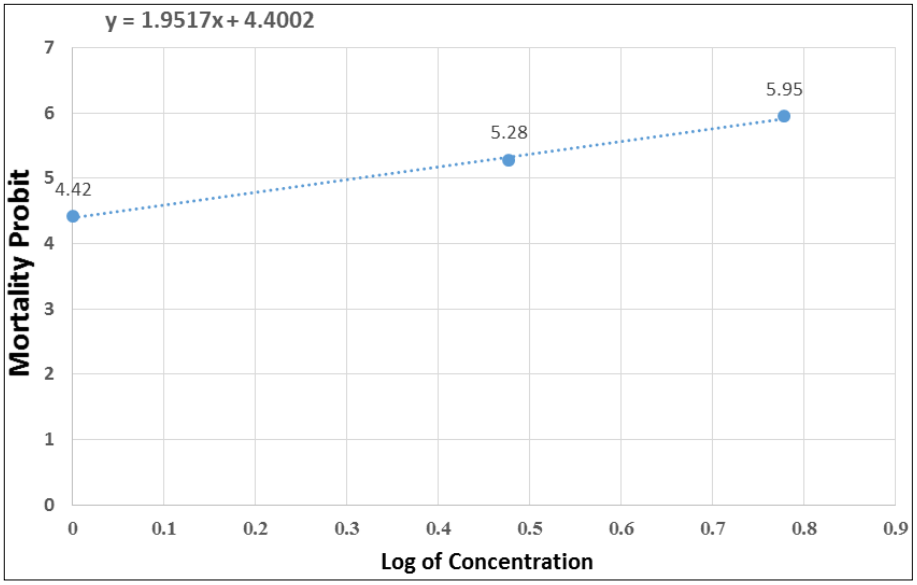

Fig 1: Plot of log-concentrations versus probits for calculation of LC_{50} of *C. carpio* exposure to malathion pesticide.

Behavioral and morphological changes

In treatment (2) when the fish is insert in the aquarium it shows an abnormal behavior to escape from the effect of the malathion pesticide. The fishes were agitation showing fast movement, move toward the surface, open their mouth and breath and later try to jump. After 96 hours The symptoms appeared on the fishes show lessen activity and began to swim irregularly as well as the rise and fall in the bottom of the aquarium and not response to external stimulant, in compare with treatment (1) control group the fish was swim in a regular circle way.

After 96 hours noticed on the fish secreted excess mucus all over the body. Feeding were affected and consumption of food in fish was impaired and reduced. After 14 days the caudal bending was noticed in which greatly retarded the normal swimming pattern figure (2). In treatment (3) the irregular swimming and difficult of breath were noticed in the first 96 hours but later the activity and swimming and

normal breath were almost near to control, the secretion of mucus was noticed in treatment (3) and (4) but less than treatment (2), the caudal bending was noticed in two fishes in treatment (3) and was absent in treatment (4), no notice in food consumption in these groups after 96 hours. In treatments (5) and (6) fish behavior was just like in treatment (1).

Fig 2: Caudal bending in Cyprinus carpio after exposed to a sublethal concentration of malathion.

Discussion

The variance in LC_{50} values in this study and the other studies was considered within the known differences that's ecological (physical and chemical properties), the type of water used in the experiments that have an effect on the toxicity of the pesticide to the fish, Also the period of exposure, fish size, fish's age, weight, length and hereditary content (Mitchell *et al*., 1987; Al-Atar, 1998) [17, 1]. All these factors could change the metabolism of the fish, stability of the pesticide and its presence in the water, dissolved substances in the water that decrease the pesticide concentration by adsorption process that lead to change the percent of taken pesticide by the fish (Murty,1988) [18].

Fish are relatively sensitive to changes in their environment and have a relatively long lifespan compared to other aquatic organisms. These animals can therefore give indication of the general health of the specific habitat or aquatic ecosystem in which they occurred.

In the present study the change in fish behaver after exposed to 0.5 mg/l malathion was and the irregular swinging and trying to escape from the effect of the pesticide that could be due to the shock caused by the pesticide stress to increase the airing of the gills, Similar, observations have also been made by Joseph *et al*, (1987) [15] and Lata *et al* (2001) [16], according to Sakshena and Parashari (1982) [24], the movement to the surface and breath of air is to recompense the oxygen lack from the medium to meet the extra energy to cope up the toxicity. Abnormal movements and loss of equilibrium, followed by hanging vertically in water are due to inhibition of AChE activity, leading to accumulation of acetylcholine in cholinergic synapses ensuing hyper stimulation, Inhibition of AChE activity is a typical characteristic of organophosphate compounds (Holmstedt, 1963; Padilla *et al*., 1996; Timchalk *et al.*, 2002) [13, 20, 27]. An excess secretion of mucus in fish forms a nonspecific response against toxicants, thereby probably reducing toxicant contact. It also forms a barrier between the body and the toxic medium, so as to minimize its irritating effect, or to scavenge it through epidermal mucus (Patil and David, 2010) [21]. Similar observations were made by Rao *et al*. (2003) [22] and Parma de Croux *et al*. (2002) [6]. Caudal bending is a sort of paralysis, which might be due to the inhibition of muscular AChE, resulting in blockage of neural transmissions. Bending of caudal base occurs because the caudal portion is the thinnest structure and can be conferred any sort of orientation due to paralysis of caudal musculature by AChE inhibition (Halappa and David, 2009) [12]. Decrease in appetite is a common response of fish to stress, and intermittence of feeding for longer periods can have a clear impact on growth and reproduction (Rice, 1990; Barbieri, 2007) [23, 3].

Conclusions

There was an effect on behavioral and morphological characteristics of *C. carpio* fish exposed to sub lethal concentration of Malathion pesticide. Results show their protective role of vitamin C. To reduce the effect of the Malathion pesticide.

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