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Short Communication

Acute Toxic Effect of Technical Grade Insecticides on Behavior, Catalase Activity and Total Protein Contents of Fish, *Ctenopharyngodon idella*

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ABSTRACT

The main objective of this experiment was to see the behaviour, catalase (CAT) activity and total protein contents (TPC) in *Ctenopharyngodon idella* under binary mixtures of insecticides viz. endosulfan(ES)+chlorpyrifos(CPF) and endosulfan(ES)+bifenthrin (BIF). The fish behaviour was observed from both treated and control groups. Fish under exposure of insecticides mixtures exhibit abnormal behaviour and tried to jump out from water, come to surface and gulp air, showed increased opercular movement and erratic movements, hyper-activity and swimming very fast. Various tissues such as hepatic, neural, renal, cardiac, gills and muscle tissues were studied for the determination of CAT activity and TPC after exposure of 24 and 96 h. Results showed that both treatments caused a significant ($P<0.01$) decline in CAT activity and TPC of *C. idella* as compared to control group. CAT activity and TPC decreased with the passage of time. Among both treatments, ES+CPF caused greater decline in CAT activity and TPC. It was concluded that insecticides in mixture form are toxic to fish and response of biomarkers depends on the type of toxicants and exposure duration.

As the world population continues to grow, pollution becoming an ever-growing problem. Pollution deteriorates the environment and drastically affects the organisms. Pesticides are used to protect the crops from pest. Through surface runoff these pesticides reached in water bodies and affect the non-target species. Fish are very sensitive to change in water quality parameters (Vinodhini and Narayanan, 2008). The environmental stress can induced the behavioral modifications in organisms that ultimately affect the survival of species. Behavioral alterations are important indicators of contaminated ecosystem. So, fish are biological indicators and shows susceptibility to aquatic ecosystem (Byrne and O'Halloran, 2001).

Chlorpyrifos is an organophosphate pesticide. It is frequently used to control insect pests in agriculture crops and most detected insecticide in aquatic ecosystem. It is reported to be involved in the mechanism of genotoxicity, impairment in heptic function and neurochemical changes (Slotkin *et al*., 2005). Endosulfan is an organochlorine pesticide and extensively used to control pests in different crops in Pakistan. It is investigated that endosulfan to be convoluted in different mechanisms like haematological and hepatic toxicity (Da-Cuna *et al*., 2011). Bifenthrin is detected in surface water bodies in areas which are extensively used for agricultural purposes. Bifenthrin is highly toxic to aquatic inhabitants particularly fish. It disrupts the Na^+-K^+ ATPase pump in nerve cells as well as other channels such as Ca^{2+} and Cl^- are also blocked. Mixtures of contaminants cause enormous toxicity which ultimately affect the survival of species (Fernandez-Alba *et al*., 2001).

Xenobiotics exposure results in the production of ROS (reactive oxygen species). An imbalance in the ratio of pro-oxidants and antioxidants cause oxidative stress. Pollutants like insecticides, heavy metals and herbicides are poisonous as they cause oxidative stress in aquatic biota by the formation of ROS. In aquatic species, catalase (CAT) is very imported antioxidant enzyme; its activity fluctuate according to oxidative potential (Roberts and Oris, 2004; Pi *et al*., 2010). CAT being antioxidant enzyme removes

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ROS which affect the different biological membranes and inactivate different enzymes. In fish, the activity of CAT is considered as a sensitive indicator of oxidative stress. In bony fishes, different tissues as hepatic, gills, muscle and renal tissues are frequently employed in ecological, toxicological and pathological studies as these tissues have high metabolic potential and can amass contaminants (Terra *et al*., 2008). The aim of this research work was to see the behavior, CAT activity and total protein contents of *Ctenopharyngodon idella* exposed to binary mixtures of pesticides.

Materials and methods

The experiment was carried out in the Toxicology laboratory at Fisheries Research Farms, University of Agriculture, Faisalabad, Pakistan. The experimental fish *Ctenopharyngodon idella* (Age 90-day; Average weight, 9.39±0.19) were purchased from Fish Seed Hatchery, Faisalabad and kept under laboratory conditions for seven days. The chlorpyrifos (98% pure), bifenthrin (98.5% pure) and endosulfan (98.65% pure) of technical grade (Powder form 1 g) were dissolved in 95% analytical grade methanol (100ml) to prepare the individual insecticide solutions while insecticides mixtures (1:1 ratio) were prepared in deionized water by dilutions.

Fish were kept in binary mixture of insecticides as endosulfan (ES)+chlorpyrifos (CPF) and endosulfan(ES)+bifenthrin(BIF) for 4 days. The 96 h LC_{50} values of ES+BIF and ES+CPF were calculated as 4.23 and 4.60 μgL^{-1}, respectively (Ambreen and Javed, 2015). The each experiment was carried out with three replicates, separately, in 70-L glass aquaria having a number (n=10) of fish. Throughout the experiment the water pH, total hardness and temperature (Thermo-sensor) were maintained as 7.00±0.02, 222.30±0.90 mg L^{-1} and 27.01±0.03°C, respectively. Dissolved oxygen was monitored as 5.06±0.11 on daily basis.

The fish behavior was observed from control and treated (ES+CPF and ES+BIF) groups at interval of 12 hours. The changes in behavior were studied such as jumping, opercular movement, balance, movements during swimming and gulping of air.

After each 24 and 96 h interval the sampling was done and fish was scarified and required organs of grass carp were removed to determine the activity of CAT in them. The removed organs were weighted and rinsed with 0.2 M phosphate buffer (pH 6.5) to remove the RBCs. By using the blender it was homogenized in cold buffer (1:4 w/v). After that centrifugation of organ homogenates were carried out for fifteen minutes at 10,000 rpm and 4ºC. The upper liquid substance was separated and stored at cooler temperature of 80 ºC.

The CAT was studied in different organs viz. hepatic, neural, renal, cardiac, gills and muscle of fish by following the protocol of Chance and Mehaly (1997). Total protein contents were estimated in selected tissues by using the Biuret Method (1949).

Data were analyzed by appropriate method of Steel *et al*. (1996).Two-way ANOVA was used to determine the statistical difference between treated and control fish at P<0.05.MS excel was used to draw graph.

Results and discussion

The results showed that *C. idella* under exposure of binary mixture of pesticides showed abnormal behavior and tried to jump from test media and showing erratic and jerky movements to as well as variation in operculum movement was also observed (Fig. 1). The movement of operculum and body balance was maximum as the exposure was given but it declined as the duration of exposure increases. The comparisons among both the pesticides mixtures showed that ES+CPF treated fish have more pronounced indications as compared to ES+BIF treated fish. However, throughout the experiment control group showed no changes in behavior. No mortality was observed during experimental trail.

Behavioral changes in fish give us lot of information because any alteration in behaviour is correlated with physiological biomarker in aquatic environment. In the present research work, the effect of pesticides on fish behaviour was observed by loss of balance, increased

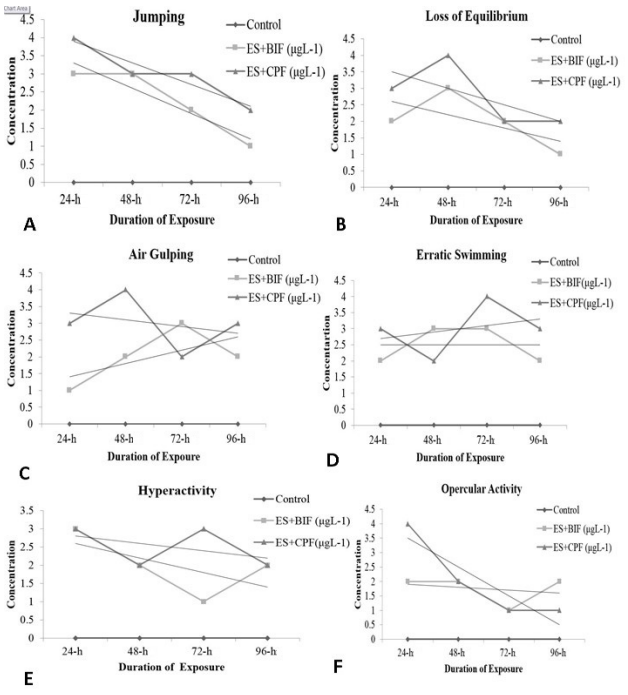

Fig. 1. Behavioral responses of fish against insecticide mixtures.

Table I. Acute effects of pesticides mixtures on CAT activity (U/mL) and protein contents (mg mL^{-1}) in different organs of grass carp.

Tissues	Treatments	CAT activity		Protein contents	
		24 h	96 h	24 h	96 h
Hepatic	Control	149.17±5.28A^{a}	149.14±4.40B^{a}	3.97±0.61A^{a}	3.95±0.56B^{a}
	ES+BIF	140.86±3.30A^{b}	105.86±3.71B^{b}	3.72±0.45A^{b}	2.31±0.33B^{b}
	ES+CPF	136.94±3.77A^{c}	99.82±2.72B^{c}	3.21±0.38A^{c}	1.98±0.21B^{c}
Gills	Control	138.12±4.55A^{a}	138.01±5.37B^{a}	2.86±0.31A^{a}	2.86±0.33B^{a}
	ES+BIF	131.92±3.59A^{b}	92.87±2.83B^{b}	2.75±0.32A^{b}	1.84±0.20B^{b}
	ES+CPF	125.67±3.91A^{c}	90.71±2.64B^{c}	2.53±0.25A^{c}	1.46±0.15B^{c}
Renal	Control	131.96±4.60A^{a}	131.67±4.49B^{a}	1.72±0.21A^{a}	1.73±0.28B^{a}
	ES+BIF	118.64±3.53A^{b}	83.72±2.50B^{b}	1.48±0.15A^{b}	0.67±0.05B^{b}
	ES+CPF	118.34±2.49A^{c}	80.58±1.40B^{c}	1.25±0.18A^{c}	0.27±0.04B^{c}
Neural	Control	124.56±3.32A^{a}	124.39±3.64B^{a}	1.25±0.18A^{a}	1.24±0.11B^{a}
	ES+BIF	110.90±2.11A^{b}	75.41±1.88B^{b}	1.02±0.12A^{b}	0.43±0.03B^{b}
	ES+CPF	105.92±2.48A^{c}	68.34±1.73B^{c}	0.91±0.09A^{c}	0.08±0.02B^{c}
Muscle	Control	113.21±3.21A^{a}	113.15±3.36B^{a}	1.03±0.10A^{a}	1.04±0.17B^{a}
	ES+BIF	99.76±3.22A^{b}	61.87±1.90B^{b}	0.96±0.05A^{b}	0.32±0.02B^{b}
	ES+CPF	83.28±2.75A^{c}	52.72±1.19B^{c}	0.80±0.05A^{c}	0.18±0.01B^{c}
Cardiac	Control	76.98±2.93A^{a}	76.54±2.04B^{a}	0.84±0.08A^{a}	0.86±0.12B^{a}
	ES+BIF	68.91±2.00A^{b}	29.73±1.76B^{b}	0.78±0.06A^{b}	0.24±0.02B^{b}
	ES+CPF	57.26±1.86A^{c}	32.91±1.38B^{c}	0.59±0.03A^{c}	0.03±0.01B^{c}

Capital letters represent the significant ($P<0.05$) difference among the treatments within the same column while small lettered superscripts show the difference between exposure duration within the same row.

opercular movement and erratic movement. Hii *et al.* (2007) reported the irregular behavioral patterns of fish Asian swamp eel after acute exposure to endosulfan. Marigouder *et al*. (2009) also observed the behavioral changes of *L. rohita*. *C. gariepinus* under exposure of several levels of chlorpyrifos showed inept behavior such as try to jump out of water, erratic and jerky swimming, frequent surfacing and gulping of air and large secretion of mucus on the gills and body followed by exhaustion and ultimately death (Nwani *et al*., 2013).

The activity of CAT and total protein contents were significantly decreased ($P<0.01$) in hepatic, renal, neural, cardiac, gills and muscle tissues of grass carp under exposure of binary mixture of pesticides as compared to control group. The activity of CAT was reduced with the passage of time. Among both the mixture, ES+CPF was found to be more toxic as it adversely affect the enzymatic activity as well as protein contents than ES+BIF mixture (Table I).

Mixture of pesticides disturbs the physiology of fish which ultimately leads to death. When the balance between oxidants and antioxidants is disrupted, oxidative stress occurs due to high formation of reactive oxygen species (ROS) which leads to breakdown of proteins, nucleic acid, lipids and carbohydrates finally damages the structure of cell. An alteration in cell structure indicates oxidative damage. However, organisms are endower with antioxidant enzyme system which neutralizes the effect of ROS (Achuba *et al*., 2003). Activation of antioxidant enzyme system is considered as warning signs of contamination. CAT is main antioxidant enzyme which is involved in detoxification of ROS and degradation of H_2O_2 to oxygen and water. The activity of CAT is controlled by various factors including unnecessary formation of O^{-2} (Pandey *et al*., 2001).

These findings were also supported by Ballesteros *et al*. (2009) who reported that CAT activity was significantly declined in hepatic, neural, gills and muscle tissues of *Jenynsia multidentata* under exposure of endosulfan for 24 h. Similarly, Sayeed *et al*. (2003) investigated the reduced CAT activity in neural, hepatic, renal and gill of *C. punctatus* after exposure to deltamethrin for 48 h. Decline in CAT activity ultimately declined the rate of reaction which resulted from the excessive production of hydrogen peroxide.

The TPC were significantly decreases as the duration of exposure increases. Our obtained results are similar to Jha and Verma (2002) who reported the decreased TPCs in tissues of *Clarias batrachus* under insecticides mixture (agrafun, endosulfan and malathion) for 96 h, 7, 14 and 21

day exposures. Singh *et al*. (2010) stated that after 24 hr exposure to cypermethrin there was a significant decrease in TPCs in muscle and liver of *Colisa fasciatus.* A decline in TPCs of *Danio rerio* exposed to λ-cyhalothrin was reported by Ahmad *et al*. (2012). According to Somaiah *et al*. (2014) phenthoate caused reduction in TPCs in several organs of *L. rohita*. In the same way, Jain (2014) observed a substantial decrease in total protein contents in the gills of *C. gachua* under exposure of methyl parathion. Ramesh *et al*. (2014) inspected that quinolphos altered the rate of metabolic reactions in various tissues of *C. carpio* by fluctuating the level of TPCs. Lihos exposure reduced the TPCs in neural, hepatic and renal tissues of *C. punctatus* (Naveed *et al*., 2010). According to Vega *et al*. (2002) a decline in tissues TPCs of fish may be due to compensation of stress.

Conclusion

The present study concluded that in aquatic ecosystem, pesticides not only affect the behaviour of fish as well as have negative effect on biochemical parameters. These biochemical parameters can also be used to assess the aquatic pollution.

Statement of conflict of interest

The authors declares there is no conflict of interest.

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