Research Article

Characterization of Catalase form Carnivorous Fish *Channa Striata* Exposed to Binary Insecticides Mixture (Deltamethrin + Endosulfan)

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Abstract | In this experiment toxic effect of deltamethrin(DM)+endoslfan(END) mixture on catalase (CAT) activity in gills of carnivorous fish C*hanna striata* was observed. Fish were exposed to sub-lethal concentration (1/3rd of LC_{50}) of DM+END mixture for 14-day. CAT was partially purified by using ammonium sulphate precipitation technique and kinetic characterization was also performed against different pH and temperature. CAT activity was measured spectrophotometrically at A_{240} nm. Results showed that highest CAT activity was noted from the crude extract of control fish gills (140.66±0.71UmL^{-1}) as compared to DM+END exposed fish (99.23±0.71UmL^{-1}). After desalted the CAT activity in gills of both control and insecticides exposed fish was calculated as 73.45±0.71 and 42.77±1.36UmL^{-1}, respectively. The highest specific activity was observed in crude CAT extract from gills of control fish (112.53±0.28 Umg^{-1}) as compared to exposed fish (82.69±1.12Umg^{-1}). After desalted specific activity of gills CAT was lower in exposed fish (112.20±1.41Umg^{-1}) in relation to control (170.80±1.41 Umg^{-1}). Highest fold purification of CAT was noted in the gills of control *C. striata* (1.52±0.01) as compared to exposed fish (1.48±0.01). The percentage recovery of CAT for control and DM+END exposed fish was calculated as 52.21±1.41 and 43.10±1.41, respectively. Results further indicated that after each step of partial purification total protein contents and percentage recovery decreased from crude extract to desalted sample while fold purification was increased. The maximum activity of purified CAT was recorded at 6.0 pH and 30°C temperature for gills of *C. striata*. As the temperature was raised further the catalase activity decreased.

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Introduction

The environment is plagued with different kinds of pollutants. Pesticides are one of such pollutants which play an important role in controlling different types of pests that cause damage to the crop plants and to improve agricultural production. Insecticides, fungicides and herbicides constitute the major source of potential environmental hazards not only to birds, fish, and other animals but also to humans when they become a part of food chains (Abd-Alla et al., 2002). Long term exposure to these pollutants causes countless abnormalities and reduces the life span of organisms (Hussain et al., 2011; Naz et al., 2011; Khan et al., 2012).

Endosulfan (EDS) is one such organochlorine (OC) compound that has been classified as highly toxic by the majority of environmental protection agencies (Sutherland et al., 2004). However, endosulfan (EDS)

has been registered and released for use as a pesticide in the cultivation of soya, cotton, coffee, tobacco, and tea among others in several developed and developing countries (Bedor et al., 2010). EDS is considered to be toxic to all kinds of organisms (Xu et al., 2007; Weber et al., 2010). One pyrethroid which is used more commonly than other synthetic pyrethroids and has found wide acceptability for agricultural purposes is deltamethrin. They are extensively used in agriculture, for controlling pests, insects and vectors of endemic diseases, protecting seeds during storage and fighting household insects because of their low environmental persistence (DeSai et al., 2003). Deltamethrin toxicity in fishes showed that it causes varied effect including histopathological, oxidative stress, haematological, neurotoxin, biochemical changes as well as immunological effects. Also, deltamethrin has found to be highly toxic in fishes even in very low concentration (Pawar et al., 2009). The neurotoxic effect of the synthetic pyrethroids, deltamethrin is attributed to the blocking of sodium channels and inhibiting the GABA receptors in the nervous filaments which results in an excessive stimulation of the central nervous system that sometimes can lead to brain hypoxia (El-Sayed et al., 2007).

Fish have to face the toxicity of different pesticides entered in natural aquatic habitats due to industrial development of man. These enter in food chain by accumulation in body tissues of fish which is consumed by a large population of human. Several studies indicated that pesticides had toxic effect on enzymes in certain fishes. Variations in activity of enzyme are used as markers to identify the tissue injury, a diseased condition, or environmental stress. The rate of increase of enzyme activity and the rate of leakage caused by injury depends on the concentration of an enzyme (Roy et al., 2011). Pesticides induce oxidative stress by generating free oxygen radicals. In this situation organisms boost up defense system by producing antioxidant enzymes viz. glutathione peroxidase, superoxide dismutase and catalase (Guven et al., 2008). If the antioxidant system not able to eliminate oxidants or neutralize the excess of ROS, the fish will be at high risk of oxidative damage and oxidative stress. It is examined that waterborne pollutants induces oxidative stress and cellular damage in fish and other aquatic organisms (Box et al., 2007). The use of biochemical biomarkers of environmental contamination allows a sensitive assessment of the xenobiotic effects in aquatic organisms in order to detect early alterations in the environment prior to any irreversible harm being caused to the ecosystem (Huggett et al., 1992; De-Caprio, 1997). The objectives of present work were to check the activity of catalase in gills in *C. striata* exposed to insecticides mixture

Materials and Methods

Experimental animal

Fish *Channa striata* commonly known as Snakehead murrel was collected from natural breeding grounds and shifted to the wet laboratory at Fisheries Research Farm, University of Agriculture Faisalabad. *C. striata* were acclimatized to laboratory condition for 14 days. After the acclimation period, fish were moved to 100-liter glass aquarium each containing a group of fish (n=10). The 96-hr LC_{50} of insecticides, deltamethrin (DM) +endosulfan (END) mixture (1:1) for *C. striata* was calculated as 1.374μgL^{-1} by Anum (2017). Fish were exposed to the sub-lethal concentration (1/3rd of LC_{50}) of DM+END mixture for 14 days. During chronic trail, water temperature, pH and total hardness were kept constant as 30°C, 7.25 and 250 mgL^{-1}, respectively.

Isolation of catalase extract

After sampling, the fish was dissected and gills were separated. Phosphate buffer (pH 6.5) was added in the extracted gills by the ratio of 1:4 (w/v) and homogenized it for 15 minutes with the help of pestle and mortar. Homogenized material was passed through Whatman filter paper no. 1. Filtrate obtained from above step was centrifuged in refrigerator centrifugal machine at 10,000 rpm for 15 minutes. Both sediments and supernatants were separated and stored at 4 °C for further analysis.

Partial purification of CAT

Crude enzyme was partially purified with the help of ammonium sulfate precipitation by following the methods of Zia et al. (2007). The purification of CAT enzyme from gills consists of Salting In and Salting Out method. Salting In procedure crude extract of CAT was saturated with 60% solid ammonium sulfate by dissolving 42g in 100 ml of sample and refrigerated it for 4 hours at 4°C. After 4 hours, centrifuged at 10000 rpm and 4°C 15 minutes. The supernatant obtained from salting In procedure was saturation up to 80% solid ammonium sulfate by adding 56g/100 ml of CAT extract by shaking it and kept at 4°C overnight. After that, centrifuged it at 10000 rpm for 15 minutes and obtained both supernatant and residues. The residues

were dissolved in minimum of phosphate buffer (pH 6.5).

Desalting of residues

Residues obtained from salting Out procedure were subjected to dialysis with the help of dialysis bag. Dialysis bag had semi permeable membranes that allow movement of salts, lower weight molecules and ions. Precipitated proteins samples obtained through salting out process in residue from dialyzed against low ionic strength phosphate buffer (pH 7.4). All of the samples obtained, supernatant, sediments and desalting sample were subjected to enzyme essay and protein contents estimation.

CAT assay

Catalase activity was determined by its ability to decrease the H_2O_2 concentration at 240 nm (Chance and Mehaly, 1977).

Estimation of total protein contents

To estimate protein contents of a sample, Biuret method (Gornall et al., 1949) was used.

Kinetic Characterization of catalase

Optimum pH and temperature: Optimum pH was determined by assaying the purified catalase enzyme from gills of wild *C. striata* at different pH ranging from 4-12(4.0,4.5,5.0,5.5,6.0,6.5,7.0,7.5,8.0,8.5 and 9.0). To obtained optimum temperature for purified CAT enzyme from gills of wild *C. striata* was assayed at different temperature ranging from 5-50°C (5,10,15,20,25,30,35,40,45 and 50) keeping the pH optimum at which catalase showed highest activity.

Statistical Analyses: Obtained data were presented as Mean Standard Deviation (Mean±SD). Analysis of variance was employed to calculated statistical difference between exposed and control fish (Steel et al., 1997). The value of P<*0.05* considered as significant.

Results and Discussion

Activity and specific activity of CAT

The results showed that highest catalase activity was noted from the crude extract of controlled *C. striata* gills (140.66±0.71UmL^{-1}) as compared to stressed *C. striata* gills (99.23±0.71UmL^{-1}). After desalted the gills of controlled *C. striata* and DM+END stressed gills about 42.77±0.71 and 99.23±1.36 UmL^{-1}, respectively Figure 1.

Figure 2 showed that highest specific activity was observed in crude CAT extract from gills of control fish (112.53±0.28 Umg^{-1}) as compared to DM+END exposed fish (82.69±1.12Umg^{-1}). After desalted specific activity of gills CAT was lower in insecticides mixture exposed *C. striata* (112.20±1.41Umg^{-1}) in relation to control (170.80±1.41 Umg^{-1}).

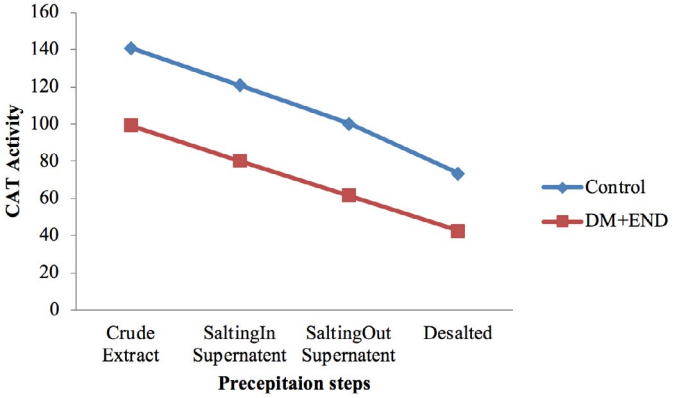

Figure 1: *Partial purification of CAT from gills of Channa striata under insecticides mixture exposure.*

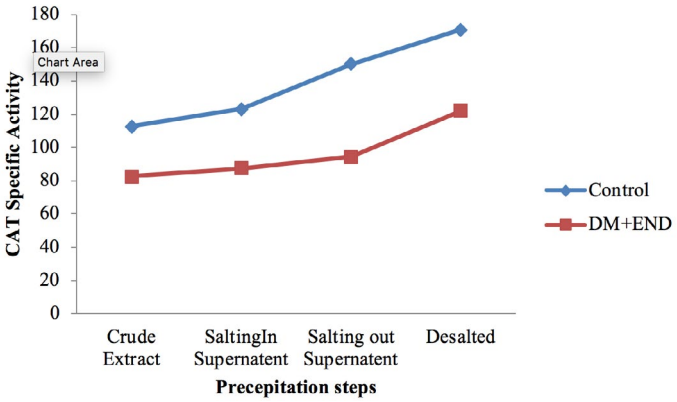

Figure 2: *Partial purification of CAT specific activity from gills of Channa striata under insecticides mixture exposure.*

Total protein contents, fold purification and percentage recovery

Total Protein contents in gills of exposed fish were lowest in relation to control. In results of partial purification highest fold purification of CAT was noted from control *C. striata* (1.52±0.01) as compared to exposed fish (1.48±0.01) gills. The percentage recovery of CAT for control and DM+END exposed fish was calculated as 52.21±1.41 and 43.10±1.41, respectively. Results further indicated that after each step of partial purification total protein contents and percentage recovery decreased from crude extract to desalted sample while fold purification was increased (Table 1).

Kinetic characterization of CAT

Effect of pH and temperature on CAT activity: The maximum activity that purified CAT showed was recorded at pH and temperature at 6.0 and 30°C for gills of C. striata. As the temperature was raised further the

Table 1: *Partial purification of CAT from gills Channa striata exposed to pesticides mixture.*

Fractions	Control			DM+END		
	Protein(mgL^{-1})	Fold Purification	%recovery	Protein(mgL^{-1})	Fold Purification	%Recovery
Crude Extract	1.25±0.01a	1.00±0.02d	100±0.41a	1.20±0.01a	1.00±0.01d	100±2.12a
SaltingIn Supernatent	0.95±0.07b	1.10±0.01c	85.85±2.83b	0.92±0.01b	1.06±0.03c	80.77±1.41b
SaltingOut Supernatent	0.65±0.01c	1.34±0.01b	71.12±1.41c	0.53±0.01c	1.14±0.01b	61.92±0.71c
Desalted	0.43±0.01d	1.52±0.01a	52.21±1.41d	0.35±0.01d	1.48±0.01a	43.10±1.41d

Means sharing similar letter in a row or in a column are statistically non-significant (p>0.05).

CAT activity decreased (Figure 3, 4). Enzymes are proteins in nature and can be isolated and purified from all kinds of living organisms. Majority of enzymes are sub mingled with other proteins and bio-molecules when isolated and therefore needed to purify by using very precise technique so that properties of purified enzyme can be described clearly. Present research work was designed to compare purified catalase enzyme characterization extracted from gills of normal and DM+END mixture exposed C. striata.

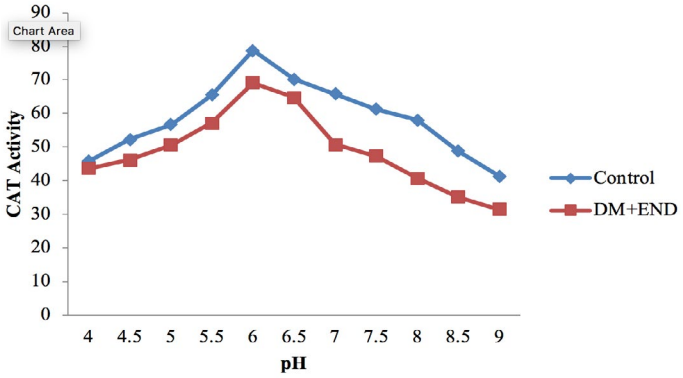

Figure 3: *Effect of different pH on CAT activity in gills of DM+END exposed C. striata.*

Oxidative stress in aquatic organisms, particularly fish, has a great importance for environmental and aquatic toxicology. Because oxidative stress is induced by many chemicals, including some pesticides, these pollutants may stimulate ROS and alteration in antioxidant systems (Kadry et al., 2012). It is well documented that DM may prompt oxidative stress (Sayeed et al., 2003; Tu et al., 2012). As itis known that the antioxidant enzymes CAT, SOD and GSH-Px are the first line of defense against oxidative stress which convert superoxide anions (O^{-2}) into H_2O_2 and then into H_2O and O_2 (Kadry et al., 2012; Stara et al., 2012). This view was in agreement with Abdelkhalek et al. (2015) who recorded significant decrease in SOD, CAT and GSH-Px levels in liver, kidney and gill tissues of tilapia fish upon exposure to DM and Hamed (2015) who documented marked depletion in the hepatic SOD and CAT levels of catfish upon exposure to malathion. This decrease in the SOD activity may be the result of excessive free radical production, such as the superoxide anion and hydrogen peroxide, direct damage of its protein structure by pesticide or a direct action of pesticide on the synthesis of the enzyme (Yonar et al., 2015). Tripathi and Shasmal (2011) Chlorpyriphos significantly decreased the specific activity of CAT in the gill of the fish *Heteropneustes fossilis*. Vineela and Reddy, 2014 CAT activity is gradually decreased in *Catla catla* exposed to Lihocin. Enzymes are important biological compounds as they minimize the activation energy of all metabolic reactions occurring in living organisms. Enzymes are protein in nature composed of amino acids and highly specific in nature so their activity affected by any change in pH, temperature, substrate concentration and pressure (Boeuf et al., 2000).

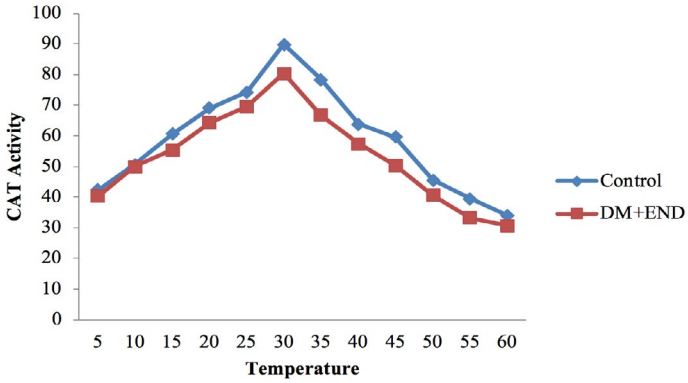

Figure 4: *Effect of different temperature on CAT activity in gills of DM+END exposed C. striata.*

Ahmed et al. (2000) also reported a decreased CAT activity in gills of *Channa striata exposed to* paper mill effluent. Atli et al. (2010) also reported the change in gills CAT activity of fish exposed to sub-lethal concentrations (25 µgL^{-1}) of carbosulfan. Bainy et al. (1996) analyzed the decreased gills CAT level in fish. Diana et al. (2007) observed the decreased gills CAT level in *Carassius auratus gibelio* exposed to deltamethrin. Faheem et al. (2012) CAT activity decreased in gill of *Oreochromis niloticus* exposed to aquatic pollutants ($CdCl_2$). Catalase activities decreased in the

gills of African cat fish (*Clarias gariepinus)* exposed to sub-lethal concentrations of butachlor (Farombi et al., 2008). Prusty et al. (2011) CAT activity decreased in gills of fishes *Labeo rohita* exposed to sub-lethal concentration (1/3rd of LC_{50}) of fenvalerate was exposed for 15 days. CAT activity was decreased in gills of gilthead seabream *Sparus aurata* exposed to sub-lethal concentration of malathion (Rosety et al., 2005). CAT reduced in gills of African catfish; *Clarias gariepinus* exposed to deltamethrin (Hamed, 2016).

Decreased CAT level gills of common carp exposed to zeta-cypermethrin (Stara et al., 2013). Yonar and Sakin (2011) studied the effect deltamethrin at concentrations of 0.018 and 0.036 μgL^{-1} on common carp for 14 days. They observed decrease SOD, CAT, and GSH activity and significantly increased levels of malondialdehyde in liver and gill. The activity of CAT was found to be reduced in the fish *Labeo rohita* exposed to endosulfan and fenvalerate when compared to control (Suneetha, 2014). Significant decrease in gills CAT level of *C. punctatus* exposed to alphamethrin was observed by Tripathi and Singh (2013). Insecticidal stress caused a reduction in CAT activity and reduced glutathione levels in zebrafish gills exposed to dimethoate (Ansari and Ansari, 2014).

Conclusions and Recommendations

These results suggest that an immediate adaptive response to the oxidative stress appeared, demonstrating alterations in the antioxidant defense mechanism in the gills of deltamethrin intoxicated fish. Therefore, present study was conducted in order to conserve freshwater fisheries in the natural waters. Both cultured and wild fish have become the victim of pesticides pollution caused by organic and inorganic chemicals.

Author's Contribution

Sana Rana executed this research work.Sajid Abdullah planned this research work. Huma Naz performed statistical analyses. Khalid Abbas help in writing article.

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