Introduction

Pesticides and their metabolites released from industries and agricultural practices pose serious threat to aquatic life. Aquatic animals, particularly fishes are greatly susceptible to these toxic chemicals because they can amass in fish organ and transferred to humans through food chain by the process of bio-magnification.

The toxicity of pesticides to fish depends upon different factors such as biochemical processes of organisms and chemical nature of toxic compounds (Jaroli and Sharma, 2005). Different abiotic factors such as pH and temperature may alter the activities of enzymes (Ugolev and Kuzmina, 1993). Temperature is one of the important stressing factors for fish because it strongly affects the metabolic rate in pokilotherms (Clarke and Fraser, 2004).
Endosulfan is a persistent organochlorine insecticide and poses severe threats to the aquatic animals (Tripathi and Verma, 2004) that include respiratory changes, physiological, biochemical and molecular alterations result in tissue damage (Dar et al., 2015); for instance, it can also change the metabolism in fishes even lead to death in some cases (Tripathi et al., 2004).

One of the synthetic type two pyrethroid insecticides deltamethrin, has been largely been used in agriculture and industrial sector and proved to be extremely toxic to aquatic animals including fish (Ural and Saglam, 2005; Kopruçu et al., 2006) under laboratory conditions.

Many classes of pesticides may provoke the formation of reactive oxygen species (ROS) and induce oxidative stress (Elia et al., 2002; Sayeed et al., 2003). To neutralize the toxic effect of ROS fishes have antioxidant defense enzymes like superoxide dismutase, catalase and glutathione peroxidase (Orbea et al., 2002). These enzymes could save the fish's tissues from oxidative damage under normal conditions and can be used as sensitive biomarkers of oxidative stress in fish exposed to aquatic toxicants (Ahmad et al., 2000). Most of the available literature deals with the effects of single pesticides to fish but there is no information regarding to the effects of pesticides in mixture form. Therefore, current work was conducted to assess the effect of insecticides mixture (deltamethrin+endosulfan) on catalase activity in hepatic tissue of Channa striata.

Materials and Methods

Experimental fish and conditions
Carnivorous fish, Channa striata called “snakehead murrel”, were collected from natural breeding grounds and shifted to the Toxicology laboratory at Fisheries Research Farm, University of Agriculture Faisalabad. C. striata were acclimatized to laboratory condition for 14 days and then fish were moved to 100-L glass aquaria each containing a group of fish (n=10). The 96-hr LC₅₀ of insecticides, deltamethrin (DM)+endosulfan (END) mixture for C. striata was 1.374 µgL⁻¹ as calculated by Abdullah (2018). Fishes were exposed to the sub-lethal concentration (1/3rd of LC₅₀) of DM+END mixture for 14 days.

Isolation and partial purification of CAT
For isolation of enzyme extract, hepatic tissues were-weighed and phosphate buffer (pH 6.5) added by the ratio of 4:1 (w/v). The sample was homogenized for 15 minutes by using pestle and mortar. Tissues homogenate was filtered and centrifuged in refrigerator centrifugal machine at 10,000 rpm for 15 minutes. Both sediments and supernatants were separated for further analysis. The extracted CAT (crude) was then partially purified by ammonium sulphate precipitation using the method of Shin et al. (1993). Ammonium sulfate precipitation was performed in two steps including Salting-in (60%) and Salting-out (80%) steps. Precipitated enzyme sample obtained through salting-out process in residue form was dialyzed against low ionic strength phosphate buffer (pH 7.4) with the help of dialysis bag. All of the obtained sample supernatants, sediments, and desalting sample were subjected to enzyme assay and protein contents estimation. Catalase activity was determined by following the method of Chance and Mehaly (1977) at 240 nm.

Estimation of protein contents
Protein contents of the sample were estimated by using Biuret method (Gornall et al., 1949).

Kinetic characterization of CAT
Optimum pH was determined by assaying the purified CAT from hepatic tissues 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, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5 and 12.0). To obtain optimum temperature for purified hepatic CAT of C. striata was assaying at different temperatures ranging from 5-50°C (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50) keeping the pH at which purified catalase showed highest activity (Nakamura et al., 2000; Al-Bar, 2012).

Statistical analysis
Data obtained in this study were represented as Mean Standard Deviation (Mean±SD). ANOVA was applied to calculate statistical difference (p<0.05) in CAT activity between both control and exposed group (Steel et al., 1997). MS excel was used to draw the graph.

Results

Activity of hepatic CAT
The result showed that lowest CAT activity was calculated in hepatic tissue to END+DM exposed C. striata (91.62±0.71 UmL⁻¹) in comparison to crude extract control (97.56±1.41 UmL⁻¹). Desalting result also confirmed that CAT activity was the lowest in END+DM exposed fish hepatic tissue as compared to controlled. The result also showed that enzyme activity decreased gradually from crude extract to desalted sample (Figure 1).

Figure 1: Partial Purification of hepatic CAT activity (Unit mL⁻¹) in C. striata exposed to END+DM
Specific activity of hepatic CAT

The lowest hepatic CAT specific activity was calculated in exposed C. striata (35.23±0.71 Unit mg⁻¹) as compared to control (37.52±1.41 Unit mg⁻¹). After desalting specific activity of hepatic CAT remain lower in exposed fish as 134.30±0.71 Unit mg⁻¹ in relation to control (148.02±0.41 Unit mg⁻¹). Results also indicated that the activity and specific activity is antagonistic to each other at each step of partial purification of enzyme (Figure 2).

Total protein contents, Fold purification and Percent recovery

Total protein contents were significantly lower in hepatic tissues of END+DM exposed fish when compared with control. The highest fold purification was calculated in hepatic tissues of control C. striata (2.35±0.01) as compared to the exposed fish (2.22±0.01). Hepatic CAT in control C. striata had highest percent recovery as 81.77±1.41% as compared to stressed (69.94±0.71%). It was noted that total protein contents and percentage recovery decreased after every step of partial purification while fold purification was increased (Table 1).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Precipitation Steps</th>
<th>Control</th>
<th>END+DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (mg mL⁻¹)</td>
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<td>2.65±0.01Aa</td>
<td>2.60±0.00Bb</td>
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<td>1.05±0.01Bc</td>
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<td></td>
<td>Desalting</td>
<td>0.34±0.01Ad</td>
<td>0.33±0.03Bd</td>
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<tr>
<td>Fold purification</td>
<td>Crude extract</td>
<td>1.00±0.02Aa</td>
<td>1.00±0.02Bb</td>
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<tr>
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<tr>
<td>% recovery</td>
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<td>100.00±1.41Aa</td>
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<tr>
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<td>Desalting</td>
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<td>69.94±0.71Bb</td>
</tr>
</tbody>
</table>

Capital letters represent the significant (P<0.05) difference at different steps of purification within the same column while small lettered superscripts show the difference between treatments within the same row.

Kinetic characterization

The activity of partially purified hepatic CAT increased as pH and temperature increased. The optimum pH and temperature for hepatic CAT was noted as 7.5 and 25°C, respectively (Figure 3-4).

Discussion

Results of present study showed that the CAT activity in hepatic tissue of exposed fish was decreased in contrast to control. Stara et al. (2013) reported the lower level of CAT in liver of Cyprinus carpio exposed to zeta-cypermethrin in relation to control. Ahmed et al. (2016) reported higher liver CAT activity in control fish as compared to binary metal mixture treated O. niloticus. Salvo et al. (2012) recorded the decreased CAT activity in liver of common carp under sub-lethal exposure of endosulfan. Hamed (2016) also recorded the decreased liver CAT activity in the African catfish (Clarias gariepinus) under deltamethrin...
exposure. These findings are also in accordance to Sayeed et al. (2003) who reported decline of hepatic CAT activity in Channa punctatus exposed to deltamethrin. Lihocin exposure gradually decreased CAT level in liver of Catla catla (Vineela and Reddy, 2014). Thenmozhi et al. (2011) observed decreased CAT in the liver of Labeorobita exposed to tomatohalogen. Significant decrease in liver CAT of Channa punctatus exposed to pyrethroid insecticides was reported by Triphiti and Sing (2013). Suneeath (2014) recorded significant inhibition in liver CAT of Labeorobita under exposure of endosulfan and fenvalerate when compared to control. Deltamethrin exposure caused depletion in hepatic CAT levels of tilapia (Abdelkhaelek et al., 2015). Depletion in hepatic CAT level of catfish upon exposure to malathion was reported by Hamed (2015). Cypermethrin decreased liver CAT in Tor putitora (Ullah, 2014). Dinu et al. (2010) recorded temporary inhibition in liver CAT activity of Carassius auratus gibelio exposed to deltamethrin. Rana et al. (2018) also reported the decreased CAT activity in Channas triata exposed to sub-lethal dose of DM+END.

The existence of toxicants significantly affects the antioxidant enzymes (catalase) because toxicants can bind with site of catalase and inhibit its activity. The inhibition in catalase activity is may be due to direct binding of toxicants to thiol (–SH) groups of enzyme which consequently increased the ROS (Faheem et al., 2012).

The unit of enzyme in one milligram of protein is known as specific activity of enzyme and it is a indicator of purification of enzyme. In present work, specific activity of CAT increased as from crude extract to dialysis. Specific activity in hepatic tissue of exposed fish decreased as compared to control. Ahmed et al. (2016) also recorded the decreased specific activity of purified hepatic CAT in stressed fish as compared to control.

In present study, it was noted that total protein contents and percentage recovery decreased after every step of partial purification while fold purification was increased. Some authors reported the similar results for percent recovery and fold purification of CAT in different fish species (Sarwar, 2013; Akram, 2014; Ahmed et al., 2016; Rana et al., 2018).

Optimum temperature and pH for hepatic catalase of Channa striata was noted as 25°C and 7.5, respectively. Ahmed et al. (2016) noted the optimum pH and temperature as found 7 and 25°C, respectively for purified CAT. Tariq (2013) obtained similar results in C. mrigala, Sarwar (2013) in C.idel and Akram (2014) in C.catla. Rana et al. (2018) also reported the maximum activity of purified CAT was recorded at pH 6.0 and 30°C temperature for C. striata.

Conclusion and Recommendations

This research work concluded that the insecticides mixture exposure could alter the antioxidant enzymes activity in fish. The biochemical technique such as partial purification is a simple and convenient method to purify the catalase and can be successfully used for evaluating the water pollution. It was also concluded that the other environmental factors such as pH and temperature could also affect the catalase activity in fish.

References


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