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Research Article

Comet Assay: Quantification of Damaged DNA in *Catla catla* Exposed to Endosulfan+Chlorpyrifos

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Authors' Contributions
HN performed the research and wrote the article. SA planned and supervised the study. KA provided lab facilities for research. MAT and LS helped in writing the article. GN helped in statistical anlyses.

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Fish, Acute effect, Blood, Insecticides mixture, Comet assay

Abstract | In an acute toxicity trail, LC_{50} and LC_{100}value (96 hr) of endosulfan (END)+chlorpyrifos (CPF) mixture for *Catla catla* was computed. The genotoxicity of END+CPF mixture in RBCs of fish was also evaluated by comet assay. To check the genotoxicity of END+CPF mixture, blood of fish was sampled after 24-hr of intervals. Some fishes were also kept in clean water known as negative control (NC) and for positive control (PC) cyclophosphamide was injected into fish. The tolerance limits of *C. catla* against END+CPF was computed as 1.35±0.01µgL^{-1}(LC_{50}) and 2.25±0.02µgL^{-1}(LC_{100}). Genotoxic results showed that END+CPF mixture caused significant damage to nuclei (22.58±5.37%) and GDI (0.73±0.15%) in RBCs of fish as compare to control. Aduration-specific damage to DNA was observed in RBCs of *C. catla*.

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Introduction

From last few years, pesticides have been extensively applied in agriculture to control the pest. In results of this natural aquatic system such as lakes, rivers, streams and wetlands have been contaminated all over the world (Ngidlo, 2013). Aquatic pollution by these pesticides cause the unwarranted mortalities of aquatic animals in general and especially fish (Gupta *et al.,* 2013). Fish is an important animal to examine the genotoxic effects of pollutants present in water bodies because fish can amass these toxicants in body organs (Bhatnagar *et al.,* 2016).

Organochlorine (endosulfan) and organophosphate (chlorpyrifos) are indiscriminately used in agricultural to save crop from pests (Rusyniak and Nanagas, 2004 Kumari *et al.,* 2007). These pesticides cause liver failure (Poet *et al.,* 2003), genotoxicity (Mehta *et al.,* 2008), neuro-chemical and neuro-behavioral alterations (Verma et al., 2009; Ojha *et al.,* 2011).

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Environmental contaminants induced genotoxicity can be assessed by using biomarkers such as comet assay. Comet assay is the sensitive methods to quantify the geno-toxic results of agrochemicals on integrity of DNA in different species of fish (Frenzilli et al., 2009; Nagarani *et al.*, 2012). According to Kim *et al.* (2002), it is most reliable technique due to its efficiency for detecting damage at DNA level even in particular cell induced by physical and chemical toxicants. So, keeping in view above mentioned problems associated with insecticides, this work was carried out to assess the genotoxic effects of dual pesticides mixture on RBCs of *Catla catla* using comet assay.

Materials and Methods

Acute toxicity assay was accomplished in Toxicology laboratory at Fisheries Research Farms, UAF, Pakistan. Fish were acclimatized for 14 days in cemented tank. Commercially available feed (at 3% wet body weight) was used to fed fish during acclimatization; however fish were kept in fasting condition during toxicity trails. The test chemicals, endosulfan (END) and chlorpyrifos (CPF) were dissolved, separately, in methanol to prepare the stock-I solutions while to prepare the mixture solution of END+CPF further dilutions was made in the deionized water.

Toxicity trail

To conduct the acute toxicity tests, each group of *C. catla* (n=10) were placed in glass aquarium (70-L) and separately exposed to 14 different levels (0.15-2.10 μgL^{-1}) of END+CPF for 96 hr. Each toxicity tests were carried out with three replicates. After 12 hr of interval fish mortality was observed and deceased fish were separated immediately.

Comet assay

Fish were exposed to LC_{50}value of END+CPF for 96 hr. Some fishes were also placed same conditions in clean water known as negative control (NC) and for positive control (PC) cyclophosphamide was injected into fish. Blood from fish was sampled after 24-hr interval to see the genotoxic effects of CPF+END, PC and NC by comet assay.The blood was collected in eppendorf which contain anticoagulant (Kousar and Javed, 2015). Sing *et al.* (1988) procedure was followed to complete assay. Jose *et al.* (2011) criterion was followed to identify the different types of damaged cells.

Data analyses

Data collected from fish mortality was used to draw the concentration response curve by Probit analyses (Finney, 1971). Mann-Whitney U-test (non-parametric) was applied on data collected from DNA damaged (Steel *et al.*, 1996) and graphs were draw in MS excel.

Results and Discussion

Toxicity trail

The tolerance limits in term of LC_{50} and LC_{100} value (96 hr) of *C. catla* against CPF+END was estimated as 1.35±0.01 and 2.25±0.03μgL^{-1}, respectively. Fish mortality against different concentration was given in Figure 1. Several researchers also calculated the lethal concentrations of insecticides for different fish species (Verma and Saxena, 2013; Haloi *et al.*, 2014; Ambreen and Javed, 2015; Naserabad *et al.*, 2015; Naz *et al.*, 2017; Naz *et al.*, 2019).

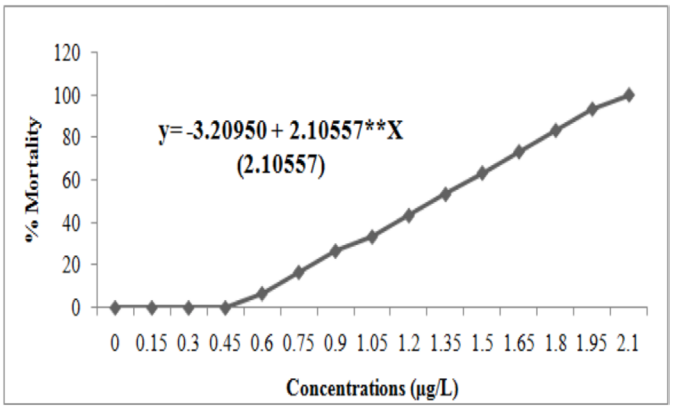

Figure 1: *C. catla* mortality (%0 against different concentration of END+CPF mixture.

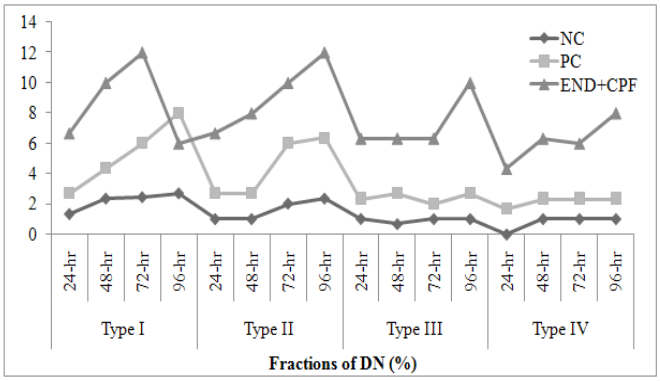

Figure 2: Different types of damaged nuclei in blood of *C. catla* exposed to END+CPF mixture.

Comet assay

Different types of DNA damage in RBCs of *C. catla* was as follow NC<PC<CPF+END (Figure 2). Regarding different treatments the damaged nuclei (DN) and genetic damage index (GDI) in RBCs of fish was higher in CPF+END mixture exposure than that of PC and NC. A duration-specific damage to DNA was observed in RBCs of *C. catla* (Figure 3-4). Ambreen *et al.* (2018) also confirmed the DN and GDI in RBCs of grass carp under chlorpyrifos+endosulfan exposure. Duration specific DNA damaged in RBCs of goldfish induced by monocrotophos was also noted by Zhao *et al.* (2015). Shukla *et al.* (2010) also noted the duration- dependent increase in DNA damage of *Mystusvittatus* exposed to dichlorvos. Malathion expo-

sure also caused DNA damage in blood of common carp (Moradi *et al.*, 2012). Blood of *T. mossambica* also demonstrated the DNA damage of monocrotophos (Banu *et al.*, 2001). Genotoxic potential of some insecticides in RBCs of fish was also noted by some other authors (Simoniello *et al.*, 2009; Gadhia *et al.*, 2016).

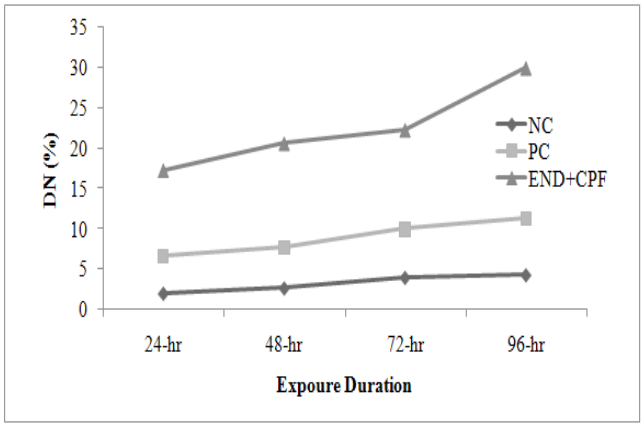

Figure 3: Percentage of damage nuclei in blood of *C. Catla* exposed to END+CPF mixture.

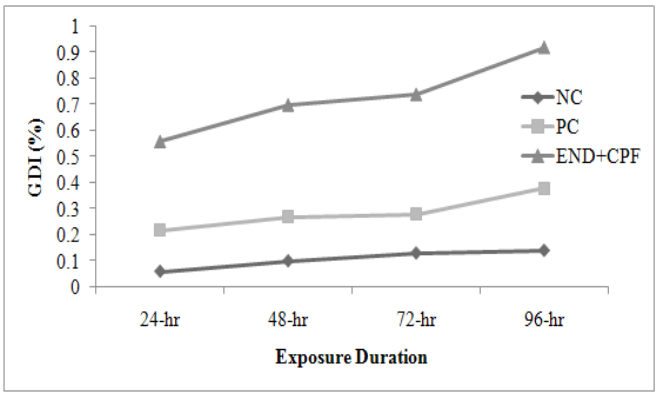

Figure 4: END+CPF mixture induced genetic damage index in blood of *C. catla*

Conclusion

In conclusion, insecticides mixture has potential to cause damage to DNA in blood of *Catla catla*. Comet assaycan be successfully applied as a good biomarker and screening approach for identifying the harmful impacts of these toxicants on DNA molecule.

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