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Genotoxic Response of *Oreochromis niloticus* Exposed to Tertiary Mixture of Pesticides

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Abstract

Background: At present the aquatic habitats of Pakistan become heavily polluted due to presence of heavy metals and pesticides. This research was carried out to check the percentage of DNA damage, Genetic Damage Index and Cumulative Tail Length of comets formed in the erythrocytes of *Oreochromis niloticus* following exposure to a tertiary mixture of pesticides (chlorpyrifos, endosulfan and bifenthrin) with the Comet assay.

Methods: Acute toxicity (96-hour LC50) of chlorpyrifos + endosulfan + bifenthrin mixture was determined for *Oreochromis niloticus* (180-day old), and then four sublethal concentrations (1/3rd, 1/4th, 1/5th , and 1/6th of the LC50) were calculated. To control the possibility of temperature variation, fingerlings of O. niloticus were treated with four experimental pesticides concentrations used for duration of 90 days under constant conditions of laboratory (with negative and positive control). On day 14, 28, 42, 56, 70, and 84 fish peripheral blood cells were collected following exposure to assess DNA damage.

Results: DNA damage was observed to be statistically significant ($p<0.05$) throughout the exposure period due to the various test concentrations. In fish erythrocytes, a dose/concentration-dependent response was observed, with the greatest DNA damage occurring at 1/3rd of the LC50 exposure. Comparing DNA damage in *Oreochromis niloticus* peripheral blood erythrocytes across all sampling days revealed a continuous rise in the quantity of damaged DNA with increase in time of exposure.

Conclusion: Present investigation represented an unprecedented approach to study genotoxic effects of pesticides on fish. The widespread application of pesticides (chlorpyrifos, endosulfan, bifenthrin) in agriculture sector exerts adverse effects on various non-target organisms via trophic transfer that ultimately pose a serious threat for human beings. Current findings suggested minimized and sensible use of pesticides to avoid genetic threats to aquatic fauna and to maintain sustainable agriculture and aquaculture.

Introduction

Aquaculture uses a variety of water sources, majority of which are highly contaminated with various forms of pesticides [1, 2]. Heavy metals, pesticides, inorganic and organic particulate matter, suspended solids and various dyes are all possible pollutants in water [3, 4]. Degradation of water quality would result in a significant depletion of aquatic biota on an organism level. It also decreases the value of aquatic animals [5]. Water quality degradation is primarily caused by human activities, which pose global threats to aquatic fauna and flora. The concentrations of many contaminants like persistent organic pollutants and heavy metals in a variety of fish and shellfish species exceeded than the recommended concentration. So, use of such products is unfit for human consumption [6]. According to a study, human tissues contain a huge amount (over 90%) of persistent organic pollutants, like chlorinated hydrocarbons that originate mainly from seafood [7].

There are four major classifications of pesticides according to their use, with regard to agricultural applications, including insecticides, herbicides, fungicides and rodenticides [8]. Pesticides are divided into ten chemical groups: pyrethroids, phthalimides, triazines, phenoxyalkonates, phenylamides, dipyridyl, carbamates, benzoic acid, organochlorines and organophosphates [9]. The flow of pesticides into water sources can be due to water run-off, subsurface runoff, absorption into the soil and spray drift [10]. Overall, only one percent of applied pesticides got to their intended locations, but roughly 99 percent went into the surrounding environment, ground water and waterways [11].

Chlorpyrifos is an organophosphate pesticide, which may trigger a number of health problems in animals, including immunological and neurochemical and neurobehavioral effects, liver dysfunctions, genotoxicity, endocrine abnormalities and carcinogenesis [12-14]. Concentration of chlorpyrifos in water has now increased many times due to bioaccumulation and biomagnifications in water, which has an adverse effect on fish [15, 16]. Organochlorines are hydrophobic, long-lasting and persistent in nature. Endosulfan is an organochlorine pesticide and a major environmental pollutant. Endosulfan leads to DNA damage and genetic change in the reproductive system of an organism [17]. It can cause DNA damage and the termination of cell division in endothelial cells [18]. The presence of organochlorine and organophosphate pesticides causing environmental problems in the ecosystem, led to the discovery of a new class of pesticides, "pyrethroids" in the 1970s. Therefore, use of organophosphate and organochlorine pesticides has been steadily declining [19]. Several in-vivo and in-vitro investigations provide the same results that pyrethroid exposure also exhibited substantial genotoxicity evidenced by the prevalence of micronuclei and structural chromosome and DNA damage [20-22]. Pyrethroids have low toxic effects on mammals and birds. A high toxicity in fish and shrimp is well recognized [23]. One of the most commonly occurring pyrethroids, which aquatic animals (fish and invertebrates) are constantly exposed to is bifenthrin in surface waters and water supplies [24]. The bifenthrin genotoxicity was clearly visible in the brain of exposed fish, as shown by a huge rise in comet endpoints [25].

Fish, among other vertebrates, are very important for evaluating the genotoxic effects of various pollutants, and as a result, they are commonly used in bio monitoring studies all over the world [26, 27]. *Oreochromis niloticus* (Nile Tilapia) is one of the warm water fish species that is very important for lower and middle income countries, particularly [28]. Since pesticides can interfere with the DNA of living cells, they can induce genotoxicity such as formation of micronuclei, base modifications, cross linkages, DNA strand breaks and deprivation [29]. The genotoxic potential of contaminants discharged into aquatic systems must be quantified. DNA integrity can be anticipated as a responsive and efficient biomarker for genotoxicants, teratogens, carcinogens and mutagens in the environment [30, 31]. Pesticides induce breakage of DNA strands by forming free radicals (OH-, O-2 and H2O2), thus disrupting phospho-diester linkages within the DNA [32]. Comet assay is an effective technique at detecting incomplete excision repair sites, alkali-labile sites, inter strand cross linkages, single-stranded DNA breaks and double-stranded DNA breaks [33]. Comet assay is a very excellent tool for locating DNA damage in-vivo as well as in-vitro studies [34]. Comet assay is also used in risk assessment of various xenobiotics by estimating DNA damage at single cell level [35]. Therefore, it was necessary to evaluate the potential of genotoxic effect of pesticides on the fish that would help in maintainable protection of fish species in their natural habitats.

Methods

Species and Required Chemicals:

Tilapia, *Oreochromis niloticus* fingerlings were procure from domestic market of Faisalabad and shifted to the University of Agriculture's Fisheries Research Farms in Faisalabad, Pakistan. For two weeks, 180-day-old fish fingerlings of comparable size and weight were acclimated in cemented tanks under constant laboratory conditions and fed a pelleted diet. Regular syphoning of feces and other waste materials was performed to reduce the ammonia content of the water. To make the stock-I mixture (1g/100 ml), chlorpyrifos, bifenthrin and endosulphan were dissolved individually in methanol

(95% analytical grade) methanol act as carrier solvent. However, stock solution of three pesticides was mixed and diluted in deionized water for preparation of required concentration (stock-II).

Finding out Sub-Lethal Concentrations:
After preparation of solutions, acute toxicity test was performed. Four sublethal values of pesticide mixture (chlorpyrifos + endosulfan + bifenthrin) were calculated from the median lethal concentration that is 3.712µgL-1 for *Oreochromis niloticus*. From this value the four sub-lethal values were as follows: 1.24µgL-1 (1/3rd of LC50), 0.93µgL-1 (1/4th of LC50), 0.74µgL-1 (1/5th of LC50) and 0.62µgL-1 (1/6th of LC50).

Single Cell Gel Electrophoresis (Comet Assay): *Oreochromis niloticus* (n=12) were individually subjected to 04 selected test concentrations (separately) in the glass aquaria with a 70L water volume. Instantaneously, one set of fish was treated as "Negative Control" as it is placed in pesticides free media. The second set or group of fish was given Cyclophosphamide (20gg-1). Throughout the 90-day exposure duration, the fish were fed a small amount of food daily. During experiment temperature of water (30oC) and, pH (7.75) while total hardness were set at constant value of 225mgL-1. The experience was sustained for the 90 days and blood (peripheral blood) slides were prepared and subjected to Comet assay [36] on days 14, 28, 42, 56, 70, and 84 of exposure. Three replications were performed for each sub-lethal test value/concentration. The DNA destruction was measured visually by classifying cells into the following 05 groups of 'comets' based on length of their tail (determined by TriTek CometScoreTM). Following categories of damaged DNA were observed: All the cells with intact nuclei were considered as Type-0, Cells having low level DNA damage were considered as Type-1, cell having medium level damage were considered as Type-2, cells with high level damage were considered as Type -3 while cell with completely disintegrated nuclei were considered as Type-4.

Percentage (%age) of DNA Damage:
It was calculated through given formula
%age of DNA Damage = DNA damage of Type 2+ Type 3+Type 4

GDI (Genetic Damage Index):
The formula used to calculate GDI is given below:

CTL (Cumulative Tail Lengths): The software (TriTek CometScoreTM program) was utilized to determine the length of tail formed as a part of comet. The length of tail represents the extent of disintegration of DNA fragment under the effect of electric field. However, cumulative length of comet tail (m) was calculated through summation of all tails [37].

Data Analyses:
MSTATC computer software was used to conduct statistical analysis, and the results are expressed as Means ±SD. Means were computed for variations by employing Duncan Multiple Range and significance level of $p<0.05$ was acknowledged as statistically important [38].

Results

Acute toxicity studies and sub-lethal doses:
Lethal concentration (LC50) of a pesticide mixture was determined using Probit analyses of data obtained in an acute toxicity bioassay. The LC50 of pesticides mixture for *Oreochromis niloticus* was 3.712gL-1 after 96 hours. Due to the need for live samples in genotoxicity evaluation, sub-lethal concentration levels were chosen for the Comet assay. Fish were individually revealed for oxidative stress detection, as a result four sub-lethal test concentrations were calculated 1/3rd of the LC50 (1.24gL-1), 1/4 the of LC50 (0.93gL-1), 1/5 the of LC50 (0.74gL-1), and 1/6 the of LC50 (0.62gL-1).

DNA Damage Assessment:
Significant differences exist in the number of cells with damaged and undamaged nuclei. Percentage of damaged cells in peripheral erythrocytes of *Oreochromis niloticus* following exposure to different concentrations of the negative control, positive control, 1/3rd, 1/4th, 1/5th, and 1/6th of the LC50 also varied significantly. The proportion of damaged nuclei varied according to the duration of exposure to the tertiary pesticide combination (Table 1). In *Oreochromis niloticus*, time and dose dependent variation appeared in percentage of damaged nuclei, genetic damage index and comet tail lengths which are shown in Table 2. Due to the different test concentrations, DNA damage was found to be statistically significant ($p<0.05$) during the exposure duration. The percentage of DNA damage increased ($p<0.05$) following exposure to 1/3rd, 1/4th, positive control, 1/5th and 1/6th of the LC50. This sequence indicates dose dependent increase in DNA damage. After 56 days of exposure the percentage of cells with damaged nuclei in *Oreochromis niloticus* was significantly elevated, followed by 42, 70, 28, 84, and 14 days. Evaluating DNA damage in *Oreochromis niloticus* across all sampling days showed a rise in damage from the 14th to the 56th day of exposure, accompanied by a decrease on days 70 and 84. However, after 28 and 84 days of exposure, no major change in these parameters was found. During exposure period of 56 and 84 days the maximum i.e. 1.63±0.01 and minimum (1.31±0.02) GDI values were observed. Nevertheless, none of these tests

revealed any statistically significant differences after 14, 28, 42 and 84 days of exposure. Moreover, the total comet tail observed in blood cells of fish differed significantly with time of exposure. Among six treatments, 1/3rd of LC50 exposure resulted in significantly greater DNA damage (72.5±60.74 percent), as compared to other treatments. Likewise, chlorpyrifos + endosulfan + bifenthrin mixture resulted in different GDI values ranging from 0.02±0.01 (negative control) to 2.22±0.02 (1/3rd of LC50), respectively. A 1/3rd of the LC50 exposure resulted in a substantially greater overall cumulative comet tail length in this fish's erythrocytes than a positive control (Table 2; Figure 1).

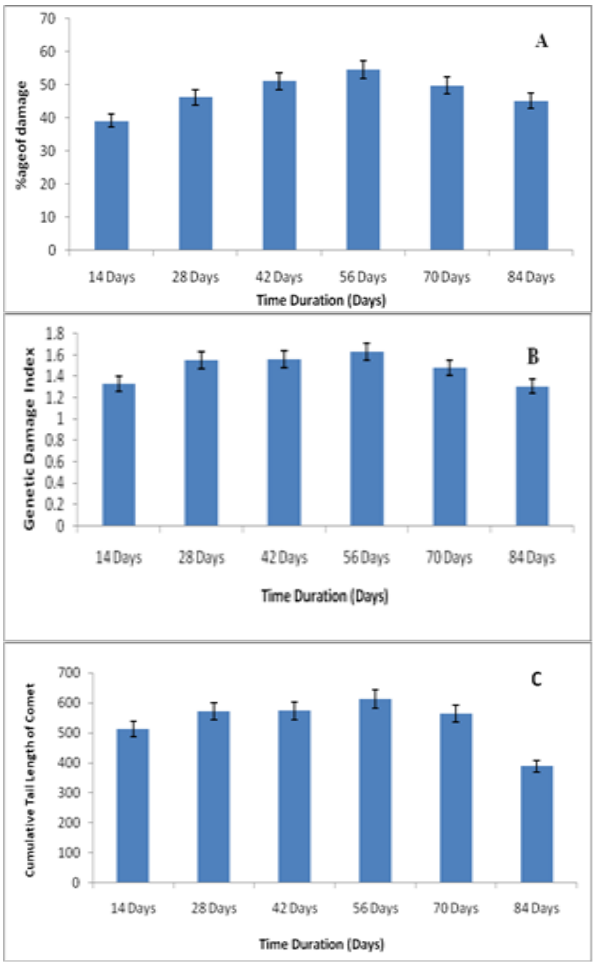

Figure 1: Percentage of damage nuclei (A), genetic damage index (B) and cumulative tail length (µm) of comets (C) induced in peripheral blood erythrocytes of fish exposed to mixture

Discussion

Population of human has rapidly increased during the last few decades, leading to mechanization, sub-urbanization and industrialization. Day by day, water bodies are getting exposed to contaminants or pollutants released by human actions like farming, industrialization and domestic actions [39]. The use of pesticides is essential for controlling pests and for increasing food production for the growing population. However, extensive use of pesticides has several implications, like genotoxicity, bioaccumulation and immune system effect, oxidation of bio molecules, enzyme inhibition, chromosomal and behavioral effects, retarded growth and alterations in biochemical parameters of blood in non-target species [40]. Different animal models such as fish, amphibians, birds, and mammal species have been evaluated against pesticides for their genotoxic effects. These animals have shown variable amounts of DNA damage upon various contaminant exposures [41].

Significant ($p < 0.05$) DNA damage were noticed throughout the contact time in this trial due to the various dose levels. Concerning the various treatments (controls, 1/3rd of LC50, 1/4th of LC50, 1/5th of LC50, 1/6th of LC50), the percentage of DNA damage was considerably greater ($p<0.05$) following exposure to 1/3rd of LC50, 1/4th of LC50, positive control, 1/5th of LC50, 1/6th of LC50, suggesting dose dependent damage in DNA. [42] noticed considerably greater DNA damage in blood erythrocytes of Oncorhynchus mykiss exposed for 60 days to different carbosulfan concentration compared to the positive control. [43] also observed specific-specific differences in following study where different metal concentrations (17, 25, 33, and 50 percent of LC50) were applied to the blood cells of *Cirrhinis mrigala*, *Labeo rohita*, *Ctenopharyngodon idella* and *Catla catla* for 30 days. After this exposure period the comet tail length, Genetic damage index and percentage of damaged cells were significantly elevated in the blood cells of fish. Exposure to contaminated water caused increased DNA damage in the RBCs of *Cyprinus carpio* [44]. It has been documented that a combination of pesticides (chlorpyrifos, endosulfan, and thiram) induces considerably more DNA damage [45]. Pesticides have been shown to suppress antioxidant thiram) induces considerably more DNA damage [45]. Pesticides have been shown to suppress antioxidant defenses and induce oxidative stress in freshwater species [46]. It is observed that the DNA damage may have been caused by DNA-DNA/DNA-protein cross linking, DNA single and double strand breaks or inhibition of repair enzymes caused by pesticides and metals or their metabolites interacting with DNA [47]. Extensive use of chlorpyrifos increases the pesticide load in the aquatic ecosystems thereby initiating an antagonistic effect on non-targeted organisms including fish [48]. Therefore, the long-lasting effect of chlorpyrifos on different species of fish has been widely observed by many researchers. According to [49] even a low dose of endosulfan is very lethal to fish.

Comparing DNA damage in *Oreochromis niloticus* peripheral blood erythrocytes across all sampling days

Exposure Duration	Treatments	Undamaged Nuclei (%)	Damaged Nuclei (%)						
		Type-0	Type-I	Type-II	Type-III	Type-IV	Damaged Nuclei (%) (II+III+IV)	*GDI	**CTL (µm)
14 Days	Negative Control	98.00±0.00 a	2.00±0.00 d	0.00±0.00 f	0.00±0.00 e	0.00±0.00 f	0.00±0.00 f	0.02±0.00 f	3.46±0.05 f
	Positive Control	34.00±2.00 d	14.67±1.15 c	20.67±1.15 bc	10.67±1.15 d	20.00±2.00 bc	51.33±1.15 c	1.68±0.04 c	148.21±0.09 e
	1/3rd of LC_{50}	12.67±1.15 f	23.33±3.06 b	24.00±2.00 a	18.00±0.00 a	22.00±2.00 ab	64.00±3.46 a	2.13±0.08 a	845.32±0.14 a
	1/4th of LC_{50}	24.67±1.15 e	22.67±1.15 b	20.00±2.00 c	14.67±1.15 bc	18.00±2.00 c	52.67±2.31 bc	1.79±0.06 b	813.15±0.08 b
	1/5th of LC_{50}	37.33±1.15 c	23.33±3.06 b	16.67±1.15 d	14.00±2.00 c	8.67±1.15 de	39.33±3.06 d	1.33±0.06 d	758.47±0.06 c
	1/6th of LC_{50}	46.00±2.00 b	26.67±3.06 a	10.67±1.15 e	10.00±2.00 d	6.67±1.15 e	27.33±1.15 e	1.05±0.01 e	509.83±0.05 d
28 Days	Negative Control	97.33±1.15 a	2.67±1.15 f	0.00±0.00 c	0.00±0.00 e	0.00±0.00 e	0.00±0.00 e	0.03±0.01 f	3.40±0.02 f
	Positive Control	24.00±2.00 d	16.67±1.15 e	20.67±1.15 a	18.67±1.15 c	20.00±2.00 b	59.33±1.15 b	1.94±0.04 c	157.74±0.12 e
	1/3rd of LC_{50}	10.67±1.15 e	22.67±1.15 ab	20.67±1.15 a	25.33±1.15 a	20.67±2.31 ab	66.67±2.31 a	2.23±0.09 a	879.66±0.05 a
	1/4th of LC_{50}	23.33±1.15 d	17.33±2.31 de	14.67±1.15 b	24.00±0.00 a	20.67±2.31 ab	59.33±1.15 b	2.01±0.05 bc	825.25±0.05 b
	1/5th of LC_{50}	28.67±2.31 c	20.67±1.15 b	15.33±1.15 b	20.67±1.15 bc	14.67±1.15 c	50.67±1.15 c	1.72±0.07 d	804.20±0.04 c
	1/6th of LC_{50}	40.67±1.15 b	18.00±2.00 cde	16.67±3.06 b	14.67±1.15 d	10.00±2.00 d	41.33±1.15 d	1.35±0.03 e	766.56±0.05 d
42 Days	Negative Control	96.67±1.15 a	3.33±1.15 e	0.00±0.00 f	0.00±0.00 e	0.00±0.00 f	0.00±0.00 e	0.03±0.01 f	3.34±0.04 f
	Positive Control	31.33±1.15 c	14.67±1.15 b	20.67±1.15 e	15.33±1.15 d	18.00±2.00 bc	54.00±2.00 c	1.74±0.05 cd	130.60±0.20 e
	1/3rd of LC_{50}	14.67±1.15 e	9.33±1.15 d	34.00±2.00 ab	19.33±1.15 bc	22.67±2.31 a	76.00±2.00 a	2.26±0.09 a	912.16±0.05 a
	1/4th of LC_{50}	18.00±2.00 d	12.00±2.00 c	32.00±2.00 b	20.67±1.15 abc	17.33±1.15 c	70.00±0.00 b	2.07±0.04 b	816.35±0.04 b
	1/5th of LC_{50}	32.00±2.00 bc	12.00±2.00 c	22.00±2.00 de	21.33±2.31 ab	12.67±2.31 d	56.00±2.00 c	1.71±0.09 d	796.00±1.00 c
	1/6th of LC_{50}	34.00±2.00 b	15.33±1.15 ab	22.67±1.15 cde	18.67±1.15 c	9.33±1.15 e	50.67±1.15 d	1.54±0.06 e	785.58±0.07 d
56 Days	Negative Control	98.00±0.00 a	2.00±0.00 f	0.00±0.00 f	0.00±0.00 e	0.00±0.00 f	0.00±0.00 f	0.02±0.00 f	3.38±0.09 f
	Positive Control	30.67±1.15 c	17.33±1.15 a	20.00±2.00 e	14.00±2.00 d	18.00±2.00 c	52.00±2.00 e	1.71±0.04 d	124.53±0.09 e
	1/3rd of LC_{50}	4.67±1.15 f	14.67±1.15 bc	35.33±1.15 b	20.67±1.15 b	24.67±1.15 a	80.67±1.15 a	2.46±0.03 a	1018.52±0.12 a
	1/4th of LC_{50}	16.67±1.15 e	11.33±1.15 d	40.00±2.00 a	20.67±1.15 b	11.33±2.31 d	72.00±0.00 b	1.99±0.03 c	902.38±0.08 b
	1/5th of LC_{50}	18.67±1.15 de	13.33±3.06 cd	30.67±1.15 c	16.67±1.15 c	20.67±1.15 b	68.00±2.00 c	2.07±0.03 bc	825.14±0.10 c
	1/6th of LC_{50}	38.67±1.15 b	6.67±1.15 e	24.67±1.15 d	22.67±3.06 a	7.33±1.15 e	54.67±1.15 de	1.53±0.02 e	800.07±0.05 d
70 Days	Negative Control	98.00±0.00 a	2.00±0.00 e	0.00±0.00 e	0.00±0.00 f	0.00±0.00 f	0.00±0.00 f	0.02±0.00 e	3.41±0.11 f
	Positive Control	30.67±1.15 c	17.33±1.15 ab	21.33±1.15 d	16.00±2.00 cde	14.67±3.06 b	52.00±2.00 d	1.67±0.08 c	118.68±0.08 e
	1/3rd of LC_{50}	14.67±1.15 e	8.67±1.15 d	24.67±3.06 c	32.00±2.00 a	20.00±2.00 a	76.67±2.31 a	2.34±0.07 a	983.93±0.06 a
	1/4th of LC_{50}	22.00±2.00 d	15.33±1.15 bc	32.00±2.00 b	19.33±1.15 b	11.33±1.15 c	62.67±1.15 bc	1.83±0.06 b	855.23±0.13 b
	1/5th of LC_{50}	22.00±2.00 d	17.33±1.15 ab	40.00±2.00 a	14.00±2.00 e	6.67±1.15 e	60.67±1.15 c	1.66±0.05 c	810.67±0.07 c
	1/6th of LC_{50}	38.67±1.15 b	14.67±1.15 c	24.00±2.00 c	15.33±2.31 de	7.33±1.15 de	46.67±1.15 e	1.38±0.02 d	614.62±0.12 d
84 Days	Negative Control	98.00±0.00 a	2.00±0.00 d	0.00±0.00 f	0.00±0.00 f	0.00±0.00 e	0.00±0.00 e	0.02±0.00 f	3.42±0.04 f
	Positive Control	28.00±2.00 d	17.33±1.15 b	20.00±2.00 e	14.67±1.15 bc	20.00±0.00 a	54.67±1.15 b	1.81±0.03 b	136.82±0.06 e
	1/3rd of LC_{50}	19.33±1.15 f	9.33±1.15 c	42.67±2.31 a	18.00±2.00 a	10.67±1.15 b	71.33±2.31 a	1.91±0.05 a	617.00±1.00 a
	1/4th of LC_{50}	24.67±1.15 e	19.33±1.15 ab	38.67±1.15 b	13.33±2.31 cd	4.00±2.00 d	56.00±2.00 b	1.53±0.05 c	592.78±0.07 b
	1/5th of LC_{50}	31.33±1.15 c	20.00±2.00 a	34.67±1.15 c	10.67±1.15 e	3.33±1.15 d	48.67±3.06 c	1.35±0.07 d	514.14±0.03 c
	1/6th of LC_{50}	40.67±1.15 b	19.33±2.31 ab	22.00±2.00 de	11.33±1.15 de	6.67±1.15 c	40.00±2.00 d	1.24±0.03 e	467.92±0.06 d

Table 1: DNA damage in peripheral erythrocytes of Oreochromis niloticus exposed to chlorpyrifos + endosulfan + bifenthrin mixture

Concentrations	Undamaged Nuclei (%)	Damaged Nuclei (%)						
	Type-0	Type-I	Type-II	Type-III	Type-IV	Damaged Nuclei (%) (II+III+IV)	*GDI	**CTL (µm)
Negative Control	97.67±0.60 a	2.33±0.60 d	0.00±0.00 d	0.00±0.00 e	0.00±0.00 f	0.00±0.00 e	0.02±0.01 f	3.40±0.03 f
Positive Control	29.78±0.46 c	16.33±0.00 b	20.56±0.44 c	14.89±0.44 d	18.44±1.00 b	53.89±0.46 c	1.76±0.02 c	136.10±0.05 e
1/3rd of LC_{50}	12.78±0.00 f	14.67±0.78 c	30.22±0.72 a	22.22±0.74 a	20.11±0.53 a	72.56±0.74 a	2.22±0.02 a	876.10±0.38 a
1/4th of LC_{50}	21.56±0.44 e	16.33±0.53 b	29.56±0.44 a	18.78±0.73 b	13.78±0.53 c	62.11±0.97 b	1.87±0.01 b	800.86±0.03 b
1/5th of LC_{50}	28.33±0.53 d	17.78±0.85 a	26.56±0.44 b	16.22±0.53 c	11.11±0.47 d	53.89±0.85 c	1.64±0.02 d	751.44±0.39 c
1/6th of LC_{50}	39.78±0.44 b	16.78±0.79 ab	20.11±0.76 c	15.44±0.79 cd	7.89±0.35 e	43.44±0.35 d	1.35±0.02 e	657.43±0.03 d

Table 2: Dose dependent DNA damage in peripheral erythrocytes of Oreochromis niloticus exposed to chlorpyrifos + endosulfan + bifenthrin mixture.

revealed a consequent rise in the DNA damage at 14th - 56th day of exposure, accompanied by a decrease after 70 and 84 days exposure. Maximum damage to DNA observed at 14th day of exposure DNA damage on the 14th day at fractions of the herbicide's LC50 concentrations (1/10th, 1/8th, and 1/5th) [50]. The time based deterioration in damage might be due to DNA damage repair, the loss of severely damaged cells, or a combination of the two. Subsequently, the same group demonstrated that comparable doses of chlorpyrifos caused improvements in the gill structure and respiration rate of Cyprinus carpio. [51] also observed a dose-dependent response of Major Carps to individual pesticides, chlorpyrifos, bifenthrin and endosulfan in terms of nuclear change in their blood cells. Similarly, [52] also investigated the genotoxic effect of mixture (chlorpyrifos + endosulfan + bifenthrin) on Cyprinus carpio (peripheral erythrocytes) at various concentrations and durations. Of all exposure concentrations, 1/3rd of the LC50 caused the highest DNA damage. A concomitant increase for percentage of GDI, genetic damage index and damaged nuclei in fish peripheral erythrocytes was observed by an increase in the period of pesticide mixture exposure, from day 15 to day 30. The genotoxic effect not only decreases fish population fitness, but also poses a risk to mankind through the food supply chain [53].

Present investigation represented an unprecedented approach to study genotoxic effects of pesticides on fish. The widespread application of pesticides (chlorpyrifos, endosulfan, bifenthrin) in agriculture sector exerts adverse effects on various non-target organisms via

trophic transfer that ultimately pose a serious threat for human beings. Although an acute and chronic toxicity of single pesticide has extensively been studied in various species of fish, but data regarding toxic effects of pesticides mixtures on fish is still lacking. The results of present investigation revealed that the exposure of pesticide mixture (chlorpyrifos + endosulfan + bifenthrin) exert severe genotoxic effects on fish at different concentration levels. Current findings suggested minimized and sensible use of pesticides to avoid genetic threats to aquatic fauna. Moreover, pest control methods other than chemical control must be practiced maintaining sustainable agriculture and aquaculture.

Competing Interest

The authors declare that there is no conflict of interest.

Author Contributions

FA designed and conducted research, and recorded data. MAHH statistically analyzed the data. SA and SK drafted the manuscript. FL and MJ proof read the manuscript.

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