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PESTICIDE INDUCED HEMATOLOGICAL, BIOCHEMICAL AND GENOTOXIC CHANGES IN FISH: A REVIEW

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ABSTRACT

Pesticides are widely used in agricultural advancement sector of entire world for increasing crop yield. However, its exposure is not limited only to target organisms instead it is affecting various non-target organisms among which fish being the most prominent one. In severe cases acute amount of various pesticides caused death of fish while lethal changes observed in case of lower amount of these pesticides. Changes in hematological parameters like red blood cells, white blood cells or plasma and serum level alterations leading to histological changes involving liver, kidneys, gills, muscles, brain, intestine in many species of fish exposed to different pesticides. Moreover, genotoxicity was also observed in many cases induced by different categories of pesticides. Extensive and continuous usage of these toxicants affecting the aquatic systems at severe level as a result getting bio-accumulated in food chain. This article emphasized over the pesticidal induced hematological and serum level alterations observed in fish.

Keywords: Pesticides, fish, toxicity, hematological changes, serological changes.

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1. INTRODUCTION

Industrialization and novel technologies are vital for expedition of success and comfort in present time, but they are decreasing valuable natural water resources. Increase in industries, modernization and urbanization eventually lead us to the contamination of our surroundings. Fresh water is enormously substantial to sustain life as it is use for human consumption, production industries, irrigation of crops and for supporting biodiversity. But these ecosystems are at the stake of extreme sufferers of biodiversity as it is vulnerable to environmental contaminants including pesticides (Gupta et al. 2008; Geist 2011; Pisa et al. 2015; El-Murr et al. 2015; Javed and Usmani 2015). Pesticides are the prominent pollutant as these are widely used for purpose of protection of crops but unluckily these chemicals are not specific to targeted organisms. Continuous and haphazard introduction of these chemicals can make innumerable irregularities in a variety of aquatic organisms and contamination of water bodies (Zaluski et al. 2015; Gul et al. 2017). It is well-recognized that these constituents react with blood components and genomic material so lead to changes at cellular level in exposed species. Several abnormalities in living organisms like apoptosis, hypospadias, developmental testicular anomalies as well as reproductive status and organ of fish observed due to contamination by residues of pesticides used in agricultural products (Witeska et al. 2014; Gibbons et al. 2015; Qureshi et al. 2016).

Several threats to freshwater ecosystems are change in climate, nutrient fluctuation, acidification, loss of habitat, exploitation and biological invasions and chemical contamination. Basis of chemical stress is undifferentiating and common use of pesticides and heavy metals, mainly in the agricultural sector, which leads to pollution of water bodies, so it is dangerous for aquatic life (Kalavathy et al. 2001; Barbieri 2009; Schäfer et al. 2011). Water contamination with enormous quantities of pesticides cause fish mortality or starvation due to deterioration of food organism. Furthermore, various toxicants observed to influence the growth parameters, reproduction, and tissue damage (Srivastav et al. 2002).

Fish mimic to situation of water quality and pollution since they existed at the lowest level of food chain of aquatic bodies. They can obtain and retain chemicals like heavy metals and pesticides through submissive phenomena so pollutant in their environment can be identified. Fish consume greater amount of algae, phytoplankton and different aquatic plant infected with pesticide, which consequently lead these chemicals to gradually accumulate in tissues and organs of fish. Metabolism can regulate little amount of these chemicals while remaining one get bio-accumulated in the organs and organs system of fish. Hematological and serological parameters are significant for measurement of pathophysiological condition of fish. These parameters extensively used as indicators of infection or stress caused by contaminants because hematological profile exhibit the internal body situation before any prominent disease identification (Ali and Rani 2009). Gills, skin or alimentary canal mainly absorbs different pollutants so they can diffuse into other organs and tissues ultimately affecting physiological and natural phenomena of fish (Banaee et al. 2008). Gills are the most affected organs due to pollutants as these are entirely exposed organs to water. Entrance of toxicants in body is done through gills so consumption of oxygen increases. As a result, it is significant parameter to observe any toxic stress in aquatic environment (Panigrahi et al. 2014).

1.1. Effects on Aquatic System

Addition of unwanted substances into the aquatic sources cause alterations in the physical, chemical and biological features leading to the ecologic imbalance (Yadav et al. 2018a). Effluents containing pesticide and heavy metals contributed greatly to water pollution creating threat for aquatic life (Ramana et al. 2001; Gupta et al. 2015). Pesticides are those stressors which get accumulated in the organ of fish either by the food chain or by absorption through the body surface so rigorously influenced the life-supporting system at molecular and biochemical levels. Many of the pesticides show bioaccumulation capacities with broad spectrum impacts and pressures on aquatic life (Censi et al. 2006; Maurya and Malik 2016a, Yadav et al. 2018b). Occurrence of pesticides in water bodies caused by various ways but three important routes were assessed owing to which it found its significant way to aquatic systems (Kosygin et al. 2007; Sarkar et al. 2008). These significant paths include water pathways, organic substrates like vascular hydrophytes, branches, mosses, algae, leaf litter and inorganic substrates comprising materials from sediments with different sizes (Murthy et al. 2013).

Humans living near water bodies use water for decontamination and waste removal of society. Biological and physical processes affecting water quality cause water pollution leading to deleterious influences on health and composition of aquatic sources. Indiscriminate use of pesticides to improve agricultural practices may have impacts on non-target organism's especially aquatic lives which can ultimately pose a serious threat to the health of human communities (Ambreen and Javed 2018). Enhanced amount of toxic substances and pollutants in fresh water have threatened numerous freshwater flora and fauna including fish. Likewise, it is detrimental for human health causing disorders and fourteen thousand deaths on daily basis (Reddy and Behera 2006). Direct emission of domestic and industrial wastes into water bodies without any processing leads to water contamination. Various pollutants like heavy metals, pesticides, herbicides, radioactive matter and corrosive material are causing pollution of water bodies. Change in the physicochemical parameters of aquatic sources influence the metabolism and homeostasis of aquatic life and disturbance in food web consequently (Pisa et al. 2015).

1.2. Effects on Blood Parameters

Hematological research of fish have gain great significance because these factors were used as an effective and sensitive index for evaluation of physiological and pathological alteration caused by natural or anthropogenic aspects like bacterial or fungal infection or contamination level of aquatic sources. Hematological parameters therefore considered as important tool for identifying functional status of the body in response to different stressors (Ali and Rani 2009). Pesticides usually made relatively rapid alterations in hematological parameters of fish (Rezania et al. 2018). Therefore, hematologic index can be used effectively for monitoring the health and response of fishes and aquatic organisms to different toxicants exhibiting the ecologic position of the habitat and common technique to decide the sub-lethal effects of the contaminant (Pimpao et al. 2007). Rios et al. (2002) studied that blood parameters in fish got affected by features like sex, age, size, reproductive stage, health and external aspects like seasonal dynamics, water temperature, quality of environmental, food and stress (Hrubec et al. 2008). Pesticides influence numerous characteristics of fish with prominent effects on the blood parameters. Various studies revealed the different toxic effects of different pesticides on the hematological parameters of fish species (Table 1; Fig.1 to Fig. 9).

Hematological parameters are good indicator of severe impacts of many toxic compounds mainly pesticides and industrial effluents containing heavy metals so these parameters are sign of internal homeostasis and physiological condition of exposed organisms. Prominent decrease in red blood cells, hematocrit and hemoglobin observed mainly on exposure to fipronil, which exhibits the anemic condition of fish. Reduced hemoglobin was maybe due to its oxidation to methemoglobin, less gaseous exchange and free radical-induced damage. In addition, reduced values of blood parameters are marker for the poor role of hematopoietic tissues, inappropriate osmoregulatory mechanisms and enhanced damage to RBCs in blood forming organs (Jenkins et al. 2003; Ghaffar et al. 2019). In another case, Ghaffar et al. (2019) exposed *Labeo rohita* to 0.03-0.15mg/L of fipronil for nine days. Indices of erythrocytes, lymphocytes, and monocytes reduced whereas total leukocyte counts, and neutrophils increased prominently. Erythrocytes exhibited a variety of nuclear irregularities. Moreover, Ghaffar et al. (2018) investigated the toxic impacts of fipronil on *Cyprinus carpio* treated with different concentrations (0 - 0.10mg/L) for 12 days. Fish in high doses treated groups exhibited severe irregularities in clinical-hematological and biochemical parameters. Erythrocyte counts, hemoglobin, and hematocrit were reduced mainly and mean corpuscular volume, total leukocyte count, neutrophils, monocytes, and lymphocytes were mainly increased.

Babu et al. (2016) carried out the experiment to assess the hematological influence of Cypermethrin (0.015-0.04mg/L) on *Anabas testudineus*. Decrease in RBC counts, hemoglobin levels, hematocrit levels and platelet counts observed. WBC counts increased after 7 days but WBC counts reduced with the increased cypermethrin at 14th and 21st days. Similarly, Ghaffar *et al*. (2015b) studied the same effects over blood parameters induced by triazophos in *Labeo rohita* (0.010-0.200ppm). Nevertheless, Ghaffar et al. (2015a) studied the effects of butachlor in *Labeo rohita* (0-1.0mg/L). Significantly increased morphological and nuclear changes like pear shape erythrocyte, microcyte, tear shape erythrocyte, erythrocytes with micronuclei, lobed, blebbed and notched nuclei and cells with nuclear remnants were observed.

Pesticides like DDT, BHC, aldrin, dieldrin, chlordane, permethrin, cypermethrin, karate, delmethrin sulfane, endosulfan etc. have affected the blood parameters like histological variation in white blood cells and red blood cells, amount of hemoglobin and packed cell volume of various species. Hematological changes were observed in *Cyprinus carpio* and *Puntius ticto* (Satyanarayan et al. 2004), *Tor putitora* (Ullah et al. 2014a) and *Oreochromis mossambicus* induced by potassium chlorate and potassium dichromate (Sivanatarajan and Sivaramakrishnan 2013), in *Onchorhynchus mykiss* (Saeedi et al. 2012) and *Cyprinus carpio* (Svoboda et al. 2001) due to Diazinon, in *Mystus keletius* due to methyl parathion (Sampath et al. 2003) and in *Labeo rohita* due to cypermethrin (Adhikari et al. 2004). Similarly, chlordane caused chronic malfunctioning of hemopoietic system of *Labeo rohita*. Similar effects were seen in *Heteropneustes fossilis, Channa punctatus* and *Labeo rohita* induced by the dimecron (Anandkumar *et al.* 2001) and endosulfan (Bhatia et al.2002, 2004; Devi et al. 2008) and furadon (Bhatkar and Dhande 2000). Various other similar results were observed in many other studies with different pesticides (Joshi et al.2002; Johal and Grewal 2004; Gautam and Kumar 2008). Therefore, hematological parameters are good indicator of severe impacts of pesticides indicating the internal homeostasis and physiological condition of exposed organisms (Jenkins et al. 2003).

Table 1: Hematological changes observed in various fish species induced by different pesticides

Pesticide Used	Dose	Fish Species	Hematological Findings	References
2, 4-dichlorophenoxyacetate	100mg/L	*Carassius auratus*	Reduction in lymphocytes	Kubrak et al. (2013)
Aldrin, dieldrin, DDT, BHC and chlordane	Sublethal dose	*Cyprinus carpio* and *Puntius ticto*	Fluctuation in level of Hb. Decrease in RBC. Rise of PCV (in case of aldrin and dieldrin) and reduction (in case of DDT, BHC and chlordane.	Satyanarayan et al. (2004)
Atrazine	428µg/L	*Cyprinus carpio*	Increase in MCV and decline of RBC, Hb, PCV, WBC, Lymphocyte, Neutrophil and monocytes	Khalil et al. (2017)
Atrazine	30mg/L	*Cyprinus carpio*	Decline of WBC, lymphocytes, Hb and HCT. Rise in monocyte	Blahova et al. (2014)
Butachlor	0.5–50µg/L	*Cyprinus carpio*	Rise in MCV, MCHC, WBC and decline of RBC, Hb and PCV	Saravanan et al. (2017)
Butachlor	0-1.0mg/L	*Labeo rohita*	Decreased RBC, Hb, HCT, and lymphocyte. Increased TLC. Morphological and nuclear changes like pear shape erythrocyte, microcyte, tear shape erythrocyte, micronuclei, lobed, blebbed and notched nuclei	Ghaffar et al. (2015a)

Butachlor	0.39mg/L	*Oncorhynchus mykiss*	Rise in neutrophils and decline of RBC, Hb, WBC, Lymphocytes	Ahmadivand et al. (2014)
Captan	0.26–0.68mg/L	*Ctenopharyngodon idella*	Rise in level of MCV, MCH, neutrophils, monocytes, eosinophils and decline of RBC, Hb, PCV, MCHC and WBC	Mohammadalikhani et al. (2017)
Carbaryl	Sublethal dose	*Channa punctatus*	Decrease in RBC, HB and HCT	Johal and Grewal (2004)
Chlorinated pesticides	Heptachlor (23.24-28.93ng/L), Aldrin (11.98-17.35 ng/L)	*Heteropneustes fossilis*	Decline level of RBC, Hb, PCV. Rise in WBC. Altered level of MCH, MCV, and MCHC	Maurya et al. (2019)
Chlorpyrifos	0.25-1.25ppb	*Oreochromis mossambicus*	Decline in RBC, Hb and HCT. Rise in WBC and platelets.	Ghayyur et al. (2019)
Copper oxychloride	32.3mg/L	*Oreochromis niloticus*	Decline of Hb and HCT	Hassaan et al. (2014)
Cypermethrin	2.05×10^{-3} mg/L	*Oncorhynchus mykiss*	Fluctuations in MCH and MCHC levels	Uçar et al. (2020a)
Cypermethrin	Sublethal dose	*Tor putitora*	Decreased RBC, Hb, HCT.	Ullah et al. (2014a)
Cypermethrin	1.6µL/L	*Labeo rohita*	Reduction of WBC, RBC, Hb and HCT. Increased TLC, MCV and MCH.	Adhikari et al. (2004)
Deltamethrin	15µg/L	*Oreochromis niloticus*	Reduced WBC, RBC, Hb.	Dawood et al. (2020)
Deltamethrin	0.058mg/L	*Cyprinus carpio*	Reduced RBC, PCV, Hb. Alterations in MCV, MCH, MCHC, leukocyte, lymphocytes, monocytes, neutrophil and their developmental forms	Svoboda et al. (2003)
Diazinon	1.65mg/L	*Oncorhynchus mykiss*	Reduction of WBC, RBC, PCV, Hb. Fluctuation in lymphocyte and neutrophil level	Saeedi et al. (2012)
Diazinon	26.7mg/L	*Cyprinus carpio*	Reduction of PCV, RBC, Hb, lymphocyte, leukocyte. Increase in neutrophils. Disturbed hematopoiesis and non-specific immunity.	Svoboda et al. (2001)
Dichlorvos	Sublethal dose	*Channa punctatus*	Decrease in RBC, HB and HCT	Gautam and Kumar (2008)
Endosulfan	0.0004ppm	*Channa punctatus*	Reduction of MCV, RBC, Hb and neutrophils. Increase in WBC, lymphocytes and monocyte.	Devi et al. (2008)
Envoy 50 SC	0.014–0.198ppm	*Heteropneustes fossilis*	Reduction in RBC. Dead, fused, binucleated, tear-shaped cells	Akter et al. (2020)
Ethofumesate	0.11mg/L	*Cyprinus carpio*	Rise in RBC, Hb, PCV, WBC. Fluctuations in level of neutrophils	Lutnicka et al. (2017)
Fipronil	1/10th LC_{50}	*Oncorhynchus mykiss*	Alterations in blood parameters	Uçar et al. (2020b)
Fipronil	0.03-0.15mg/L	*Labeo rohita*	Reduction of RBCs, lymphocytes, and monocytes. Increase in total leukocyte counts, and neutrophils. Nuclear irregularities in RBCs.	Ghaffar et al. (2019)
Fipronil	300–400µg/L	*Rhamdia quelen*	Decline of HCT and platelets	Fredianelli et al. (2019)
Fipronil	0 - 0.10mg/L	*Cyprinus carpio*	Reduced RBCs, Hb, HCT. Increased MCV, TLC, neutrophils, monocytes and lymphocytes.	Ghaffar et al. (2018)
Fipronil and buprofezin	400µg/L; 100mg/L	*Cyprinus carpio*	RBC, Hb, HCT and MCH decreased and WBC increased	Qureshi et al. (2016)
Furadon	0.5ppm	*Labeo rohita*	RBC, Hb, HCT and MCH decreased and total leukocytes, MCV increased	Bhatkar and Dhande (2000)
Glyphosate	0.02mg/L	*Cyprinus carpio*	Increase in PCV and decrease in WBC	Kondera et al. (2018)

Glyphosate	12.21mg/L	*Anabas testudines*	Increase in MCV, MCH, WBC, platelets and reduction of RBC, Hb, PCV and lymphocytes	Samanta et al. (2019)
Glyphosate	12.21mg/L	*Heteropneustes fossilis*	Increase in MCV, MCH, MCHC, WBC, platelets and reduction of RBC and Hb	Samanta et al. (2019)
Isoprothiolane	2.7 and 27µg/L	*Cyprinus carpio*	Rise in level of WBC and decrease in RBC, HB and HCT	Saravanan et al. (2015)
Mancozeb	1mg/L	*Cyprinus carpio*	Fluctuation in levels of RBC, Hb, PCV. Rise in WBC and neutrophils. Decline in MCHC and lymphocytes	Lutnicka et al. (2017)
Methyl parathion	0-200ppb	*Mystus keletius*	Reduction of MCV, RBC, Hb and thrombocyte. Increased TLC, ESR. Anemia, inhibition of erythropoiesis and hemodilution.	Sampath et al. (2003)
Monocrotophos	2.14mg/L	*Clarias batrachus*	Reduced RBCs, Hb, PCV	Narra et al. (2017)
Paclobutrazol	1.18; 2.36mg/L	*Oreochromis mossambicus*	Decline observed in level of RBC, Hb, PCV, MCV, MCH, MCHC, WBC	Ghane et al. (2017)
Paraquat	0.37–1.12mg/L	*Mesopotamichthys sharpeyi*	Increase in RBC, PCV, MCV, MCH, MCHC, WBC. Decline in Hb	Hashemi et al. (2017)
Potassium chlorate and potassium dichromate	-	*Oreochromis mossambicus*	Decreased RBC, Hb, HCT.	Sivanatarajan and Sivaramakrishnan (2013)
Prochloraz	1mg/L	*Cyprinus carpio*	Rise in WBC and neutrophils. Decline in RBC, Hb, PCV. MCH, MCHC and lymphocytes	Lutnicka et al. (2017)
Propiconazole	0.89µL/L	*Labeo rohita*	Rise in MCV, MCHC and decline in RBC, Hb, PCV and WBC	Hemalatha et al. (2016)
Tebuconazole	2.5mg/L	*Cyprinus carpio*	Rise in Hb, PCV, WBC and neutrophils. Decline in lymphocytes	Lutnicka et al. (2017)
Thiamethoxam	0-2.0mg/L	*Labeo rohita*	Decreased RBC, Hb, HCT, and lymphocyte. Increased WBC and neutrophil. Morphological alterations like leptocytes, stomatocytes, and tear shape erythrocyte.	Ghaffar et al. (2020)
Triazophos	0.010-0.200ppm	*Labeo rohita*	Reduction in RBCs, PCV, Hb, MCHC, MCV, lymphocyte and monocyte. Rise in leukocyte count.	Ghaffar et al. (2015b)

1.3. Effects on Biochemical Parameters

Research based on serological parameters considered important for estimating the lethal impacts of chemicals in target organs of fish in laboratory and field research (Wester and Canton 1991). Gills are the crucial site for oxygen uptake in fish and delicate organs as these organs are exposed to chemical toxins increasing the stress. In similar manner liver is detoxification site and intestine by which all the contaminants pass through faced hematological and serum level alterations requiring the studies of different organs of aquatic animals exposed to different toxicants (Meyers and Hendricks 1985). Various studies have exhibited the serum level investigations as a trustworthy biomarker of stress in fish (Maurya and Malik 2016a; Maurya and Malik 2016b).

Toxicants made severe pathological alterations in fish mainly gill lesions and protein level changes which are indications of exposure to pesticides (Peebua et al. 2008; Kaoud and El-Dahshan 2010; Maurya and Malik 2016a). Prominent changes in liver after exposure to heavy metals of pesticides have altered the changes in levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) (Peebua et al. 2008). In addition, histopathological alterations in gills, liver, kidneys and gonads of fish due to agricultural sewage and industrial pollutants have been observed causing changes in the serum levels (Mohamed 2003). In another case, hematological deterioration in liver caused by lack of oxygen lead to gill degeneration, dilation of vessels and intravascular hemolysis with consequent stasis of blood. Vacuolar deterioration, hemorrhages, necrosis, degeneration of hepatocytes and pyknosis were observed in *Labeo rohita* and *H. fossilis* owing to presence of heavy metals and pesticides indicating strong link of liver damage with toxicants (Loganathan et al. 2006; El-Naggar et al. 2009; Kalita et al. 2012). Different studies exhibit the different toxic effects of various pesticides over biochemical parameters of fish (Table 2).

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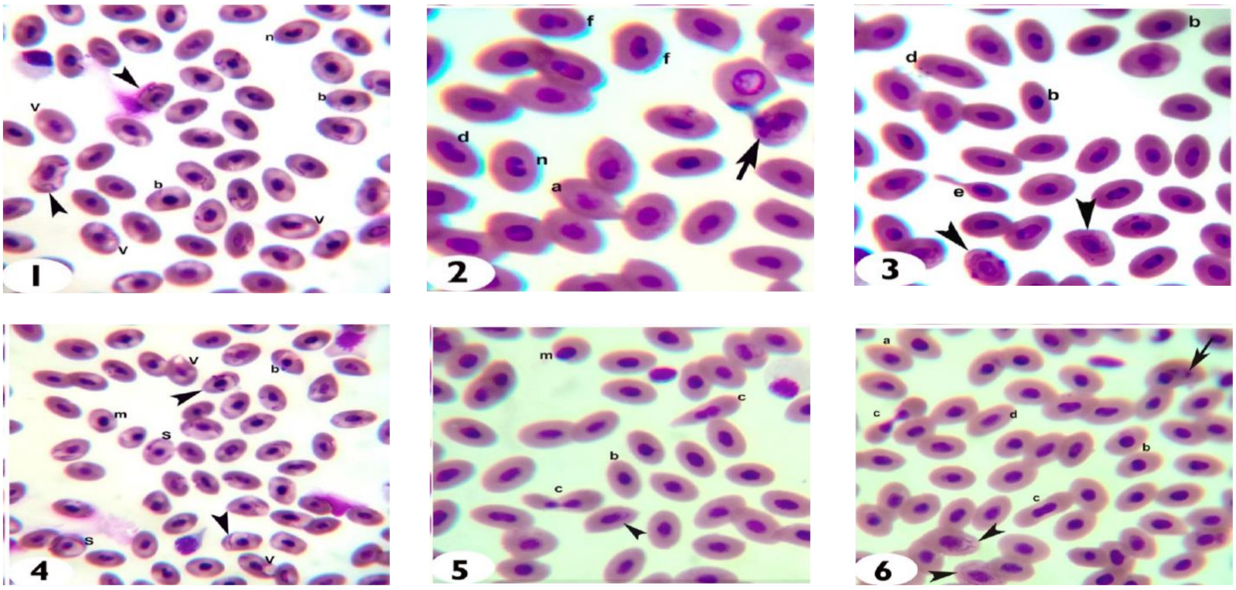

Fig. 1-6: Blood smears of butachlor treated fish showing morphological and nuclear alterations of RBCs like nuclear remnants (arrow heads), micronucleus (arrow), pear shaped (a), condensed nucleus (b), dividing erythrocyte (c), leptocyte (d), tear shaped erythrocyte (e), fragmented nucleus (f), microcyte (m), notched nucleus (n), stomatocyte (s) and cytoplasmic vacuolation (v). Wright-Giemsa stain:1000x. Ghaffar *et al. (*2015a).

Extensive research has revealed the greater impact of pesticides over the protein contents and serological changes in various tissue like gills, liver, blood, intestine and muscle of fish. Nickel chloride resulted in significant reduction in serum protein content in liver, gonads and muscles of *Anabus testudineus* and phenyl mercuric acetate leads to decrease in protein content of muscles and liver of *Channa punctatus* (Karuppasamy 2000) while the same species exhibited low protein level on exposure to oleandrin (Tiwari and Singh 2004). Reduced protein contents observed in liver of *Lepidocephalicthus thermails* by copper containing pesticides, in *Cirrhinus mrigala* by lead acetate (Ramalingam et al. 2000) and in *Cyprinus carpio* by endosulfan (Jenkins et al. 2003). Cypermethrin exposure caused prominent reduction in protein contents of *Tor putitora* and *Colisa fasciatus* (Singh et al. 2010; Ullah et al. 2014b). Malathion reduced the protein contents of rohu and *Clarias batrachus* (Khare and Singh 2002; Thenmozhi et al. 2011).

Thiamethoxan and thiodon influenced the total protein content in liver of Nile tilapia and *Clarias gariepinus* respectively (Aguigwo 2002; Bose et al. 2011). Dichlorvos exhibited greater impact in tissue glycogen, total protein and albumen content in liver, kidneys and muscles of *Oreochromis mossambicus* (Lakshmanan et al. 2013). *Clarias batrachus* have shown the pesticidal mixture induced changes in protein content (Jha and Verma 2002). Similarly, karate reduced the protein contents of common carp (Bibi et al. 2014) while monocrotophos reduced the protein, lipid and carbohydrate content in different tissues of rohu (Muthukumaravel et al. 2013).

Dioxin interacts with DNA in a complex pathway to change how genes control synthesis of protein such as vitellogenin protein for egg development (Zorriehzahra 2008). El-Murr et al. (2015) conducted experiment to assess the impacts of various concentrations of fipronil on health of *Oreochromis niloticus* by evaluation of biochemical and hematological parameters investigation. Mortality, pale gills, congestion and hemorrhages of various internal organs observed. Noteworthy reduction in level of Immunoglobulin M and lysozyme with simultaneous increase in level of serum nitric oxide was observed. Noteworthy increase in serum level of AST, ALT and cortisol in all the exposed groups was observed. Similarly, Ghaffar et al. (2015) studied the serum analysis revealed that amount of various enzymes and lipid peroxidation products were enhanced. So triazophos caused severe hemato-biochemical damage in aquatic organisms. Ghaffar *et al*. (2019) exposed *Labeo rohita* to 0.03-0.15mg/L of fipronil for nine days. Relative weight of kidneys, gills, heart, and brain were mainly decrease. Gills and kidneys exhibit severe lesions. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) enhanced mainly. So fipronil induced severe clinical signs and adverse hemato-biochemical changes in *Labeo rohita*, even at low concentrations. Therefore, serum level changes due to stressors are also important for the assessment of toxicity of xenobiotics affecting the vital proteins of aquatic organisms and fish (Maurya and Malik 2016a).

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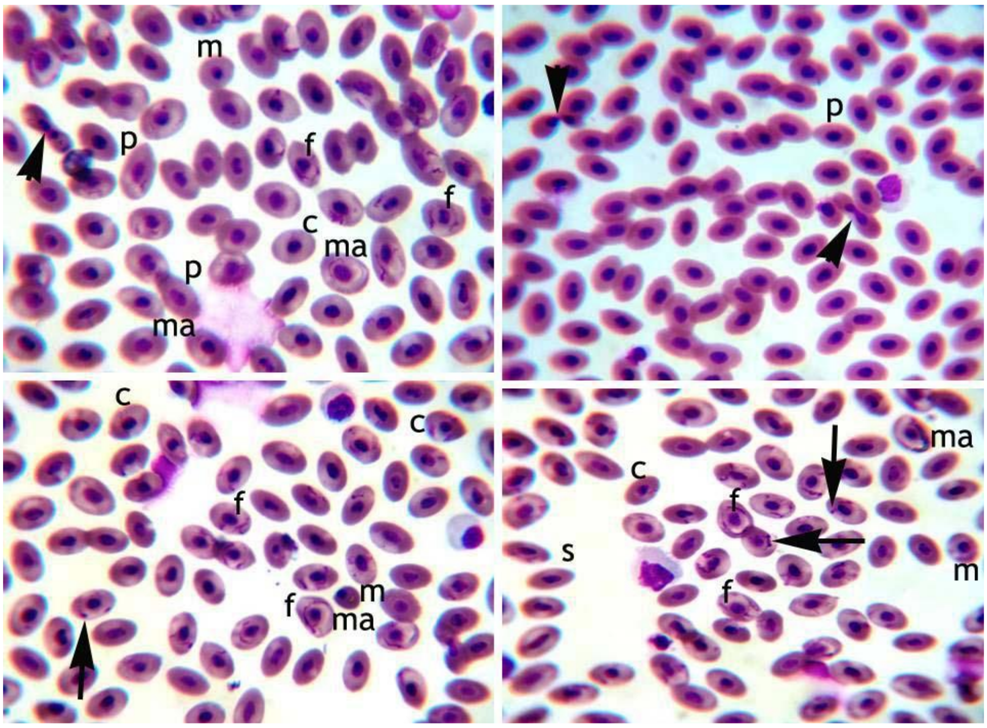

Fig. 7: Blood smear of *Labeo rohita* treated with thiamethoxam showing various nuclear and morphological changes in red blood cells, i.e., microcytes (m), macrocytes (ma), pear shaped erythrocytes (p), spindle shaped erythrocytes (s), dividing erythrocytes (arrow-heads), erythrocytes with condensed nucleus (c), erythrocyte with micronucleus (arrows) and with fragmented/nuclear remnants (f). Giemsa Stain; X1000. Ghaffar *et al.* (2020).

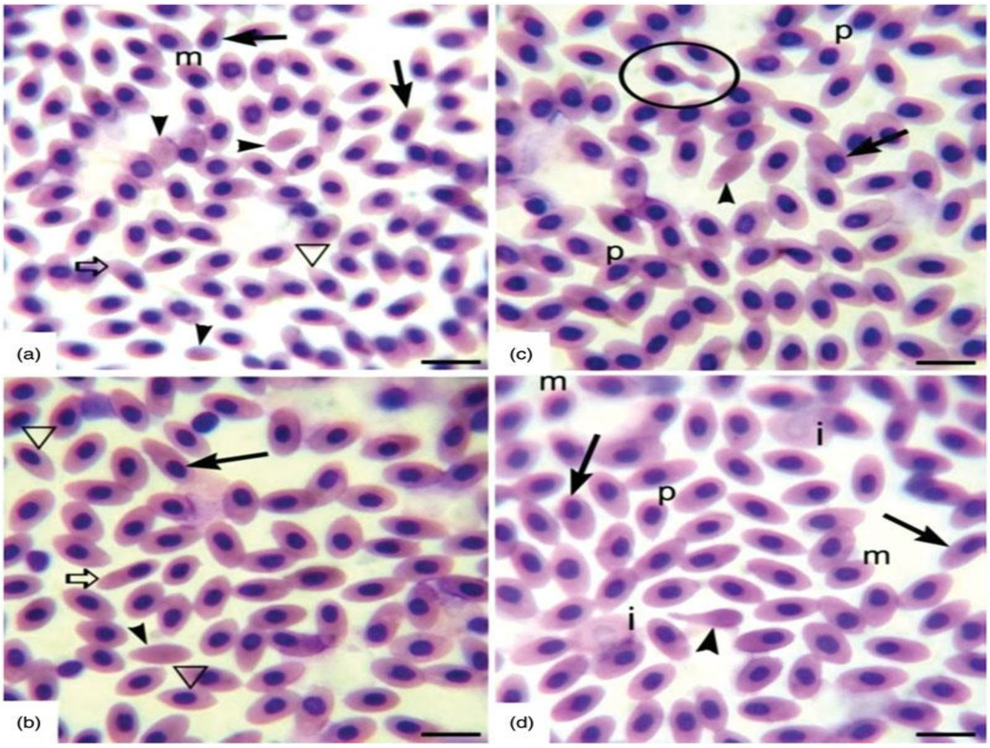

Fig. 8: *Labeo rohita* treated with fipronil exhibiting changes in red blood cells i.e., eccentric nuclei (filled arrows), erythrocytes without nuclei (filled arrow-heads), leptocytes (hollow arrows), spindle shaped erythrocytes (hollow arrow-heads), microcytes (m), pear-shaped cells (p), immature erythrocytes (i) and protruding cytoplasm (encircled). Giemsa Stain. Ghaffar *et al.* (2019).

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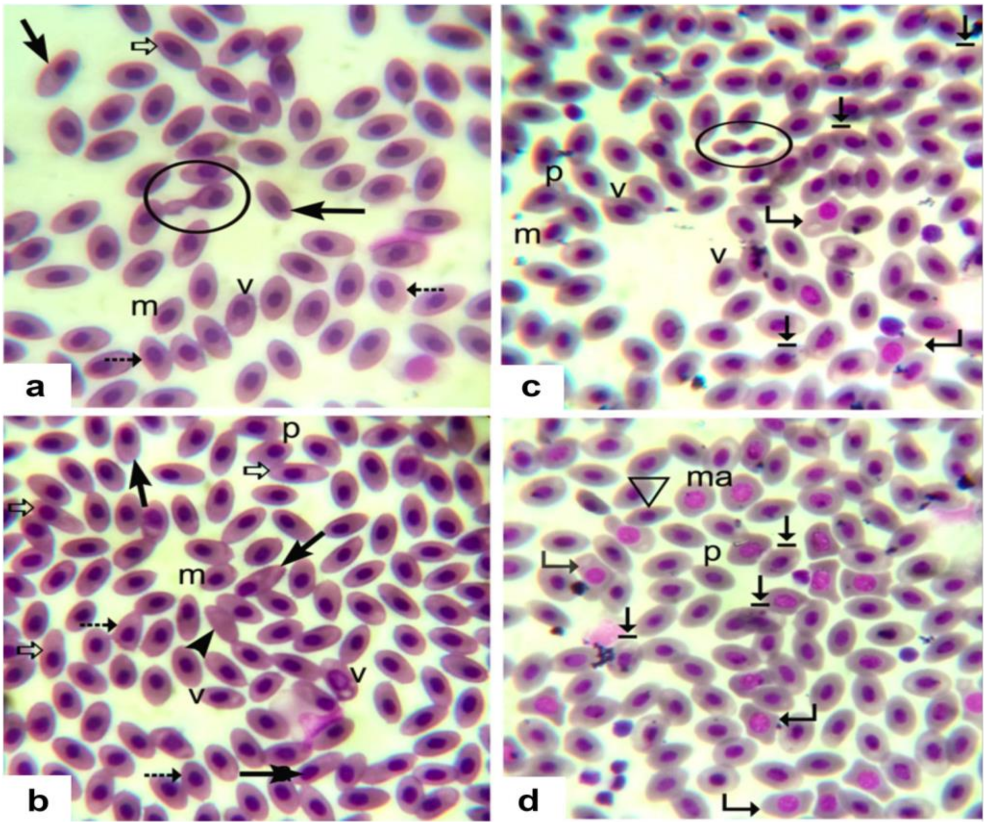

Fig. 9: Blood smear of common carp treated with fipronil showing morphological and nuclear changes in red blood cells. Filled Arrows=Eccentric nucleus; Filled Arrow heads=Erythrocytes without nucleus; Hollow Arrows: leptocytes; Hollow Arrow heads=Spindle shaped erythrocytes; m=microcytes; ma=macrocytes; p=pear shaped; V=vacuolation; Encircle=protruding cytoplasm (a) and dividing erythrocyte (c); Underlined vertical arrow: erythrocyte with micronucleus/ nuclear remnants. Arrows with bend=abnormal shaped erythrocytes; Arrow with dotted tail=Acanthocytes; d) Anisocytosis. Ghaffar *et al.* (2018).

Table 2: Biochemical changes observed in various fish species induced by different pesticides

Pesticide Used	Dose	Fish Species	Biochemical Findings	References
Fenvalerate	1/10th LC50	*Danio rerio*	Increase in AST in liver and ALT in gills	Al-Ghanim et al. (2020)
Deltamethrin	15µg/L	*Oreochromis niloticus*	Increased blood urea, bilirubin, ALP, AST and ALT. Decline in blood total protein, globulin, albumin, cortisol and glucose.	Dawood et al. (2020)
Cypermethrin	2.05×10^{-3} mg/L	*Oncorhynchus mykiss*	Fluctuations in levels of glucose, urea, creatine, AST, ALT, ALP, cholesterol, total glyceride, high density lipoprotein, total protein, albumin, LDH, low density lipoprotein-cholesterol, creatin kinase	Uçar et al. (2020a)
Diazinon	0.73–1.46 mg/L	*Clarias gariepinus*	Rise in level of glucose, AST, ALT	Al-Otaibi et al. (2019)
Deltamethrin	2 µg/L	*Hypophthalmichthys Molitrix*	Rise of bilirubin, cholesterol and potassium level and decline in total proteins and albumin	Ullah et al. (2019)
Chlorpyrifos	0.25-1.25 ppb	*Oreochromis mossambicus*	Blood glucose, cortisol and cholesterol increased. Reduced total plasma proteins and triglyceride level	Ghayyur et al. (2019)
Fipronil	0.03-0.15 mg/L	*Labeo rohita*	Relative weight of kidneys, gills, heart and brain decrease. Gills and kidneys exhibit severe lesions. ALT, AST, ALP and LDH increased.	Ghaffar et al. (2019)
Boscalid	0.1-1mg/L	*Danio rerio*	Decline in level of glucose	Qian et al. (2019)
Fipronil	300–400	*Rhamdia quelen*	Rise in level of ALP, ALT, AST and GGT	Fredianelli et al.

	µg/L			(2019)
Cypermethrin	1.8µg/L	*Brycon amazonicus*	Rise in level of sodium, glucose and chloride	De Moraes et al. (2018)
Penoxsulam	0.90 and 1.79mg/L	*Oreochromis niloticus*	Decline in level of nitric oxide and lysozyme; Rise in level of ALT, AST, ALP	Galal et al. (2018)
Prometryn	0.1, 0.5 and 2.5 mg/L	*Carassius auratus*	Decline in LDH	Mosiichuk et al. (2018)
Propanil	0.44 and 0.87 mg/L	*Oreochromis niloticus*	Rise in level of total proteins, phosphoglycerate kinase, triglycerides and decrease of cholesterol	Abubakar et al. (2018)
Fipronil	0 - 0.10 mg/L	*Cyprinus carpio*	Urea, creatinine, cholesterol, triglyceride, glucose increased but albumin reduced	Ghaffar et al. (2018)
Cypermethrin	0.5–1.5 µg/L	*Labeo rohita*	Increase in glucose level in serum	Khan et al. (2018)
Trichlorfon	0.5–2.0 g/kg	*Carassius gibelio*	Rise in serum level of catalase and superoxide dismutase	Lu et al. (2018)
Malathion	0.1–1 µg/L	*Cirrhinus mrigala*	Rise in sodium and potassium levels in serum and decline of chloride and calcium	Rani et al. (2017)
Dimethoate	0.1–1 µg/L	*Cirrhinus mrigala*	Rise in sodium and potassium levels in serum and decline of chloride and calcium	Rani et al. (2017)
Monocrotophos	2.14 mg/L	*Clarias batrachus*	Decrease in level of glucose, total proteins, albumin and globulin	Narra et al. (2017)
Chlorpyrifos	1.65 mg/L	*Clarias batrachus*	Decrease in level of glucose, total proteins, albumin and globulin	Narra et al. (2017)
Phosalone	2.5 mg/L	*Labeo rohita*	Rise in serum glucose	Kalaimani and Kandeepan (2017)
Dimethoate	1.245 mg/L	*Clarias batrachus*	Rise in serum level of glucose, creatinine, peroxidase, AST and decline globulin and albumin levels	Narra (2017)
Arsenic and urea	8-15 mg/L +0.2-0.8 g/L	*Labeo rohita*	ALT and AST activities were enhanced whereas glucose and total protein reduced	Ghaffar et al. (2016)
Propiconazole	0.89 µL/L	*Labeo rohita*	Rise in glucose and potassium level and decrease in total proteins, sodium and chloride	Hemalatha et al. (2016)
Lead nitrate	55 mg/L	*Mystus cavasius*	Decreased protein content in liver and kidney due to proteolysis. Reduced liver glycogen.	Jain and Batham (2016)
Azadirachtin	73–219 µg/L	*Ctenopharyngodon Idella*	Rise in total proteins, albumin, AST, ALT, ALP.	Gholami et al. (2016)
Glyphosate	0.10 and 0.19 mg/L	*Leporinus obtusidens* & *Rhamdia quelen*	Rise in level of ALT, AST and decline in glucose level	Loro et al. (2015)
Isoprothiolane	2.7 and 27 µg/L	*Cyprinus carpio*	Rise in level of albumen and decrease in glucose, triglycerides and cholesterol.	Saravanan et al. (2015)
Copper oxychloride	32.3 mg/L	*Oreochromis niloticus*	ALT, AST, uric acid and creatinine increase.	Hassaan et al. (2014)
Monocrotophos	0.5 mg/L	*Labeo rohita*	Reduced protein, lipid and carbohydrate content in different tissues	Muthukumaravel et al. (2013)
Prometryn	8 and 80 µg/L	*Cyprinus carpio*	Increased glucose and ALT and decline of calcium, magnesium, phosphate and creatinine	Velisek et al. (2013)
Dichlorvos	Sublethal dose	*Oreochromis mossambicus*	Impact over tissue glycogen, total protein and albumen content in liver, kidneys and muscles	Lakshmanan et al. (2013)
Propiconazole	50 and 500 µg/L	*Oncorhynchus mykiss*	Increase observed in level of ammonia, total protein, glucose, LDH and creatine kinase.	Li et al. (2011)
Dioxin	Sublethal dose	*Oncorhynchus mykiss*	LDH, AST and total protein plasma decreased. Interaction with DNA in a complex pathway to change how genes control synthesis of protein such as vitellogenin protein for egg development	Zorriehzahra (2008)
Malathion	0.91 ppm	*Clarias batrachus*	Reduced protein contents	Khare and Singh (2002)
Thiodon	4.17 mg/L	*Clarias gariepinus*	Alterations in total protein content in liver	Aguigwo (2002)

1.4. DNA Damage

Besides affecting on hematological and serological parameters, pesticides also influenced over genetic systems of fish. Genetic damage was discussed in few mentioned cases. Naphthalene-2-sulfonate caused genotoxic effects over *Channa punctatus*. Fish were exposed to LC_{50} values as 2.38g/15 g BW and 4.77 g/15g BW. For evaluating sub chronic exposure 1/10th (0.238g/L) and 1/20th (0.119g/L) of safe application rate (SAR) were reckoned. Sixty days exposure revealed the greater DNA damage in time and dose dependent manner by using comet assay and micronucleus assay. After thirty days, quitting of exposure proved to be recovery period for species. Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) was used further to evaluate the genotoxicity (Mehra and Chadha 2021).

DNA damage induced by pesticide mixture (endosulfan+chlorpyrifos) in peripheral blood erythrocytes of freshwater fish, *Oreochromis niloticus* by using Comet assay by (Ambreen and Javed 2018). According to them, dose dependent response was observed in fish erythrocytes with induction of maximum DNA damage at highest concentration (1/3rd of LC50) of pesticide mixture. Statistically significant effects for both concentrations and time of exposure in terms of DNA damage were observed in treated fish as compared to control group (Fig. 10).

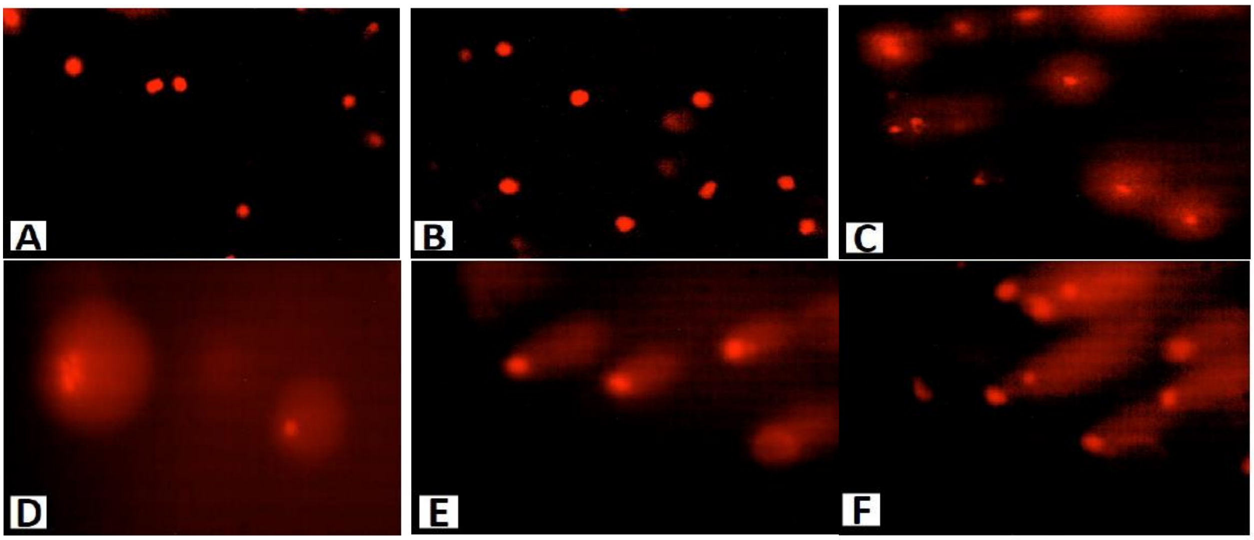

Fig. 10: Blood cells after Comet assay from control and treated group of *Oreochromis niloticus.* A, Control; B, Type 0 nuclei; C, Type I nuclei; D, Type II nuclei; E, Type III nuclei; F, Type IV nuclei (Ambreen and Javed 2018).

Karanjin is secondary metabolite derived from Karanja plants with pesticidal influences. Genotoxicity caused by the metabolite was studied using comet assay over *Cyprinus carpio* to sub-lethal concentration (0.28ppm). Gill, liver, kidney, and blood were examined to check the DNA damage by using Nano Drop. Damage DNA was examined in all observed tissues with increased exposure leading to more genotoxicity. Comet assay revealed the DNA damage in the form of tails of DNA content easily observable in Fig. 10-13. Enhanced exposure leads to more genotoxicity in *Cyprinus carpio* as compared to control groups (Tasneem and Yasmeen 2018).

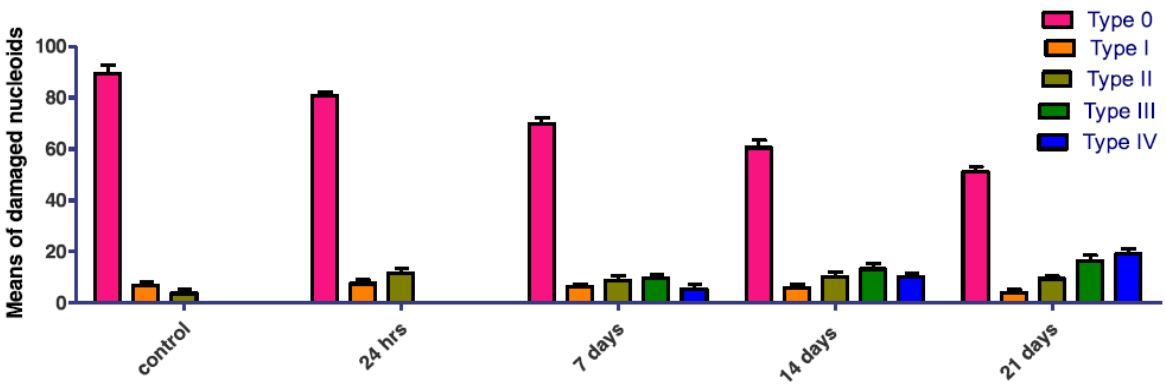

Fig. 11: Graph showing the genotoxicity in blood of *Cyprinus carpio* after exposure to Karanjin (Tasneem and Yasmeen 2018).

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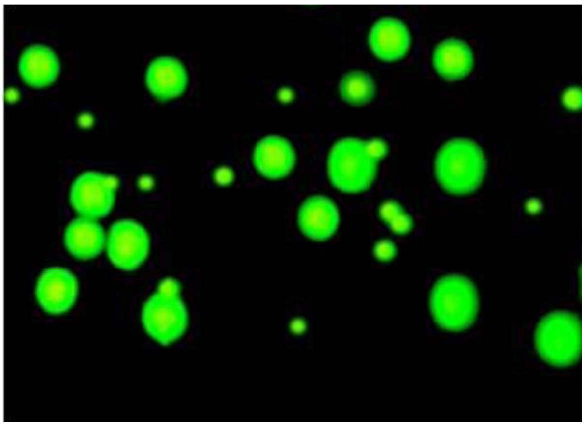

Fig. 12a: Genotoxicity level in group unexposed to pesticides indicating the no comet tail so no DNA damage observed.

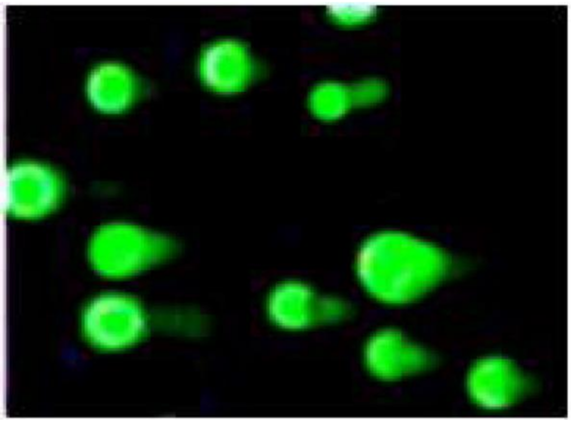

Fig. 12b: Genotoxicity level in group exposed to pesticides for 24 hours indicating the slight comet tail due to minor DNA damage.

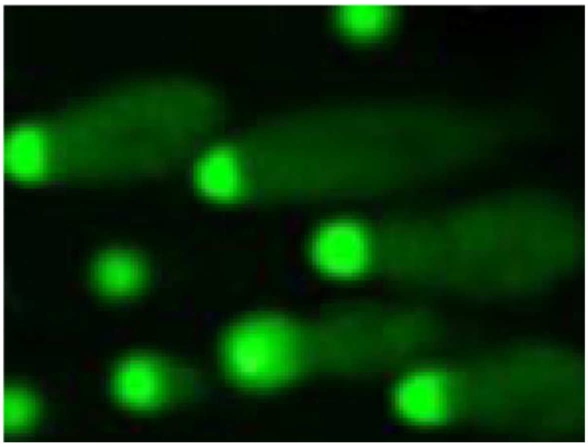

Fig. 12c: Genotoxicity level in group exposed to pesticides for 14 days indicating the greater comet tail due to high DNA damage.

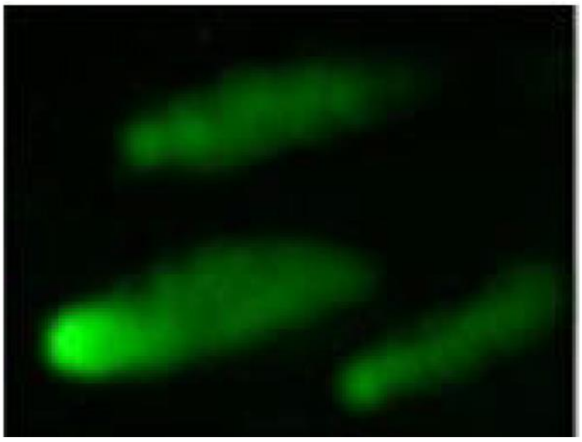

Fig. 12d: Genotoxicity level in group exposed to pesticides for 21 days indicating the easily observable comet tail due to very high DNA damage (Tasneem and Yasmeen 2018).

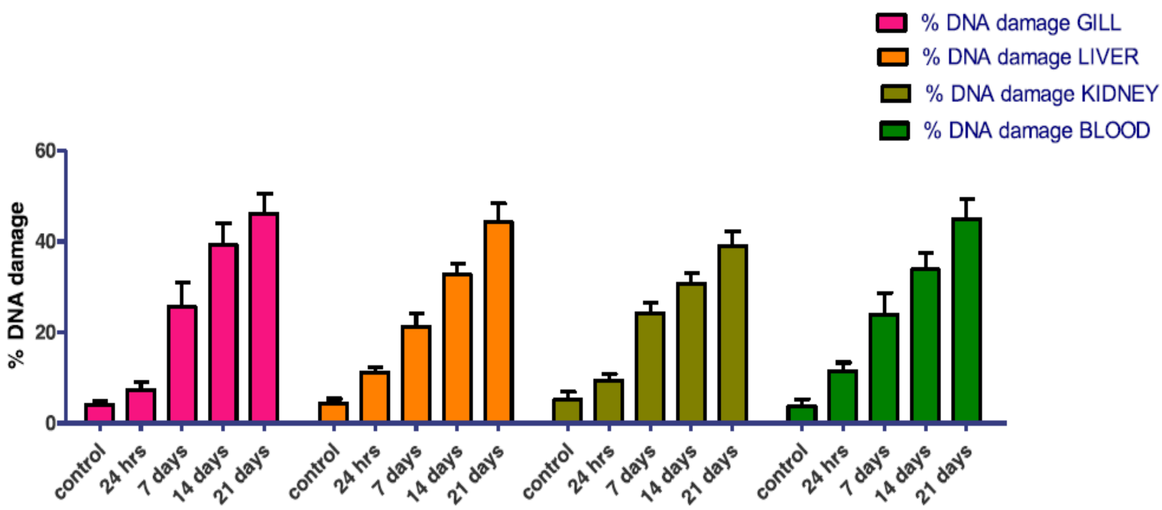

Fig. 13: Percentage of DNA damage in tissues of *Cyprinus carpio* after exposure of sublethal doses of Karanjin (Tasneem and Yasmeen 2018).

Conclusion: Although new technologies are necessary for success and comfort in present era but it must be remembered that water resources have extreme value for life. Increase in agricultural sector, industries and urbanization eventually resulted in contamination of our surroundings mainly water bodies. This article determined that pesticides have generated pronounced economic damage by mortalities of fish and on the other hand leading them unhealthy for human ingestion. Article exhibited that one should keep in mind the essential precautionary measure for fish selection for the purpose of ingestion. Researchers all over the world have been working on the harmful impacts of pesticides over various organisms including fish such as histopathological, hematological and biochemical alterations along with DNA damage, reduction in protein and lipid content in serum and tissues as discussed. So it is obvious to conclude that pesticides usage should be lessen and must be avoided for protection of aquatic life or use of bio pesticides can be done where necessary to cope with situation. As an alternative, based on variable susceptibility of fish species to pesticides, less susceptible fish can be cultured in aquatic bodies exposed to pollutants. In addition, species cultured must be the one, which are accumulating low amount of pesticides and heavy metals in their organs. Further research relevant to newly synthesized pesticides and other chemicals should be done in natural and laboratory condition, which will help in determining the harmful impacts effects of these pollutants and based on these research less toxic and environmental friendly chemicals can be used.

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