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EVALUATION OF ZIRAM-INDUCED GENOTOXICITY IN FISH USING THE COMET ASSAY

[1]SNOBER SHAH, [2]MD. NIAMAT ALI, [3]SHOWKAT AHMAD GANIE, [4]DURDANA SHAH AND [5]BILAL AHMAD BHAT

[1]*Cytogenetics and Molecular Biology Research Laboratory Centre of Research for Development, University of Kashmir, Srinagar, India*

E.mail;snobershah29@gmail.com

[2]*Cytogenetics and Molecular Biology Research Laboratory, Centre of Research for Development, University of Kashmir, Srinagar, India*

E.mail;mdniamat@hotmail.com

[3]*Cytogenetics and Molecular Biology Research Laboratory Centre of Research for Development, University of Kashmir, Srinagar, India*

E.mail; durii.amu@gmail.com

[4]*Department of Clinic Biochemistry, University of Kashmir, Srinagar, India*

Email: showkatganie@kashmiruniversity.ac.in

[5]*Division of Agricultural Econ & Statistics, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, J&K, India*
Email: bhat_bilal@rediffmail.com; bhat_bilal@skuastkashmir.ac.in

ABSTRACT

Ziram is a broad-spectrum pesticide from the dimethyl-dithiocarbamate (DTC) fungicide class. It is mostly used as a foliar spray to prevent fungal diseases in crops like peaches, apricots, almonds, and other vegetables. Ziram has a wide range of action against a variety of fungal infections, such as those that cause fruit rot, leaf spot, and downy mildew. The study examined the effects of ziram on DNA damage in the blood cells of Carassius carassius, using comet scoring and DNA damage index assessments. Exposure to sub lethal dose of ziram (0.055 mg/L) resulted in a significant increase in DNA damage compared to untreated and solvent-exposed control groups. Specifically, the ziram-treated group showed a more than tenfold increase. These findings suggest a substantial genotoxic impact of these chemicals on aquatic organisms. Furthermore, when compared to a positive control group treated with Cyclophosphamide (4 mg/L), ziram treatments approached significance in terms of DNA damage levels, highlighting their potential environmental hazards and the need for rigorous regulatory measures.

KEYWORDS; Ziram, Carassius Carassius, comet assay, genotoxicity

INTRODUCTION

The excessive use of pesticides in agriculture has led to huge increases in crop output. Pesticides used in agriculture and other chemicals from industries ultimately make their way to sources of water (Mensah *et al., 2014).* Ziram has a wide range of action against a variety of fungal infections, such as those that cause fruit rot, leaf spot, and downy mildew. (Gullino *et al.,* 2010). Ziram exposure in the environment, either by itself or in conjunction with other pesticides, may be connected to the onset of neurodegenerative illnesses like Parkinson's disease, according to epidemiological research (Fitzmaurice *et al.,* 2014). Ziram is an effective fungicide, but its possible toxicity to creatures other than its intended target has sparked worries. Fish, amphibians, and invertebrates are among the aquatic species that have been negatively impacted by it, according to several studies (Caux *et al*., 1996). The International Agency for Research on Cancer has also designated ziram as a potential human carcinogen (IARC, 1991), underscoring the necessity of additional research into its toxicological effects. The environmental and human health dangers of ziram continue to be a source of concern and this call for more research into its toxicity and methods of action in non-target species and humans (Martin *et al.,* 2016). The brain system, liver, and thyroid appear to be the principal targets of toxicity (Pandey *et al.,* 1990).

Fish are commonly employed as water pollution models because they absorb toxins and exhibit a biological, cell-based, physiological, and histologically distinct response, making them a viable biomarker with widespread applicability in environmental monitoring (Fontanetti *et al.,* 2012; Marinowic *et al.,* 2012). Fish, along with other aquatic species, have long been employed to evaluate the health of aquatic ecosystems (Saiki *et al.,* 1993; Kock *et al*., 1996). Since fish are very sensitive to environmental changes and have a significant role in assessing the potential risk associated with the pollution of novel chemicals in aquatic settings, the use of fish as a bio indicator of pollutant impacts is growing (Banaee *et al*., 2009; Monteiro *et al*., 2006; Begum, 2004). More ever they appear to be helpful in identifying DNA damage in freshwater caused by mutagens and pro-mutagens (Lemos *et al.,* 2005; Boettcher *et al.,* 2010; Rocha *et al.,* 2011; Deutschmann *et al.,* 2016). The Comet assay, requires a small amount of blood cells per fish samples and provides sensitivity for determining the minimum intensity of DNA fragmentation Singh *et al*., (1988) is the most affordable and sensitive technique for identifying genetic damage at the cellular level in an alkaline state (Tice *et al*., 2000).The Comet assay's main benefits include its quick turnaround time, short sample size required, and ability to differentiate between different cell types based on the extent of DNA damage or repair.

The year-round availability and extensive dispersion of *C. carassius*, along with its ease of maintenance in aquariums and commercial significance, make it a valuable model for eco toxicological investigations pertaining to toxicity. This species has far-reaching ecological and economic consequences. The majority of research on the

ziram effect focuses only on the fish's physiological changes and dose-response. The ziram induced genotoxic modulation is receiving comparatively less attention. Therefore, the goal of this study was to investigate the toxicological effects of ziram in one of the frequent cyprinids in Dal Lake Kashmir commonly known as crucian carp (*C. carassius*).

MATERIALS AND METHODS

In this paper, Commercial grade pesticide manufactured by Gharda chemicals Ltd, Maharashtra India, having 95% (w/w) ziram technical grade as active ingredient was used. DMSO 99% purity was purchased from Hi-Media Labs, Mumbai, India. Himeda offers a range of products that are 99.95% pure, including Agarose-normal melting, Agarose-low melting , NACL , KCL, disodium hydrogen phosphate, potassium dihydrogen phosphate , di sodium EDTA, Tris base , NAOH, sodium lauryl sarcosinate , triton-100 ,HCL , ethanol, ethidium bromide .

Experimental fish

Carassius carassius, a member of the family Cyprinidae and order Cypriniformes, was collected by a local fisherman from the Dal Lake in the vicinity of the University Of Kashmir, Jammu and Kashmir, India. Fish was fed commercial fish feed (Vijay) and given preventive treatments, such as a brief immersion in 0.05% $KMnO_4$, in order to prevent any cutaneous infection.The fish were naturally photo periodically adapted to laboratory environment for 15 days in a 60-liter glass tank that was frequently aerated and maintained at 14.63 ± 2.83°C temperature with stale, chlorine-free tap water (pH 7.5–8.7).

Test chemical

Commercial grade pesticide manufactured by Gharda chemicals Ltd, Maharashtra India, having 95% (w/w) ziram technical grade as active ingredient was used in the investigation. The stock solution was made by weighing a specific amount of ziram and diluting it with DMSO in 1:1 ratio.

Acclimatization

Fish that had been acclimatized were exposed to a sub-lethal concentration of ziram (1/10 LC_{50} ziram 0.055 mg/L) for the duration of four days. According to established testing protocols, a group of acclimatized fish was separated into three distinct groups, each consisting of 10 fish. Following the standardized OCED testing protocols (1992), a set of acclimatized fish was categorized into four groups, each containing 10 fish individuals. Group 1 of the acclimatized fish was treated with a sub-lethal dosage of ziram (0.055 mg/L). The fish in Group 2 were exposed to a positive control (cyclophosphamide, 4 mg/L) treatment, while Group 3 were subjected to a solvent control (0.1% DMS0), while group 4 received no treatment (negative control) as part of the experimental conditions. Fish were checked for aberrant behavior and outward appearance during the exposure period.

Comet assay/Single cell gel electrophoresis (SCGE)

Singh *et al*., (1988) was followed for performing Comet assay. A caudal puncture technique was used to extract 0.3 milliliters of blood. A sample of 5 µL of blood was diluted with 1000 µL of PBS. Trypan Blue Exclusion method (Anderson *et al*., 1988) was used for determining cell viability, blood samples with 84% cell viability were used for the Comet assay. Slides that had previously been coated with 1% normal melting point Agarose (NMPA) were layered with 10µL of cell suspension combined with 120µL of 0.5% low melting point Agarose (LMPA) at 37%. To harden the gel, the slides were covered and chilled at 4˚C for 10–15 minutes. Cover slip was carefully removed after the blood-LMPA layer solidified to prevent the underlying layer from being avulsed. After adding 75µL of LMPA to the layer of Agarose gel mixture and carefully covering it with a fresh cover slip, the gel was refrigerated at 4˚C for ten to fifteen minutes to solidify.After the gel set, the cover slips were gently removed. The slides were then submerged in a freshly produced cold lysing solution and cooled for at least one hour at 4˚C.The slides were gently removed from the lysis solution and placed in a horizontal submarine gel electrophoresis apparatus, with the Agarose-coated side facing upward and perpendicular to both electrodes. The slides were kept in the alkaline buffer for 30 minutes to allow the DNA strands to unwind and reveal the alkali labile spots. The electrophoresis tank was filled with cool, fresh electrophoresis buffer until the slides were completely covered and no air bubbles appeared on the Agarose gel. The power supply was turned on at 0.74V/cm (between electrodes), and the current was regulated to 300mA by increasing and decreasing the buffer level. The electrophoresis procedure lasted 30 minutes. After 30 minutes of electrophoresis, the power was turned off, and the slides were gently withdrawn from the buffer and placed on a drain tray. After coating drop-by-drop slides with neutralization buffer for five minutes and draining the buffer, the procedure was performed twice more and then several times with distilled water. Slides were drained and then submerged in 100% ethanol to dehydrate them. After air drying, slides were baked for fifty minutes.After that, the slides were kept in a dry location. Slides were rehydrated in cold distilled water for 30 minutes, then stained with 50µL Ethidium bromide and covered with a cover slip. The edges and back of the excess stain were blotted away before viewing.A fluorescent microscope with an excitation filter of 515-560 nm and a barrier filter of 590 nm was used to visualize Ethidium bromide-stained slides at a magnification of 200X.

Comet scoring

For each sample, images of 300 randomly selected cells (100 per slide) were assessed to determine DNA damage. The cells were visually evaluated based on tail length into five distinct groups, ranging from undamaged (0) to completely damaged class (type IV) (Anderson *et al.,* 1988). The statistics are reported in the form of the

frequency of cells with and without DNA damage, as well as the score and class distribution. The score was calculated by multiplying the number of nuclei in a class by the class number.

RESULT

Genotoxicity Assessment: Comet Assay Analysis of Sub-lethal Exposure to ziram

The study examined the effects of ziram on DNA damage in the blood cells of *C. carassius*, using comet scoring and DNA damage index assessments. Exposure to sub-lethal dose of ziram (0.055 mg/L) resulted in a significant increase in DNA damage compared to untreated and solvent-exposed control groups. Specifically, the ziram-treated group showed a more than tenfold increase. These findings suggest a substantial genotoxic impact of these chemicals on aquatic organisms. Furthermore, when compared to a positive control group treated with Cyclophosphamide (4 mg/L), ziram treatments approached significance in terms of DNA damage levels, highlighting their potential environmental hazards and the need for rigorous regulatory measures. Fig.1 shows that the untraeted and solvent control (DMSO) groups exhibited no comet formation, whereas clear comet formation was observed in the postive control (cyclophposphamide) group. Fig. 2 shows different classes of comets formed in ziram treated group.

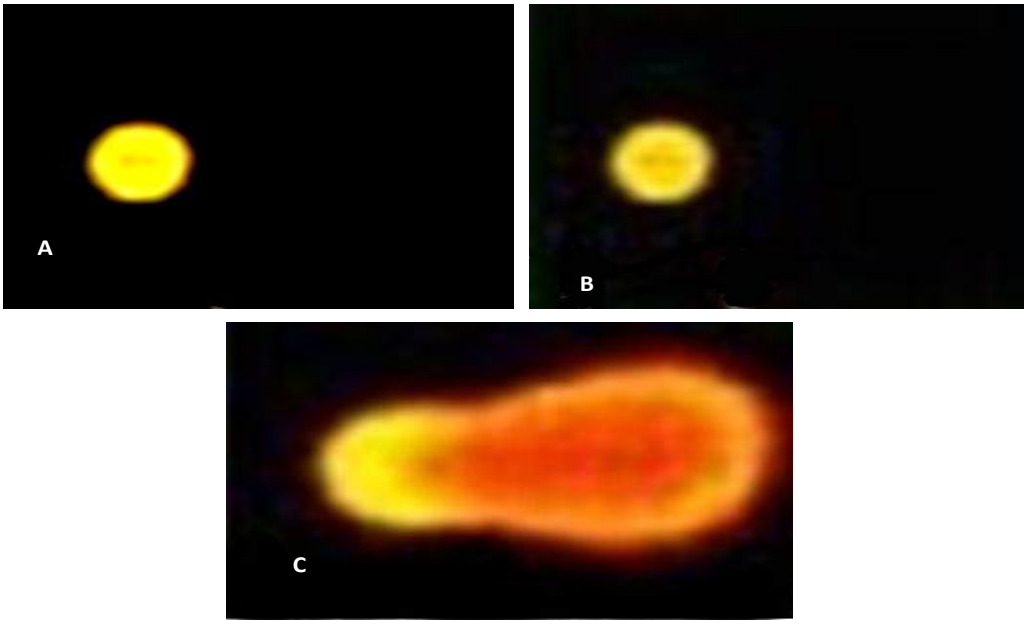


Fig.1: A Showing no comet formation in the blood cells of the *C. carrassius* as an untreated and B treated with solvent control (DMSO), C showing comet formation treated with positive control (cyclophosphamide).

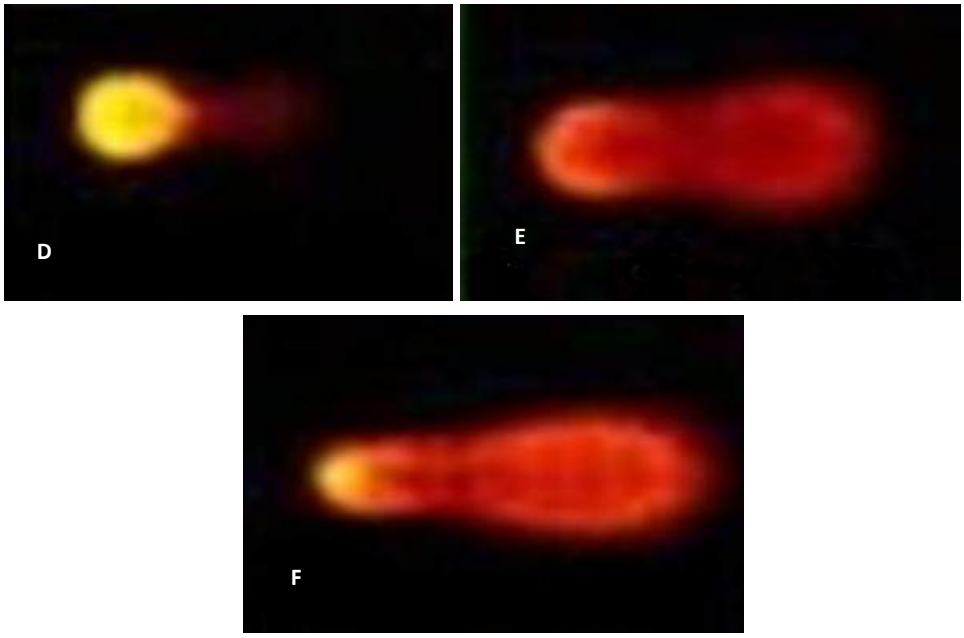


Fig.2: D, E, F showing different classes of DNA damage (comet formation) in blood of *C. carassius* treated with sub-lethal ziram (0.55mg/L)

The evaluation of total DNA damage in groups treated with ziram indicates significantly elevated levels of DNA damage, with a mean of 262 units. (Table 4). This is notably higher than the mean DNA damage observed in the positive control group, which was 171.33 units. (Table 3) These findings suggest that ziram exposure induces substantial DNA damage in the tested organisms, while the control groups remained largely unaffected, underscoring the genotoxic effects specifically attributable to ziram. (Table 1,2).

Table 1: Evaluation of Comet score and DNA Damage index in blood cells of untreated *C. carassius*.

Total cells examined	Classes of DNA Damage						Damage Index (DI)
	Total tailed cells	0	1	2	3	4	
100	12	4	3	1	1	1	12
100	13	3	2	2	2	1	16
100	17	7	8	1	1	1	17
Total damage Index							15

Table 2: Evaluation of Comet score and DNA Damage index in blood cells of *C. carassius* treated with (DMSO)

Total cells examined	Classes of DNA Damage						Damage Index (DI)
	Total tailed cells	0	1	2	3	4	
100	12	4	2	1	2	3	22
100	13	6	3	1	2	1	15
100	17	8	6	1	1	1	15
Total damage Index							17.33

Table 3: Evaluation of Comet score and DNA Damage index in blood cells of *C. carassius* using cyclophosphamide as positive control

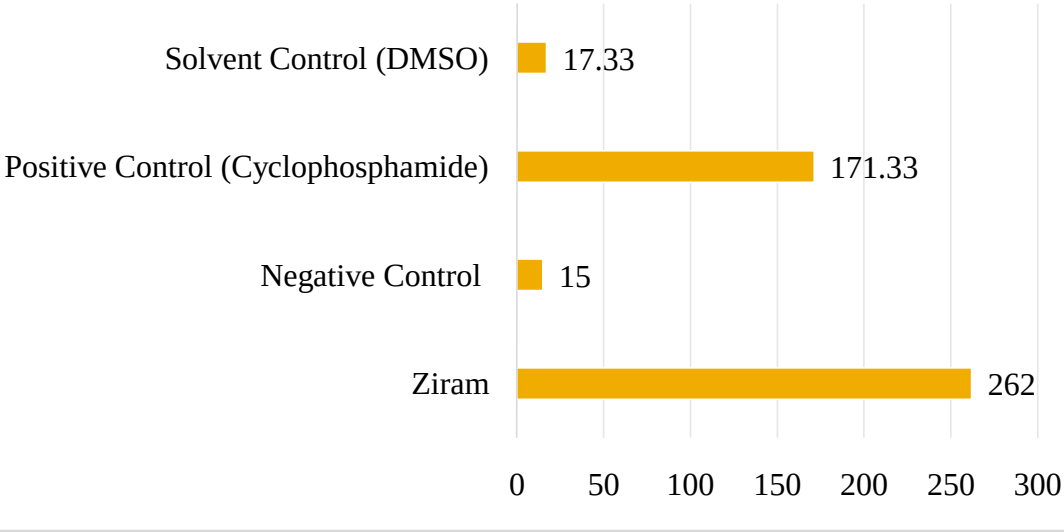
Total cells examined	Classes of DNA Damage						Damage Index (DI)
	Total tailed cells	0	1	2	3	4	
100	65	8	10	12	16	19	158
100	74	7	12	15	16	24	186
100	70	9	10	13	18	20	170
Total damage Index							171.33

Table 4: Evaluation of Comet score and DNA Damage index in blood cells of *C. carassius* exposed to sub-lethal level of ziram

Total cells examined	Classes of DNA Damage						Damage Index (DI)
	Total tailed cells	0	1	2	3	4	
100	96	11	12	17	21	35	260
100	94	9	13	15	25	34	254
100	95	7	10	13	24	41	272
Total damage Index							262

Fig 3: DNA damage index in peripheral blood cells of *Carassius Carassius* following exposure to different treatment groups.The negative control and solvent control (DMSO) exhibited negligible DNA damage,while a significant increase in DNA damage was observed in positive control (cyclophosphamide).The highest DNA damage index was recorded in the ziram treated group indicating it's strong genotoxic potential. Statistically, significant difference has been observed among different treatments ($P<0.01$).

Figure 3: DNA Damage in *C.carassius* blood cells treated in different treatment groups



DISCUSSION

A significant percentage of pesticides have genotoxic qualities, which means they can harm aquatic organisms by damaging their DNA, encouraging the growth of tumors and negatively affecting their health. The overall effectiveness of aquaculture operations may be lowered as a result of these effects. In fish, erythrocytes make up around 97% of the total blood cells. Importantly, as peripheral blood provides a thorough indicator of an organism's general health, fish erythrocytes are especially well-suited for DNA damage investigations. As extremely sensitive biomarkers for genotoxic damage, DNA strand breaks are thought to be possible precursors to mutagenic events. Since its introduction by Singh *et al.* (1988), the comet assay has attracted a lot of interest from researchers due to its versatility. This method is widely used in many academic disciplines and is renowned for its accuracy and quickness in identifying breaks in DNA strands. Because of its simplicity and sensitivity, the Comet test is a suitable approach for measuring DNA damage *in vivo, in vitro*, and *in situ* in fish organs such as the liver, kidney, and blood following exposure to various pollutants in water. (Dhawan, Bajpayee and Parmar, 2009). This experiment does not require active cell division. As reported by Buschini *et al*., (2003), Collins (2004), and Bucker *et al*., 2006) that there is also no need for metaphases or specialized knowledge of chromosomal numbers. The comet test is therefore a crucial tool in environmental monitoring investigations, exposing the genotoxic implications of exposure to environmental pollutants, as explained by Belpaeme *et al*., (1998). According to Mitchelmore and Chipman (1998) the comet assay is a critical biomarker for evaluating genotoxicity in fish. This technique measures breakage in DNA strands. Fish are important for aquatic habitats because they contribute to different trophic levels. They respond directly to even very low quantities of harmful compounds in the water. In

the current experiment, the sensitivity of *C.carassius* fish to ziram was assessed. Three high-quality slides were chosen for each treatment group, including control. A single observer independently coded and scored all of the slides.During the comet scoring, the following criteria were followed: measurements near the edges of the slides were avoided, no cells were scored near or in a trapped air bubble, only slides with a low background were used, and comets with no heads and images with nearly all DNA in the tail or with a very wide tail were excluded from evaluation because they could represent dead cells (Hartmann and Speit, 1995).

The control group exhibited the lowest Genetic Damage Index (GDI) across all examined groups. Of all the groups under investigation, the control group had the lowest Genetic Damage Index (GDI). In comparison to the negative control group, the treatment group had approximately ten times as many cells with DNA damage. However, with respect to positive control group, the treated group showed a slightly greater degree of DNA damage.The mean score, showing the degree of damage in the blood cell analysis, was somewhat greater in ziram treated groups (μ=262) compared to the positive control (μ = 171.33). On the other hand, the treated group's mean score was around ten times higher than the values found in the negative control group (μ=15). Furthermore, when comparing the positive control (4 ppm Cyclophophamide) to negative control, noteworthy outcomes were seen. The results of this study about DNA damage that was discovered are consistent with earlier studies on *Carassiusauratus* blood cells exposed to glyphosate that were carried out by Cavas and Konen (2007). Similar results were found in a study conducted by Cavalcante, Martinez, and Sofia (2008), wherein exposure to glyphosate caused DNA damage in *Prochilodus lineatus* gill cells and blood. Furthermore, increasing heavy metal concentrations were linked to rise in DNA damage in *Oreochromis mossambicus* blood cells, according to Ahmed *et al*., (2011).More evidence for these findings came from Kousar and Javed (2015), who found that longer the exposure to heavy metals, the more DNA damage was seen in the red blood cells (RBCs) of four different fish species. Kopjar *et al.* (2008) reported that erythrocytes of Cobitis elongate exposed to industrial effluents showed large comet tails of DNA.The link between different environmental stressors, like glyphosate and heavy metals, and the induction of DNA damage in a variety of fish species is highlighted by this body of evidence. The current study's findings support the established evidence that Malathion damages *Channa punctatus* DNA because they are in line with those published by Kumar *et al*., (2010).

CONCLUSIONS

Ziram exposure induces significant DNA damage in fish, as evidenced by increased comet tail formation in the Comet assay. These results indicate the genotoxic potential of ziram and support the use of fish as sensitive bioindicators for environmental monitoring of pesticide contamination.. Such studies are invaluable for discerning the impact of contaminants on genomic integrity within these ecosystems and are essential tools for creating

practical plans to keep an eye on and lessen the negative effects of pollution in aquatic environments, therefore preserving the health of the ecosystem and the general welfare.

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REFERENCES

1. Ahmed, M. K., Habibullah-Al-Mamun, M., Hossain, M. A., Arif, M., Parvin, E., Akter, M. S., Khan, M. S and Islam, M. M. (2011). Assessing the genotoxic potentials of arsenic in tilapia (*Oreochromis mossambicus*) using alkaline comet assay and micronucleus test. *Chemosphere, 84*(1), 143–149.
2. Anderson, D., Yu, T.W., Phillips, B.J. and Schmezer, P. (**1988)**. The effect of various antioxidants and other modifying agents on oxygen-radical-generated DNA damage in human lymphocytes in the Comet assay. Mutation Research/Fundamental and *Molecular Mechanisms of Mutagenesis,* 307(1): 261–271.
3. Banaee, M. A.R Mirvaghefi. K. Ahmadi. And R. Ashori. (2009).The effect of Diazinon on histophatological changes of testis and ovaries of common carp (*Cyprinus carpio*). *Scientific Journal of Marine Biology,* 1(2): 25-35.
4. Begum, G. (2004). Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish *Clarias batrachus* (linn) and recovery response. *Aquatic Toxicology*, 66: 83–92.
5. Belpaeme, K., Cooreman, K., and Kirsch–Volders, M. (1998) Development and validation of the in–vivo alkaline comet assay for detecting genomic damage in marine flatfish. *Mutation Research* 415(3): 167–184.
6. Boettcher, M., Grund, S., Keiter, S., Kosmehl, T., Reifferscheid, G., Seitz, N., Suares Rocha, P., Hollert, H., Braunbeck, T. (2010). Comparison of in vitro and in situ genotoxicity in the Danube River by means of the comet assay and the micronucleus test. *Mutat. Res.* 700 (1–2), 11–17.
7. Bucker, A., Carvalho, W. and Alves-Gomes, J. A. (2006). Avaliation of mutagenicity and gentotoxicity in Eigenmannia virescens (Teleostei: Gymnotiformes) exposed to benzene. *Acta Amazonica*, 36(3): 357- 364.

8. Buschini, A., Carboni, P., Martino, A., Poli, P. and Rossi, C. (2003). Effects of temperature on baseline and genotoxicant-induced DNA damage in haemocytes of Dreissena polymorpha. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 537(1): 81-92.

9. Caux, P. Y., Menard, L and Kent, R. A. (1996). Comparative study of the effects of TCDD, Oxon and Pentachlorophenol on the pituitary-thyroid axis in the newt Cynopspyrrhogaster. *Environmental Toxicology and Chemistry: An International Journal*, 15(4), 533-535. https://doi.org/10.1002/etc.5620150422

10. Cavalcante, D. G. M., Martinez, C. B. R and Sofia, S. H. (2008). Genotoxic effects of roundup on the fish Prochilodus lineatus. *Mutation Research*, 655, 41–46.

11. Cavas, T., Konen, S. (2007). Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (*Carassius auratus)* exposed to a glyphosate formulation using the micronucleus test and the comet assay. *Mutagen.* 22(4), 263-268

12. Collins, A. R. (2004). The comet assay for DNA damage and repair: Principles, applications, and limitations. *Molecular Biotechnology*, *26*(3), 249–261.

13. Deutschmann, B., Kolarevic, S., Brack, W., Kaisarevic, S., Kostic, J., Kracun-Kolarevic, M., Liska, I., Paunovic, M., Seiler, T.B., Shao, Y., Sipos, S., Slobodnik, J., Teodorovic, I., Vukovic-Gacic, B., Hollert, H., 2016. Longitudinal profile of the genotoxic potential of the River Danube on erythrocytes of wild common bleak (Alburnus alburnus) assessed using the comet and micronucleus assay. *Sci. Total Environ.* 573 (15), 1441–1449.

14. Dhawan, A., Bajpayee, M and Parmar, D. (2009). Comet assay: a reliable tool for the assessment of DNA damage in different models. *Cell Biology and Toxicology*, 25, 5–32

15. Fitzmaurice, A.G., Rhodes, S.L., Cockburn, M., Ritz, B., Bronstein, J.M. (2014). Aldehyde dehydrogenase variation enhances effect of pesticides associated with Parkinson disease. *Neurology 82, 419e426.*

16. Fontanetti, C.S., Nogarol, L.R., Souza, R.B., Perez, D.G., Maziviero, G.T., Nogueira, L. and Pina, A. (2012). Bioindicators and biomarkers in environmental pollution assessment: Fish as a model organism. *Environmental Monitoring and Assessment.* 184(3): 1409–1421.

17. Gullino, M. L., Tinivella, F., Garibaldi, A., Kemmitt, G. M., Bacci, L and Sheppard, B. (2010). Mancozeb: Past, present, and future. Plant Disease, 94(9), 1076-1087. https://doi.org/10.1094/PDIS-94-9-1076

18. Hartmann, A and Speit, G. (1995). **Comparative investigations of the genotoxic effects of chemicals in the single cell gel (SCG) assay and the micronucleus test.***Mutation Research*, *346*, 49–56.

19. IARC (1991). Ziram. In IARC monographs on the evaluation of carcinogenic risks to humans: Occupational exposures in insecticide application and some pesticides (Vol. 53, pp. 241-252). *International Agency for Research on Cancer.*

20. Kock, G., Triendl, M., Hofer, R. (1996). Seasonal patterns of metal accumulation in Artic char (Salvelinus alpinus) from an oligotrophic Alpine lake related to temperature. Can. *J. Fish Aquat. Sci.* 53, 780–786

21. Kopjar, N., P. Mustafic, D. Zanella, I. Buj, M. Caleta, Z. Marcic, M. Milic, Z. Dolenec and M. Mrakovcic. (2008). Assessment of DNA integrity in erythrocytes of Cobitis elongate affected by water pollution: the alkaline comet assay study. *Folia. Zool.* 57:120-130.

22. Kousar, S. and Javed, M. (2015). Diagnosis of metals induced DNA damage in fish using Comet Assay. *Pakistan Veterinary Journal* ,vol.35,no.2,pp. 168-172.

23. Kumar, R., Nagpure, N. S., Kushwaha, B., Srivastava, S. K., & Lakra, W. S. (2010). Investigation of the genotoxicity of malathion to freshwater teleost fish *Channa punctatus* (Bloch) using the micronucleus test and comet assay. *Archives of Environmental Contamination and Toxicology, 58*, 123–130. https://doi.org/10.1007/s00244-009-9354-3.

24. Lemos, N.G., Dias, A.L., Silva-Souza, A.T., Mantovani, M.S. (2005). Evaluation of environmental waters using the comet assay in Tilapia rendalli. *Environ. Toxicol. Pharmacol*. 19 (2), 197–201.

25. Marinowic, D.R.; Mergener, M.; Pollo, T.A.; Maluf, S.W.; da Silva, L. B. (2012). In vivo genotoxicity of the pyrethroid pesticide beta-cyfluthrin using the comet assay in the fish *Bryconamericus iheringii*. *Z. Naturforsch C*. 67(5–6), 308–311.

26. Martin, C.A., Myers, K.M., Chen, A., Martin, N.T., Barajas, A., Schweizer, F.E., Krantz, D.E. (2016). Ziram, a pesticide associated with increased risk for Parkinson's disease, differentially affects the presynaptic function of aminergic and glutamatergic nerve terminals at the Drosophila neuromuscular junction. *Exp. Neurol*. 275 (1), 232e241.

27. Mensah, P.K., Palmer, C.G., Muller, W.J. (2014). Lethal and Sub-lethal Effects of Pesticides on Aquatic Organisms: the Case of a Freshwater Shrimp Exposure to Roundup®. In: Pesticides: Toxic Aspects. *InTech Publica-tions, Rijeka, Croatia*, pp. 163–185.

28. Mitchelmore, C. L and Chipman, J. K. (1998). Detection of DNA strand breaks in brown trout (Salmo trutta) hepatocytes and blood cells using the single cell gel electrophoresis (comet) assay. *Aquatic Toxicology*, 41, 161–182.

29. Monteiro, D.A., Alves de Almeida, J., Rantin, F.T and Kalinin, A.L. (2006). Oxidative stress biomarkers in the freshwater characid fish, *Brycon cephalus*, exposed to organophosphorus insecticide Folisuper 600 (*methyl parathion*). *Comparative Biochemistry and Physiology, Part C: Toxicology & Pharmacology*, 143, 141–149.

30. OECD, Guidance Document for Aquatic Effects Assessment. Organization for Economic Cooperation and Development, 1992. (n.d.). *Environment Monograph 92. Environment Directorate,* Paris, France.

31. Pandey M, Raizada R.B and Dikshith T.S (1990) 90-day oral toxicity of Ziram: a thyrostatic and hepatotoxic study. *Environmental Pollution* 65: 311–322.

32. Rocha, C., Cavalcanti, B., Pessoa, C., Cunha, L., Pinheiro, R.H., Bahia, M., Ribeiro, H., Cestari, M., Burbano, R. (2011). Comet assay and micronucleus test in circulating erythrocytes of Aequidens tetramerus exposed to methylmercury. In vivo 25 (6), 929–934ntaminated wetland in Louisiana, USA. Rev. *Environ. Health* 11 (4), 191–203.

33. Saiki, M.K., Jennings, M.R., Brumbaugh, W.G. (1993). Boron, molybdenum and selenium in aquatic food chains from the lower San Joaquin River and its tributaries, California. Arch. *Environ. Contam. Toxicol*. 24, 307–319

34. Singh, N. P., McCoy, M. T., Tice, R. R. and Schneider, E. L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental cell research*, 175(1): 184-191.

35. Tice, R.R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J.C., Sasaki, Y.F. (2000). Single Cell Gel/Comet Assay: Guidelines for In Vitro and In Vivo Genetic Toxicology Testing. *Environ. Mol. Mutag*. 35, 206-221.