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Original article

Effect of pesticides on erythrocytes of indigenous fish *Labeo rohita*

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ABSTRACT

Single cell gel electrophoresis was used to observe the effect of bifenthrin and chlorpyrifos on *Labeo rohita*. The present work was planned to study the genotoxic effect of pesticide mixture on the major indian carp, *L. rohita* at sub-lethal concentration 33% LC_{50}. First of all, 96-hr LC_{50} was determined for *L. rohita*, then fish was given sub-lethal environment for 70 days. Erythrocytes from blood were taken after each fortnight for the assessment of time dependent DNA damage (% damage), aggregate tail length of comets and genomic destruction. There was increase in damage up to 56 days and after that a slight decrease was observed in next fourteen days. After statistical analysis, it was concluded that the Single Cell Gel Electrophoresis may be implemented in ecofriendly observer courses in evaluation of toxicity.

1. Introduction

Pesticides have prominent place and gained responsiveness owing to their character in considerable dropping level of freshwater biota and ecological unit presentation (Araldi et al., 2015; Malaj et al., 2014; Elina et al., 2016). As to increase the agri-production, different types of pesticides are introduced for the control of pests. But these are not biodegradable and after entering in the aquatic medium proving unadorned menace to the non-target fauna (Ruiz-Suarez et al., 2015; Bantu et al., 2017). Pesticides may enter by many ways in aquatic system for example after their use on crops if there is rain it can be washed from leaves and flow in aquatic habitats. The possible genotoxic effect of insect killer on the creatures (other than pests) is of universal importance.

From past centuries, organo-phosphate is best commonly preferred pesticides but are involved in toxicity as alkylating partisans and possible genotoxicants. Asian countries including China and Indio-Pakistan have increased the production of these from the past few years (Yen et al., 2011). One of organophosphate is chlorpyrifos which is commonly used not only as the pest regulator in agriculture but also for dynasty pest worldwide and is reported as one of the fourteen supreme in routine dynamic integral part of the agricultural practices (Yen et al., 2011). There are different ways for chlorpyrifos to enter in water including, Runoff events, erosion and leaching (Jin et al., 2015). Other extensively used pesticide, pyrethroid is also reported to be genotoxic to organisms including fish (Ambreen and Javed, 2015). Bifenthrin is not only

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used in agriculture but also in public health control programs (including mosquito control). It is reported as carcinogen, genotoxic, harm the central and peripheral nervous system (Ponepal et al., 2010). It is proven that chemicals, interact with DNA which may result into genic variability and alteration (Malling, 2004). Monitoring experts as of Food and Drug Administration (FDA) and European Medicines Agency (EMA) these days worked on altered Geno toxicity experiments like in vitro and in vivo assays (Araldi et al., 2015; Hartmann, 2004). There are different genotoxic tests, among them; Single cell gel electrophoresis is actually a complex and fast test which is practically for cells having nucleus in the recognition of DNA destruction. For observing the breakage in single strand or double strand, Single cell gel electrophoresis assay may be applied (Azqueta and Collins, 2013). Ostling and Johanson (1984) commented on Comet assay as best method however, after that usage several operational amendments were recommended, successively its expansion. The use of high pH methodology of comet survey, DNA denaturation can be detected with double strand and single strand breaks (which is associated by means of chromosomal aberrations and genomic instability) along with alkali labile sites (Singh et al., 1988; Pfeiffer et al., 2000).

Impurities commonly exist in multifarious form (Ambreen and Javed, 2015). As there is vast use of different pesticides and they leach into aquatic ecosystems and may cause damage. Fish is the most sensitive organism living in water. Rohu ever since remain important among major carps of Asia was selected as the test organism as it there remains a large need of experimentation for information on genotoxic effect of pesticides on freshwater fish.

2. Materials and methods

Labeo rohita juveniles were obtained from Govt. fish seed hatchery Punjab, Pakistan and carefully moved to laboratory. For fifteen days, fish were acclimatized and were given fish meal as diet. Stock 1 solution were prepared by dissolving pesticides (bifenthrin and chlorpyrifos) in 95% methanol (J.T Baker), and stock 2 solution were prepared by further dilution. The 96-hour LC_{50} was noted by Probit analyses method and on the basis of this value, one test concentration (33% of LC_{50}) was calculated which was further used for calculating the sub-lethal genotoxicity.

To study the DNA damage in the erythrocytes of *L. rohita*. Three groups were made, including twelve fish of equal size. One group was control group kept in tap water, second group was positive contrrol kept in cyclophosphamide (20 $\mu g g^{-1}$) and test group was exposed to 33% LC_{50} of pesticide. During experiment, water quality parameters were kept constant as temperature at 30 °C, pH at 7 and total hardness as225 mg/L. blood cells (Erythrocytes) were checked by using single cell gel electrophoresis on fortnight basis for 70 days. The SCGE was performed as by Singh et al. (1988).

The DNA destruction was calculated by optical cataloging of nuclei (un damaged = class1, medium damage = class2, high damage = class3, full damage = class 4) of "comets" (measured through TriTek CometScore™). Dim light was used during each step to stop further damage to DNA. Complete examination of DNA damage was observed as total percent sum of nuclear damage including, sum of classes (2 + 3 + 4). Data was statistically analyzed and means were compared by Duncan Range Multiple tests.

3. Results

Comet assay was used for investigating DNA damage by mixture of bifenthrin and chlorpyrifos. The detail about the damage of erythrocytes in *L. rohita* is shown in table 1. The number of damaged cells increased as the time of exposure was increased. Most of the damage to erythrocytes was observed by 33% LC_{50} whereas there was least damage in negative control group. As far as comet

Table 1
.DNA damage in erythrocytes of an indigenous fish (*L. rohita)* exposed to pesticide (mixture of bifenthrin and chlorpyrifos).

Fortnights	Treatments	Undamaged Nuclei (%)	Damaged Nuclei (%)						
		Class-0	Class-I	Class-II	Class-III	Class-IV	*Damaged Cells (%)	**GDI	***CTL (μm)
1st	Negative Control	96 ± 0.10 ^{a}	3 ± 1.01^{c}	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}	0.02 ± 0.00^{c}	3.23 ± 0.03^{c}
	Positive Control	28 ± 0.00^{b}	13.67 ± 1.15^{b}	18 ± 3 ^{a}	8.99 ± 2.19^{b}	19.99 ± 2.35 ^{a}	48.13 ± 2.09^{b}	1.63 ± 0.01^{b}	128.15 ± 0.05^{b}
	Treatment	19.43 ± 2.35^{c}	23.23 ± 1.15 ^{a}	11.98 ± 3.25^{b}	23.17 ± 2.35 ^{a}	20.01 ± 1.90^{b}	52.72 ± 2.31 ^{ab}	1.83 ± 0.08 ^{a}	572.37 ± 0.06 ^{a}
2nd	Negative Control	95.10 ± 1.01 ^{a}	3 ± 1.01^{c}	0.11 ± 0.11^{c}	0 ± 0^{c}	0 ± 0^{b}	0 ± 0^{c}	0.02 ± 0.00^{c}	3.19 ± 0.08^{c}
	Positive Control	30 ± 2.10^{b}	13 ± 2.01^{b}	19.45 ± 2.05 ^{ab}	13.89 ± 2.45^{b}	22.09 ± 2.59 ^{a}	49.01 ± 1.45^{b}	1.67 ± 0.04^{b}	130.56 ± 0.05^{b}
	Treatment	16.879 ± 2.35^{c}	19.33 ± 3.25 ^{a}	17.73 ± 2.16 ^{bc}	25.97 ± 2.90 ^{a}	22.18 ± 3.66 ^{a}	62.13 ± 0.01 ^{a}	2.06 ± 0.04 ^{a}	678.20 ± 0.05 ^{a}
3rd	Negative Control	95 ± 0.20 ^{a}	3 ± 1.01^{c}	0.11 ± 0.11^{c}	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}	0.02 ± 0.00^{c}	3.22 ± 0.04^{c}
	Positive Control	36 ± 3^{b}	14.33 ± 1.15 ^{ab}	14.67 ± 2.15^{b}	13.89 ± 2.45^{b}	22.09 ± 2.59^{b}	49.52 ± 3.61^{b}	1.42 ± 0.07^{b}	128.70 ± 0.10^{b}
	Treatment	9.87 ± 3.25^{c}	9.00 ± 2.00^{b}	18.00 ± 2.00 ^{a}	24.88 ± 2.35 ^{a}	27.01 ± 4.90 ^{a}	79.37 ± 2.35 ^{a}	2.28 ± 0.04 ^{a}	694.51 ± 0.14 ^{a}
4th	Negative Control	95.00 ± 0.10 ^{a}	3 ± 1.01^{c}	0.11 ± 0.11^{c}	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}	0.02 ± 0.00^{c}	3.42 ± 0.08^{c}
	Positive Control	28.57 ± 1.35^{b}	13.57 ± 2.31 ^{ab}	23.12 ± 3.02^{b}	18.47 ± 2.05^{b}	22.09 ± 2.59 ^{ab}	60.82 ± 4.35^{b}	1.93 ± 0.04^{b}	134.58 ± 0.07^{b}
	Treatment	9.23 ± 2.31^{c}	9.33 ± 0.45^{b}	33.00 ± 3.46 ^{a}	27.89 ± 3.45 ^{a}	21.98 ± 2.12 ^{bc}	78.16 ± 4.05 ^{a}	2.43 ± 0.01 ^{a}	681.35 ± 0.05 ^{a}
5th	Negative Control	93.33 ± 2.15 ^{a}	1.97 ± 2.15^{c}	0.11 ± 0.11^{c}	0 ± 0^{c}	0 ± 0^{b}	0 ± 0^{c}	0.03 ± 0.01^{c}	3.45 ± 0.05^{c}
	Positive Control	28.00 ± 2.30^{b}	13.57 ± 2.31^{b}	14.27 ± 0.15^{b}	19.03 ± 3.09^{b}	18.98 ± 2.65 ^{a}	54.29 ± 2.95^{b}	2.05 ± 0.06^{b}	139.54 ± 0.04^{b}
	Treatment	9.27 ± 2.15^{c}	16 ± 3 ^{ab}	33.12 ± 2.11 ^{a}	30.10 ± 4.18 ^{a}	23.89 ± 1.95 ^{a}	74.78 ± 4.56 ^{a}	2.27 ± 0.09 ^{a}	713.70 ± 0.06 ^{a}

The means with similar letters in a single column for each variable are statistically non-significant at $p < 0.05$
a = is significant, b = significantly different from a and c, bc = significantly different from a but not from b and c

Table 2
DNA damage of an indigenous fish (*L. rohita*).

		Damaged Cells (%)	*GDI
Dose Dependent Genotoxicity			
Treatments	**Negative Control**	0.00 ± 0.00^{c}	0.02 ± 0.00^{c}
	Positive Control	51.00 ± 3.95^{b}	1.74 ± 0.09^{b}
	33% of LC_{50}	67.66 ± 10.77 ^{a}	2.17 ± 0.21 ^{a}
Time Dependent Genotoxicity			
Fortnights	**1st**	33.29 ± 29.23 ^{e}	1.13 ± 1.05 ^{e}
	2nd	38.23 ± 29.43 ^{d}	1.22 ± 1.14 ^{d}
	3rd	39.63 ± 38.94 ^{bc}	1.27 ± 1.25^{c}
	4th	51.33 ± 39.86 ^{a}	1.29 ± 1.22^{b}
	5th	39.89 ± 36.22^{b}	1.32 ± 1.23 ^{a}

The means with similar letters in a single column for each variable are statistically non-significant at $p < 0.05$.
* Genetic Damage Index
a = is significant, b = significantly different from a and c, bc = significantly different from a but not from b and c

assay is concerned, there was maximum damage by 33% LC_{50} in first fourteen days, then it continued to next fourteen days and after that damage was not observed in next two fortnights (in this damage was observed by positive control) but again damage was observed in fifth fortnight. The results of second-class cell damage were also significantly varying during different fortnights, as there was higher percent damage during first thirty days. But after first two fortnights, second class cell damage decreased (table 1). Among control, treatment and positive control, the higher damage group and complete damage group were observed in treatment (33% LC_{50}).

The proportion of high damage cells were observed from 24.67 ± 1.15 – 34.33 ± 1.15 and there was continuous increase in damaging cells from first fourteen to forty-two days. There was continuous increase in DNA damage of all comet sections with the passage of time from the start to the end of the experimental period.

There was not only dose dependent but also time dependent deviations in damage cells percentage, index of genetic damage and increasing tail length of comet in erythrocytes of *L. rohita* as shown in table 2. Both the dose and time dependent damage remain higher in treatment (33% LC_{50}) followed by positive and control group and the results remain statistically different from each other. There was increase in percentage of damage cell till forty-two days and after that there was some decrease in DNA damage with the passage of exposure time as shown in table 2. As far as the indices of genetic damage is concerned, it remains high in fifth fortnight and lower at first fourteen days of experiment.

There was increase in comets tail length in first forty-two days of experiment after that there was decrease in length but again increase was observed in last fourteen days of experiment.

4. Discussion

Pesticides are used to control pests and act directly or indirectly on DNA by the parental compound/ metabolites or by producing reactive oxygen species (Oliveira et al., 2009). In the present study, the effect of pesticides was studied on fresh water fish for its time as well as dose dependent effects were observed. Here in present study, there was significant increase in damage similar results were reported by Bantu et al., 2017; Polard et al. (2011) and Nwani et al., 2010. Pesticide effect on erythrocytes of fish in relation to time and dose was studies by Nwani et al., 2013; Rani and Kumaraguru, 2013; Pandey et al., 2011 and Yong et al., 2011. This was also reported that a pesticidal mixture (chlorpyrifos, endosulfan and thiraam) may be DNA destructive. In a tropical fish, an acute herbicidal exposure may increase the cuts/ lesions of DNA of blood cells which can be observed by single cell gel electrophoresis (Arcaute et al., 2014).

This study was designed to identify the prolonged effect of pesticides on DNA damage as in past decade acute toxic effects were estimated but such methods fail to provide all related data. In the present study, results showed that pesticides affect DNA and genotoxic effect on *L. rohita*. Similar results were observed by Li et al. (2015) and concluded chlorpyrifos as a strong genotoxic contaminant which may damage DNA and cells. It is also observed that reactive oxygen species also contribute to bifenthrin + chlorpyrifos mediated DNA damage. Deshmukh, 2016 also observed similar results in china sp.

In the present study, there was decrease in DNA damage after 56 days. It might be the repair of DNA by losing the damaged cells. The tail length shows the way of DNA migration from nucleus. It must be taken in mind that small DNA fragments move far (Kumaravel and Jha, 2006).

5. Conclusion

This study provides good information to the fisheries researchers and farmers about the genotoxic effects of pesticides on fish health and ecology. This study may turn out to be a significant pointer in evaluating universal healthiness of freshwater fish. This will be also helpful in recognition of the status of genic toxicity, as an observing aspect for calculating the influences of effluence on the aquatic organisms.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

F. Ambreen M. Javed Assessment of acute toxicity of pesticide mixture for Cyprinus carpio and Ctenopharyngodon idella Pakistan Journal of Zoology 47 2015 133 139 0030-9923/2015/0001-0133

Arcaute, C.R., Soloneski, S., Larramendy, M.L., 2014. Evaluation of the genotoxicity of a herbicide formulation containing 3, 6-dichloro-2-metoxybenzoic acid (dicamba) in circulating blood cells of the tropical fish *Cnesterodon decemmaculatus*. Mutation Research 773, 1–8. https://doi.org/10.1016/j.mrgentox.2014.08.001.

Araldi, R.P., Melo, D., Mendes, T.C., Junior, T.B., Nozima, B.H.N., Ito, E.T., Carvalho, R.F. D., Souza, E.B.D., Stocco, R.D.C., 2015. Using the comet and micronucleus assays for genotoxicity studies: A review. Biomed Pharmacother 72, 74–82. https://doi.org/10.1016/j.biopha.2015.04.004.

Azqueta, A., Collins, A.R., 2013. The essential comet assay: a comprehensive guide to measuring DNA damage and repair. Arch. Toxicol 87 (6), 949–968. https://doi.org/10.1007/s00204-013-1070-0.

Bantu, N., Chaithnaya karri, K., Krishnan VK, G., Kumari, N., Vakita, R., 2017. Histological Alteration in Different Tissues of Indian major carp, *Labeo rohita* (Hamilton) exposed to Profenofos 50% EC and Carbosulfan 25% EC formulations. J Biol Today World 6 (3). https://doi.org/10.15412/J.JBTW.01060301.

Deshmukh, D., 2016. Hamatological response in a freshwater fish Channa Striatus exposed to endosulfan pesticide. Bioscience Discovery 7, 67–69. https://doi.org/10.5281/zenodo.1341004.

Elina, F.G.C.D., Spadotto, C.A., Weber, O.L.S., Villa, R.D., Vecchiato, A.B., Pinto, A.A., 2016. Environmental behavior of chlorphyrifos and Endosulfan in tropical soil in central Brazil. J Agricultural and food Chemistry 64, 3942–3948. https://doi.org/10.1021/acs.jafc.5b04508.

Hartmann, A., 2004. Use of the alkaline in vivo comet assay for mechanistic genotoxicity investigations. Mutagenesis 19 (1), 51–59. https://doi.org/10.1093/mutage/geg038.

Jin, Y., Liu, Z., Peng, T., Fu, Z., 2015. The toxicity of chlorpyrifos on the early life stage of zebrafish: A survey on the endpoints at development, locomotor behavior, oxidative stress and immunotoxicity. Fish and Shellfish Immunology 43, 405–414. https://doi.org/10.1016/j.fsi.2015.01.010.

Kumaravel, T.S., Jha, A.N., 2006. Reliable Comet Assay measurements for detecting DNA damage induced by ionising radiation and chemicals. Mutation Research 605 (1-2), 7–16. https://doi.org/10.1016/j.mrgentox.2006.03.002.

Li, D., Huang, Q., Lu, M., Zhang, L., Yang, Z., Zong, M., Tao, L., 2015. The organophosphate insecticide chlorpyrifos confers its genotoxic effects by inducing DNA damage and cell apoptosis. Chemosphere 135, 387–393. https://doi.org/10.1016/j.chemosphere.2015.05.024.

Malaj, E., Ohe, V.D., Grote, M., 2014. Organic chemicals jeopardize the health of freshwater ecosystems at the continental scale. Proceedings of the National Academy of Sciences of the United States of America 11, 9549–9554. https://doi.org/10.1073/pnas.1321082111.

Malling, H.V., 2004. History of the science of mutagenesis from a personal perspective. Environ Mol Mutagen 44 (5), 372–386. https://doi.org/10.1002/(ISSN)1098-228010.1002/em.v44:510.1002/em.20064.

Nwani, C.D., Nagpure, N.S., Kumar, R., Kushwaha, B., Lakra, W.S., 2013. DNA damage and oxidative stress modulatory effects of glyphosate-based herbicide in freshwater fish, *Channa punctatus*. Environmental Toxicology and Pharmacology 36 (2), 539–547. https://doi.org/10.1016/j.etap.2013.06.001.

Nwani, C.D., Lakra, W.S., Nagpure, N.S., Kumar, R., Kushwaha, B., Srivastava, S.K., 2010. Mutagenic and genotoxic effects of carbosulfan in freshwater fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single cell gel electrophoresis. Food Chemical Toxicology 48 (1), 202–208. https://doi.org/10.1016/j.fct.2009.09.041.

Oliveira, M., Maria, VL., Ahmad, I. & serafim, V. (2009) Contamination assessment of coastal lagoon (Ria de Aveira, Portugal) using defence and damage biochemical indicators in gill of Liza aurata an integrated biomarker approach. Environmental Pollution 157, 959-967. doi.org/10.1016/j.envpol.2008.10.019

Ostling, O., Johanson, K.J., 1984. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochemical and Biophysics Research Communication 123 (1), 291–298. https://doi.org/10.1016/0006-291X(84)90411-X.

Pandey, Atindra Kumar, Nagpure, N.S., Trivedi, Sunil P., Kumar, Ravindra, Kushwaha, Basdeo, 2011. Profenofos induced DNA damage in freshwater fish, *Channa punctatus* (Bloch) using alkaline single cell gel electrophoresis. Mutation Research 726 (2), 209–214. https://doi.org/10.1016/j.mrgentox.2011.09.011.

Pfeiffer, P., Goedecke, W., Obe, G., 2000. Mechanisms of DNA double-strand break repair and their potential to induce chromosomal aberrations. Mutagenesis 15, 289–302. https://doi.org/10.1093/mutage/15.4.289.

T. Polard S. Jean L. Gauthier C. Laplnche G. Marlina J.M. Sanchez-Perez E. Pinelli Mutagenic impact on fish of runoff events in agriculture areas in south-west France Aquatic Toxicology 101 2011 126 134 doi.org/10.1016/j.aquatox.2010.09.014

Ponepal, MC., Paunescu, O., Draghici, & Marinescu, V. (2010) Research on the changes of some physiological parameters in several fish species under the action of the talstar insecticide. Analele Universităţii din Oradea - Fascicula Biologie 17, 175-179. doaj.org/article/0491b954eb394f3389834a22b993e99f

Rani, A.P.A., Kumaraguru, A.G., 2013. DNA integrity as molecular biomarker of genotoxic effect og endosulfan in *Oreochromis mossambicus* (peters). International Journal of Engineering Science Invention 2, 58–61.

Ruiz-Suarez, N., Luis, D.B., Henriquez-Hernandez, A.L., Gonzalez-Moreo, F., Suarez-Perez, A., Camacho, M., Zumbado, M., Almeida-Gonzalez, M., Travieso-Aja, M.D. M., Luzardo, O.P., 2015. Continued implication of the banned pesticides carbofuran and aldicarb in the poisoning of domestic and wild animals of the Canary Islands (Spain). Sci Total Environ 505, 1093–1099. https://doi.org/10.1016/j.scitotenv.2014.10.093.

N.P. Singh M.T. Mccoy R.R. Tice E.L. Schneider A simple technique for the quantization of low levels of DNA damage in individual cells Experimental Cell Research 175 1988 184–191. scribd.com/document/95082787

Yen, Jerry, Donerly, Sue, Levin, Edward D., Linney, Elwood A., 2011. Differential acetylcholinesterase inhibition of chlorpyrifos diazinon and parathion in larval zebrafish. Neurotoxicology and Teratology 33 (6), 735–741. https://doi.org/10.1016/j.ntt.2011.10.004.

Yong, C., Guo, J., Xu, B., Chen, Z., 2011. Genotoxicity of chlorpyrifos and cypermethrin to ICR mouse hepatocyte. Toxicology Mechanisms and Methods 21, 70–74. https://doi.org/10.3109/15376516.2010.529192.