Research Journal of Zoology

Research A SCITECHNOL JOURNAL

Cytogenotoxic Effect Of Pesticides Induces Variability In Micronucleus And Nucleo-Cytoplasmic Abnormalities In Channa Punctatus In Vivo

Pradipta Kumar Sarangi*

Department of Zoology, Indira Memorial College, Chandiput,Gajapati,Odisha

***Correspondence to:** Pradipta Kumar Sarangi, Department of Zoology, Indira Memorial College, Chandiput, Gajapati, Odisha, Tel no: 919437986186; E-mail:sarangi.pradipta@gmail.com

Received date: Aug 26, 2021; **Accepted date:** November 17, 2021; **Published date:** November 29, 2021

Citation: Sarangi pk (2021) Cytogenotoxic effect of pesticides induces Variability in Micronucleus and Nucleo-Cytoplasmic Abnormalities in Channa punctatus in vivo. Res j Zool. Res j Zool,3(5).

Abstract

The aquatic resource is the major part of our environment , its safety is directly related to our health. In this study, fresh water fish Channa punctatus was taken as the genotoxic test model to estimate water pollution using micronucleus (MN) assay. It has been used successfully as a mutagenic assay. The fish was exposed in vivo to three different concentrations(MC,MC/2&MC/5)of eight pesticides (Dimethoate,Dichlorovos,chlorpyriphos and Malath ion,Methyl parathion,Fenvalerate,Cypermethrin and Carbaryl) at different time periods (5,10,15,20,25days). Peripheral blood samples smears were stained with Giemsa,MN frequencies were qualitatively analyzed. Qualitative analysis of the output recommends the rate of concentration, period, nature and mode of action of different Agro- pesticides causes varieties of micronuclei and nucleo-cytoplasmic abnormalities in live fish Channa punctatus erythrocyte as sensitive indicator for evaluation and assessment of aquatic pollution.

Key Words: cytogenetics, genotoxicity; pesticides; fish; channa punctatus,micronucleus; pollutants.

Introduction

In the modern world, humans are exposed to different genotoxic agents present in the environment [1] directly or indirectly through various medium mostly aquatic. The use of pesticides has been recognized as a vital part of agricultural practices throughout the world. Various industrial and agricultural activities increase pollution, [2,3,4,5] particularly in the aquatic environment, which is contaminated by various toxic chemicals from the discharge of waste waters and agricultural drainage[6,7].These are responsible for multiple effects at the organisms, including human beings, and ecosystem levels, affecting organ function, reproductive status, species survival, population size and ultimately biodiversity. Among these, carcinogenic and mutagenic compounds are the most problematic as their effect may exert a damage beyond that of individual and may be active through following generations. Epizootic neoplasm has been found in a variety of exothermic species, such as shell fish, echinoderms, jawless and bony fish [8].

Fish are excellent subjects for the study of the clastogenic, mutagenic and/or carcinogenic potential of contaminants present in water samples since they can metabolize, concentrate and store water borne pollutants [9, 10, and 11]. Since fish often respond to toxicants in a similar way to higher vertebrates, they can be used to screen for chemicals that are potentially teratogenic and carcinogenic in humans. The main application for model systems using fish is to determine the distribution and effects of chemical contaminants in the aquatic environment [12, 13] evaluated monitoring systems that use aquatic organisms to assess the genotoxicity of water in the field as well as in the laboratory.

Micronucleus assay was shown to be applicable to fresh water and marine fishes and that gill cells are more sensitive than hematopoietic cells to micronucleus inducing agents The micronucleus test, developed by [14,15],is an in vivo and in vitro short-time screening method is widely used to detect genotoxic effects . It is one of the simplest, reliable, least expensive and rapid screening system for both clastogenic (chromosome breakage and formation of acentric fragments) and aneugenic (chromosome lagging and effects on spindle) effects[15]. Clastogenic and aneugenic agents are known to affect the spindle apparatus, and can be differentiated on the basis of the relative induced micronucleus sizes or in the presence of kinetochores[16] .The micronucleus (MN) test, one of the most popular tests of environmental genotoxicity, has served as an index of cytogenetic damage[17,18,19] ().Micronuclei are cytoplasmic chromatin masses with the appearance of small nuclei that arise from chromosome fragments or from intact whole chromosomes lagging behind in the anaphase stage of cell division. Their presence in cells is a reflection of structural and/or numerical chromosomal aberrations arising during mitosis [16, 20, and 21].

The formation of morphological nuclear abnormalities (NAs) was first described in fish erythrocytes by[22] NAs, including lobbed (LB), blebbed (BL), and notched (NT) nuclei, bi nucleated (BN) cells and many others have been used by several authors as possible indicators of genotoxicity . Several studies have shown that erythrocytes of fish present a high frequency of micronuclei and nuclear abnormalities after exposure to different heavy metals under both field and laboratory conditions [6, 12, and 23]

For the determination of genotoxic effect in fish, the micronucleus test as well as the study of the abnormal shape of nuclei is a suitable measure with which the presence or absence of genotoxins can be detected in water. The detection of MN and NAs in fish help us to assess the status of water quality as well as the health of a particular species and any potential risk it might have after consumption [24]

Since micro nucleated piscine erythrocytes have been proved to be sensitive indicators of genetic damage, the purpose of our study was to evaluate the cytogenotoxic (clastogenic or aneugenic) effects of Dimethoate, Dichlorvos, Chloropyriphos meth yl parathion, Malathion,Fenvaleate, cypermethrin, carbaryl are the organophosphorous,pyrethroid and carbamate group of insecticides in Channa punctatus live fish using the MNT[25,26,27,28]

Material Methods

Material Methods

Test animal

Specimens of Channa punctatus measuring about 10-12 cm collected from the local ponds and maintained in laboratory aquaria were used for seven days before treatment.

Test Chemical

Dimethoate, Dichlorvos, Chloropyriphos, Methyl Parathion, Malathion,Fenvalerate ,Cypermethrin, Carbaryl are the organophosphorous, pyrethroid and carbamate group of insecticides belongs to different trade name and manufacture bought from the local market.

Doses and route of exposure: From among the specimens acclimatized for at least a fortnight in the laboratory aquaria, only strong and active fishes were released into different aquaria containing pesticides correspond to LC50, MC, MC/2 and MC/s doses respectively as per table-1.

Table1: List of pesticides used in the present study along with their LC50, MC, MC/2 and MC/5 concentrations.

Pesticide(Trade name)	Manufacturer	LC50(inµg/liter)	MC/2(inµg/liter)	MC/2(inµg / liter)	MC/5(inµg/lite)
Dimethoate(Roger-30E)	Rallies India Ltd., 21, D,Sukhadev Marg,Mumbai-400001,India.	100	50	25	10
Dichlorovos(Nuvan)	Hinustan Ciba-Geigy Limited, 14.J.Tata Road, Mumbai-400020, India.	500	250	125	50
Chlorpyriphos(Tafaban-20E)	Rallies India Ltd., 21,D,Sukhadev Marg,Mumbai-400001,India	10	5	2.5	1
Methyl Parathion (Metacid-50)	All India Medical Corporation, 185, Princess Street, P.B.No. 2398, Mumbai,India.	300	150	75	30
Malathion(Mal-Tox)	All India Medical Corporation, 8thRoad, AkhandJyotiBuilding, SantaCruez East Mumbai-400020, India.	250	125	67.5	25
Fenvalerate (Sumicidin)	Rallies India Ltd., 21,D,Sukhadev Marg,Mumbai-400001,India	250	125	67.5	25
Cypermethin(Polytren-20E)	Solar FARMA CHEM Ltd.Sorodhi,Valsad,Gujarat.	10	5	2.5	1
Carbaryle (Sevin)	Rallies India Ltd., 21,D,Sukhadev Marg,Mumbai-400001,India	250	125	67.5	25

MC represent the maximum tolerable concentration of the test compound at which nmber of death of animal beyond 5% was observed during the period of treatment and was determined from preliminary experiments on groups of 20 specimens in aquaria containing 100 litre of water. The test lasted for 25 days with change of water, chemical and food every alternate day. The lowest concentration leading to 50 % death after the treatment was considered as LC50 and half of this corresponds of MC. MC/2 and MC/5 represent 1/2 and 1/5 of MC. The treated specimens received an intramuscular injection of 0.02% colchicines solution at the rate of 1ml per 100 mg body weight 2 h prior to their sacrifice on completion of 5,10,15,20 and 25 days of exposure to the test chemical.

Micronucleus Test

The smear of peripheral blood drawn from the caudal vein with a heparinized syringe, was prepared and well-dried slides were stained in 10% Giemsa solution (Stock solution diluted with Sorensen's buffer at pH 6.8) for 30 min following the method of [14] Four thousand cells per animals (1000 cells per slide) were scored for micro-nuclei and nuclear anomalies.

Results

Erythrocytes of Channa punctatus have a fairly large smooth centrally placed, elliptical nuclei and sizeable cytoplasm. The ratio of nucleus to cytoplasm is about 1:5 .The smears of all the treated group of specimens, irrespective of the pesticides concentrations or the period of exposure examined in this study, revealed consistent variations from the above mentioned normal feature of erythrocytes in significantly high frequency than in control. These anomalies were similar to those described in earlier studies which utilized the Piscean micronucleus test [13, 26,29,30,31,32,33,34,,35,36,37,38,39] and thus were not artifactual. In

other words, the anomalies did not result due to technical protocols followed in this study but were, in fact, produced due to action of pesticides. The following were the types of nuclear lesions and Nucleocytoplasmic Abnormalities observed in this study as [40] and other workers [41,42,43,44,45,46,47,48,49,50,51,52].

- Micronuclei

Non- refractive cytoplasmic particles with a distinct and characteristics consistent with stain intensity of the nucleus were considered as micronuclei. The size as well as location of such particles in the cytoplasm varied from cell to cell but the shape was almost round or oval in all cells examined. In majority of cells, however, they appeared as minute dot with diameter varying from 1/5to 1/20 of the main nucleus. Furthermore, in many cells they were placed very close to the nucleus and appeared to be connected to the latter a very thin basophilic strand (fig.2.1A). Again, each affected cells usually has a single MN but cells with two or more MNs were not completely absent from the preparations (figs.1.B-C, 2. A-M , 3. A-L,4. A-G).

- Nucleocytoplasmic Anomalies
- Notched Nuclei (fig.1.D,5.B-C)

A nucleus with a well-defined slit of uniform width extending to an appreciable depth into the nucleus was considered as notched nucleus. Notches appeared to contain no nuclear material and seemed to be demarcated by the nuclear envelope.

- Blebbed Nuclei (figs.1. E-F,5.D-F)

Nuclei with relatively small evagination of the nuclear envelope were considered. As blebbed nucleus. The size of the blebs in the majority of the cells with blebbed nuclei was similar to that of the micronuclei. However, the size of different blebs varied, from cell to cell, from a slight protrusion to a slaked structure and round terminus.

- Lobedd Nuclei (fig1.G,5.G-H)

Nuclei with evaginations larger than blebs were recorded as 'lobed nuclei'. In the majority of the cells, the latter appeared as cross or 'X' Shaped.

- Conical nuclei(fig.1.H-I,6-A)

In many cells nuclei assumed a cone shape due perhaps to the presence of a well-connected micronucleus near them and space in between little lightly stained.

- Budding(Fig.5.I-L,)
- Vacuolated Nuclei (fig.1. J,6.B-I)

Nuclei with definite hole (s) devoid any visible materials wear considered as vacuolated nuclei. The size, location and number of the holes, however, varied from cell to cell .

- Fragmentation((fig.1K,6.J-K)
- The vacuole formation in the nuclei might have resulted in the fragmentation.

Disintegration (fig1.L, 6. L) And ultimately to the disintegration of the nuclei in groups of specimens exposed for longer period to higher concentration (MC) of the pesticides.

- Nuclear Anomalies and Micronuclei Identification.

The consideration should be given to the various nuclear abnormalities. Such nuclear abnormalities can get confused with Micronuclei. Various nuclear abnormalities are Binucleated cells.

- Binucleated cells

(fig.1.J, 7. A-F) Presence of two nuclei within the cell which is indicative of failed cytokinesis. It was found that higher frequency of chromosomal disjunction occurs such binucleated cells than those cells with completed cytokinesis. [7]

Broken eggs or cells with nuclear buds (fig.9.E-H) They contain nuclei with a sharp constriction at one end of the nucleus. Such nuclear bud and the nucleus are usually appearing to be attached to one another. The morphology and staining properties of the nuclear bud are same as that of the nucleus; however its size may range from a quarter or less to that of the main nucleus. [8]

- Cells with condensed chromatin

A roughly striated nuclear pattern in which the aggregated chromatin is intensely stained. When chromatin aggregation is extensive the nucleus may appear to be fragmenting [8].

- Pyknotic cells

Cells characterized by a small shrunken nucleus which contains a high density of nuclear material. They may represent as an alternative mechanism of nuclear disintegration that is distinct from the process leading to the condensed chromatin and cell death stages.[7] Diler and ergine 2010

- Karyorrhectic cells

Fig- 4J,13 JCells with nuclear disintegration and the loss of integrity of the nucleus.[7] They have nuclei that are characterized by more extensive nuclear chromatin aggregation relative to condensed chromatin cells.[8]

- Karyolytic cells

fig.4. (K-L) fig. 8.K Cells in which the nucleus is completely depleted of DNA and is apparent as a ghost like image.

- Fused nucleus Fig.7. (G)
- Twisted Fig.7.(H)
- X shaped with MN Fig. 7(I-J).
- Tear drop like nuclei Fig.7.(K)
- Sickle shaped and MN Fig.7.(L)
- Pin worm Fig.8. (A)
- Saucer Fig.8(B)
- Tadpole Fig.8(C)
- kidney Fig.8(D)
- Heart Fig.8 (E - F)
- Hooked Fig.8 (H-I)
- Deformed nucleus (Irregular Shaped Nucleus) Fig.9(A-D)
- Broken egg nucleus Fig.9 (E)-(H)
- Retractor Nuclei Fig.9 (I-L)
- Condensed nuclei Fig.10 (A-K
- Terminal nucleus Fig.10(L)
- Echinocytic nucleus Fig.11 (A-C)
- Swollen Nucleus Fig.11 (D)
- Elongated Fig.11 (E)
- Trilobeed Fig.11 (F)
- Nuclear budding Fig.11 (G)-(I)
- Apoptosis Fig.4(H-J) Fig.11 (J -K)
- Necrosis Fig.11 (L)
- Hooked Nucleus 8(G-H)3
- Microcyte Fig.12 (A),13(B)
- Stomatoocyte Fig.12(B)- (C)

- Discocyte Fig.12 (D)
- Echinocyte Fig.12(E)
- Astrocyte Fig.12(F)-(G)
- Tailed cytoplasmic pocess Fig.12(H-K)
- Twin Cell with Cytoplasmic bridge Fig.12 (I)
- cytoplasmic budFig.12(J-L)
- AnisochromiasisFig.13(A)Cytoplsmic Abnormalities(CA) pigmented periphery and a virtually colourless central region
- Fused Cell. Fig.13 (C)
- condensed chromatin lobe Fig.13 (D)- (F)
- enucleated microcyte Fig.13 (G)
- Spindle Shaped nucleus Fig.13 (H)
- Large Nucleus Fig.13 (I)
- Sickle shaped cell vacuolated bud Fig.13 (K)
- Microcyte Fig.12 (A),13(B)
- vacuolated cytoplasm Fig.13(L)
- Stomatocyte Fig.12(B- C)Slit like erythrocyte
- Discocyte Fig.12 (D)Normal erythrocyte a biconcave shape
- Echinocyte

Fig.12(E)From the Greek word echinos , *meaning* 'hedgehog' or 'sea urchin'), in human biology and medicine, refers to a form of red blood cell that has an abnormal cell membrane characterized by many small, evenly spaced thorny projections.. A more common term for these cells is burr cells

- Enucleated(EN) without nucleus 8(L)
- Nuclear bud 11(G-I)
- Nuclear bridge 5(A)
- Cell with damaged Nucleus8(J)
- Anisochromiasia(AN):Cytoplsmic Abnormalities(CA)s pigmented periphery and a virtually colourless central region13(A)

Micronucei and nucleocytoplasmic anomalies (Group microphotograph)

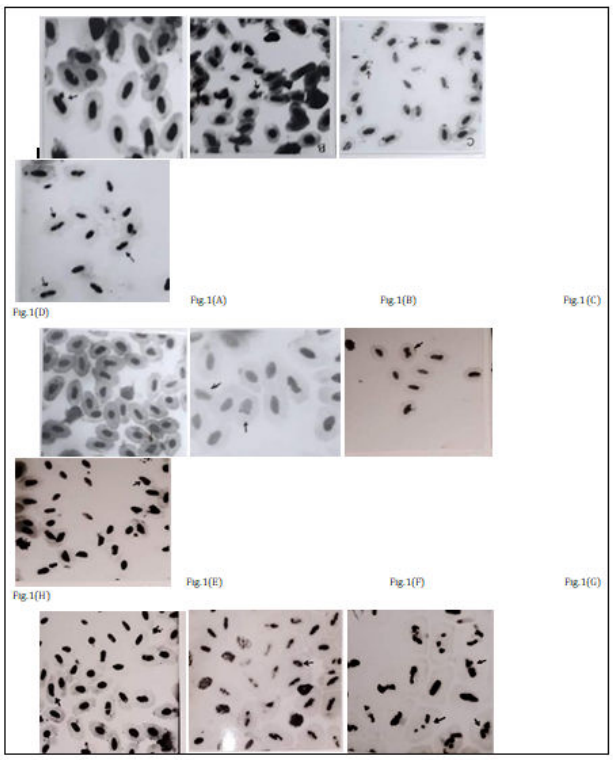
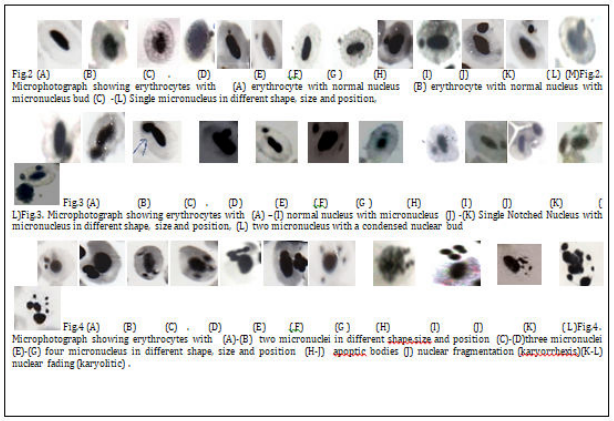
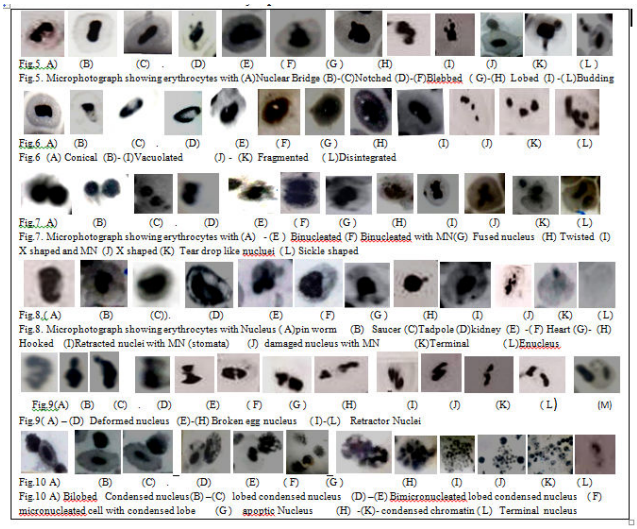


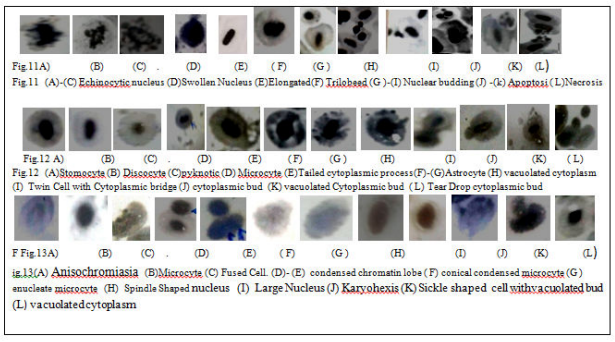
Figure 1: Microphotograph showing erythrocytes with (A)Single micronucleus.Fig. 1.(B-C) two and four micronuclei Fig.1.(D) notched nucleusFig.1 (E-F) blebbed nucleus. Fig. 1.(G) lobed nucleus.1.Fig.(H-I)conical nuclei 1.Fig. (J) vacuolated nucleui1.Fig. (K) fragmented and disintegrating nucleus.1.Fig. (L) disintegrating nuclei in erythrocytes

Micronclei(MN)



Nuclear –Cytoplasmic Anomalies





Find that chromosomes from micronuclei may trigger a chromosomal instability phenotype disaggregating at the mitosis following MN formation.Atrazine induced genotoxicity that could be

useful for investigating the effect of toxic blooms on wild fish populations[45]. Established a correlation between chemical composition of each compound,dose time period and mechanism of action of metabolites in different fish species of different location may cause variety of micronuclei and nuclear Anomalies in fish. The frequencies rates of MN, NAs in addition to Morphologically Alterd Eythrocytes(MAE) may exhibit significsant variation depending upon the nature and kind of the toxic agents[2]. [44] Said the frequencies in rates of MN and NAs may vary depending on type of pollutants, their combination and time of exposure. [5] Supported influence of factors like fish species, class, dose and concentration of the pesticide and exposure time in enhancement of Piscine micronucleus.

Discussion

Micronuclei are supernumerary nuclei visible by light microscopy in the cytoplasm of hematopoietic or sometimes even in actively dividing cells. Also known as “Howell-Jolly Bodies” in mammals[63], they are formed in dividing cells when acentric chromosome fragment (s) or whole chromosome lags behind during anaphase of clastogenic or aneuploidic events[65,66,67]. Althouth ,the test was originally developed and standardized using rodent bone marrow cells[15,65,68] ,it has been shown to work well with peripheral blood [69],Meiotic cells [70] ,liver cells[71] etc. of rodents ,red blood cells of news[72,73] and peripheral blood and kidney cells of fish [26,29,31,34,36,37,38,74].Utility of micronucleus test as in situ indicator of biological effects in wild fish, has, however, been doubted by[28,74] for two main reasons. First ,occurrence of micronuclei in extremely low frequency in the dividing cells in fish species so far investigated as compared to rodents and second, a lack of a significant correlation between the variations in nuclear morphology and the level of chemical contamination in the sediment or the bile or liver of the specimens of while croaker, Genyonemus lineatus [75] investigated. In sharp contrast to the observation of [75],we observed a very consistent and significant in the nuclear morphology and micronuclei in all the groups of specimens of Channa punctatus exposed to different pesticides as compared to the controls. We also observed a significant increase in their frequency with the increase in the concentration of the pesticides as well as the period of exposure (vide infra). This clearly suggests that the various kinds of erythrocyte nuclear lesions including micronuclei observed during this study must have originated from a genotoxic event as a result of exposure of the specimens to pesticides. A further support to our contention comes from observations of [13],. While developing suitable genotoxicity assay systems based on aquatic organisms, these authors observed high frequency of micronuclei in the gill epithelia of Carassius sp. (Funa) and Zacco platypus (Oikawa) collected from mid-stream of the river Tomio (Nova, Japan) as compared to those collected from upstream of the same river. They also observed structural chromosome aberrations as well as micronuclei in the cells of embryos Rhodeus ocellatus (Rose bitterling) grown in water containing trichloroethylene and

Evaluating Micronucleus Test’s sensitivity in freshwater fish check changing of genome, [50, 76]. Variability in micronucleus induction with different mutagens applied to several species of fish, But here we differ with[77] using a single species with eight different agro pesticides obtained more variable Micronucleus(MN) , Nuclear anomalies(NA) and Nucleo-Cytoplasmic Anomalies(NCA) [52] which is highly significant one.

Emergence of micronclei and their effects on the fate of cells under replication stress was studied by [78] is fur there supported by DNA Breaks and Chromosome Pulverization from Errors in Mitosis by [79].Many more deep study and co-relation regarding mechanism of action of mutagens, carcinogens and clastogens is essential among the workers for origin of micronucleus and nuclear anomalies in future. Hence said chromosome aberrations and micronuclei tests may prove to be powerful sensitive assays for detecting genotoxins in aquatic environment.

For the first time suggests the induction of cell death, ghost cells, cells with membrane damage and binucleted cell by cytotoxic and genotoxic effects of the Inula viscose leaf extracts on Allium cepa [80],. As a Consequence of global warming [51] found frequenc y of erythroblasts (Ebs),erythrocytic nuclear abnormalities(ENA) and erthrocyttic cellular abnormalities(ECA) were increased in response to to thermal stress in common carp Cyprinio carpio. Comparing Cellular alterations in fish exposed to ionisising radiations and pesticides, [60] in order to identified micronuclei Assay as biomarker of radiation also suggested that the erythrocyte MN assay can be aptly renamed the Erythrocytic micronucleus Cytome assay(ECMNA) as it encompasses variety of biomarkers that may find application in genotoxicity. Micronuclei and Nuclear Abnormalities increases during increased the days of exposure of sub lethal Karanjin obtained from seeds of plant Pongamiaa pinnata in Fish Cyprinio carpio[81]

Conclusion

Our study suggests, genotoxic pesticides with variation in structural organization, functional group, different mode of action and mechanism of function in same tissue of same species of fresh water live fish Channa punctatus(Bloch)induces variety of Micronuclei ,Nuclear Nucleo Cytoplasmic Abnormalities(NCA) with respect to time period and concentration dependent which differs from [76] in different species . In the subsequent trophic level at the end point of food chain the most sufferer will be the human being through biomagnifications of mutagenic ,carcinogenic,clastogenic and teratogenic pollutants[8] Hence rapid and urgent alternatives are necessary for both agricultural practices, Industrial development[82] as well as the survival of flora and fauna including human civilization in a healthy environment .

References

1. Sommer, S., Buraczewska. I., Kruszewski, M. Micronucleus Assay The State of Art and Future Directions .Int. J. Mol. Sci. 2020; 21 (4):1534.
2. Mourad,M.,Tanekhy,M.,Wassif,E.,Abdel-Tawab,H.,Mohamed,A.et al. Biochemical and Histopathological changes inNile Tlapia,Oreochromis niloticus at lake Edku.AJVS. 2017;55(2) pp.40-51.
3. Sayed,A.E-D.H.,Elbaghdady,H.A.M.,Zahram,E. Arsenic induced genotoxi city in Nile tilapia Oreochromis niloticus the role of Spirulina platensis extract. Environ.Monit.Asses. 2015; 187 pp.751.
4. Ragade Vinod, R., Kengar Ajit A., Khade Bipin, S., Shaikh, J. D., Pradhan ,P.S.et al.Effects of monochrotophos pesticide on liver, gill and kidney of fresh water fish Channa punctatus Trends in Fisheries research.2015; 4 (1) :2319–4758.
5. Salvagni C.A., Dagnone A.S., Gomes T.S., Mota J.S., Andrade G.M., Baldani C.D., et al. Serologic evidence of equine

granulocytic anaplasmosis in horses from central West Brazil. Rev.Bras.Parasitol.Vet.2010;19(3):135140.

6. Isani, G., Andreani, G., Cocchioni, F., Fedeli, D., Carpene, E. andFalcioni, G. Cadmium.et al. accumulation and biochemical responses in Sparus aurata following sub-lethal Cd exposure.Ecotox. Environ. Saf. 2009;72: 224-230.
7. Whitehead, H., Rendell, L., Osborne, R. W. , Wursig, B. et al.Culture and conservation of non-humans with reference to whales and dolphins: review and new directions. Biological Conservation.2004; 120 : 427–437.
8. Obiakor M. O.,Okonkwo ,J.C.,Nnabude p.c. and Ezeonyejiaku, C.D.Journal of animal science advances. 2012; (1): 123-133.
9. Ahmad, K.and Saleh,J. Clastogenic studies on Tandaha Dam water in Asser.J.Black Sea Mediterranean Environment.2010 ; 16(1):33-42.
10. Abdel-Gawad, F.K., El-Seehy, M.A. and El-Seehy, M.M. Clastogenicity in fish genome and aquatic pollution. World Journal of Fish and Marine Sciences.2010; 2: 335-342.
11. Al-Sabti K. Handbook of Genotoxic effects and Fish Chrom.Jozef Stefan Institute, Jamova.1991.
12. Al-Sabti K, Metcalfe CD Fish micronuclei for assessing genotoxicity in water Mutat. Res. 343: 121 -135.
13. Hayashi M, Ueda T, Uyeno K, Wada K, Kinae N, Saotome K, Tanaka N,Takai A, Sasaki YF, Asano N, Sofuni T, Ojima Y.et al. Development of genotoxicity assay systems that use aquatic organisms.Mutat. Res.1998; 399(2): 125-33.
14. Schmidt, W. The micronucleus test. Mutation Research.1975; 31(1): 9-15.
15. HeddleJ.A. A rapid in vivo test for chromosomal damage Mutation Research.1973;18: 187–190.
16. Heddle J.A., Hite ,M., Kirkhart ,B.,Mavouin,K., MacGregor, J. T.,Newell,G.W., and Salamon,M.F.et al. The induction of micronclei as a measure of genotoxicity.Mutat.Res. 1983;123:61-118.
17. Heddle J.A., Cimino M.C., Hayashi M., Romagna F., Shelby M.D., Tucker J.D., Vanparys Ph. and MacGregor J.T. et al.Micronuclei as an index of cytogenetic damage: past, present, and future. Environmental and Molecular Mutagenesis.1991; 18: 277–291.
18. Fenech M, Chang WP, Kirsch-Volders M, Holland N, Bonassi S, ZeigerE .et al.Human Micronnucleus Project. HUMN project detailed description of the scoring criteria for the cytokinesis-block micronucleusassay using isolated human lymphocyte cultures Mutat. Res.200.;534 (1-2): 65-75.
19. Udroiu I.The micronucleus test in piscine erythrocytes. Aquatic Toxicology,2006; 79: 201–204.
20. Savage,J.R.K.Micronuclei Pitfalls and problems.Atlas Genetics and Cytogenetics.2000; 1-9.
21. Bolognesi C. and Hayashi M. Micronucleus assay in aquatic animals. Mutagenesis,2011; 26: 205–213.
22. Carrasco K.R., Tilbury K.L., Myers M.S. Assessment of the piscine micronucleus test as an in situ biological indicator of chemical contaminant effects. Cana. Journ. Fishery and Aqua. Sc.i.1990; 47: 2123–2136.
23. Çavaş T., Ergene-Gözükara S. Micronucleus test in fish cells: a bioassay for in situ monitoring of genotoxic pollution in the marine environment. Environmental and Molecular Mutagenesis. 2005; 46: 64–70.
24. Talapatra, S.N. and Banerjee, S.K. Detection of micronucleusand abnormal nucleus in erythrocytes from the gill and kidneyof Labeo bata cultivated in sewage-fed fish farms. Food Chem.Toxicol.2007; 45: 210-215.
25. Abdelaziz K. B., El Makawy A. I., Abd Elsalam,A. Z. El-A and Darwish, A. M. Genotoxicity of Chlorpyrifos and the Antimutagenic Role of Lettuce Leaves in Male Mice .Comunicata Scientiae.2010; 1(2): 137-145.
26. Porichha, S.K., Sarangi P.K. and Prasad R. In Genotoxic effect of chlorpyriphus in fish.Perspectives in Cytol. and Gen. 9 Eds. G.K. Manna and S.C. Roy.1998; 631-638.
27. Sarangi , P.K, Patnaik, R, Porichha ,S.K and Prasad, R . Genotoxicity of malathion in Channa Punctatus cultured in vivo Perspective in Cytology and Genetics, (Editors G.K.Manna and S.C.Roy, AICCG Publication, Kalyani University. 2001;10:835-844.
28. Sarangi,P.K. Micronucleus Assay A sensitive indicator for aquatic pollution International Journal of Research in BioSciences.2012;1(2):(32-37).
29. Hooftman, R.N. and DE Raat, WK.Induction of nuclear anoma lie micronuclei in the peripheral blood erythrocytes of the e astern mudminnow Umbra pygmaea by ethyl methanesulphon ate. Mutation Research.1982; 104:147-152.
30. Longwell,A.C , Perry.D., HughesJ.B. and Hebert.A Frequencies of micronuclei in mature and immature erythrocytes of fish as an estimate of chromosome mutation rate-results of field surveys on windowpane flounder, winter flounder, and Atlantic mackerel. Int. Counc. Explor Sea, C M E55.1983.
31. Manna G.K., Banerjee G. And Gupta S. Micronucleus test in the peripheral erythrocytes of the exotic fish, Oreochromis mosambica.Nucleus.1985; 28(3): 176-179
32. Al Sabti,K. Clastogenic effects of five carcinogenic mutagenic Chemicals on the cells of the common carp Cyprinus carpio. L.Comp.Biochem. Physiol. C.1986; 85(I): 5–9.
33. Al Sabti, K. Comparative Micronuclei erythrocyte cell induction in three cyprinids by five carcinogenic mutagenic Chemicals.Cyto bios.1986; 47 (190-191):147-154.
34. Das, R. K.,andNanda, N. K. Induction of micronuclei in peripheral erythrocytes of fish Heteropneustes fossilis by mitomycin C and paper mill effluent," Mutation Research Letters.1986; 175, (2): 67–71.
35. Hose,J.E.,Cross,J.N.,Smith,S.G.and Diehl. Elavated circulating erythrocyte micronuclei in fishes from contaminated of sout hern California.Mar.Environ.Res.1987; 22:167-176.
36. Hose, J. E. Large scale genotoxicity assessment in the marine environment. Environ. Health Perspect.1994;102, 29–32.
37. Brunetti,R.,Majone,F.,Gola,I.and Belframe,C.(1988) The mironucleus Test:examples of applications to marine ecology.Mar.Eco.Prog.Ser.1988; 44:65-68.
38. Metcalfe, C.D. Induction of Micronuclei and Nuclear Abnormalities in the Erythrocytes of Mudminnows (Umbra Limi) and Brown Bullheads (Ictalurus Nebulosus) Bull Environ Contam Toxico. 1988; 40(4): 489-95.
39. Long,E.R., and Buchman,M.F. An evaluation of candidate measures of biological effects for the National status and Trends program.Natl.Oceanic Atmos . Tech.Memo.NOSOMA.1989; 45-97.
40. Tolbert, P. E. , Shy, C. M. , and Allen, J. W. Micronuclei and other nuclear anomalies in buccal smears: methods

development Mutation Research/Environmental Mutagenesis and Related Subjects.1992; 271(1): 69–77.

41. Ramakrishnan.S., Grebe.R.,Singh.M.,and Schimid-Schonbein.,H. Aggregation of shape altered erythrocytes: An invitro study. Current Science.1999; 7 (6).
42. Grisolia, C.K., Cordeiro, C.M.T. Variability in micronucleus ind uction with different mutagensapplied to several species of fi sh, Genet. Mol.Biol.2000;23:235–239.
43. Palhares,D.,and Grisolia ,C.K. Comparison between the micronucleus frequencies of kidney and gill erythrocytes in tilapia fish ,following mitomycin C treatment. Genetics and Molecular Biology.2002;25 281-284.
44. Barsine,J.,Dedonyte,V.,Rybakovas,A.,Andreikenaite,L.,Anderso n,O.k. et al. Investigation of micronuclei and other nuclear bnormalities in peripheral blood and kidney of marine fish treated with crude oil.Aquatic Toxicology78S(2006)S99-S104.2006.
45. Ivanova, L., Popovska-Percinic, F., Slavevska-Stamenkovic, V.,Jordanova, M., Rebok, K. et al. Micronuclei and nuclear abnormalities in erythrocytes from barbel barbus peloponnesius revealing genotoxic pollution of the river bregalnica Mac Vet Rev.2016; 39 (2): 159-166.
46. Braham. R.P., Blazer. V.S, Cossidy. H,.S., and Mazk,P.M. Micronuclei and othe erythrocyte: Nuclear abnormalities in fishes from the great lakes Basin,USA. Environmental molecular mutagenesis.2017; 58:570-581.
47. Kishino,Y.,Hasegaw. T.,Yamoto. T.,and Mori.K. Species difference in micronucleus induction of clastogenic associated with drug metabolic profileThe Journal of Toxicological Research.2019; 44 (10): 701-709.
48. Vasanth ,S.,Bupesh,G.,T, Siva Vijayakumar and Subramanian ,P. (2017)Impairment of micronucleus and DNA in the 2-Chloro Ethylamino-6-Isopopylamino-1,3,5-Triazine exposed Poecilia Sphenops. Haematology International Journal.2017; 1(2) :1-5.
49. Galindo T.P, Moreira L.M., Evaluation of genotoxicity using the micronucleus assay and nuclear abnormalities in the tropical sea fish Bathygobius soporator Teleostei, Gobiidae Genet Mol Biol. 2009;32(2):394–398.
50. Grisolia C.K.A.Comparison between mouse and fish micronucleus test using cyclophophamide, mitomycin C and various pesticides. Mutate .Res.2002;518:145-150.
51. Jiraungkoorskul, W., Kosai, P. Somphong, S, Kirtputra, P., Chawlab, J. S., (2007) Evaluation of Micronucleus Test's Sensitivity in Freshwater Fish Species.Research Journal ofEnvironmental Sciences.Volume 1 (2): 56-63.
52. Shahjahan,M.Khatun,M.S.Mun,M.M.,Islam,S.M.M.,Uddin,M.H. ,Badruzzaman,M.,khan, S. et al.nuclear and Cellular abnormalities of erythrocytes in response to thermal stress in common carp Cyprinus carpio.Frontiers in Physiology June. 2020;11(543):1-8.
53. Anifowoshe A.T., Oyebanjil, J. B., Oladipo, O. S., Oyeyemi, F. B., Abdulrahim , M. Y., Abdulkareem1, S. I., Mustapha1, M. K. et al. Histological Changes, Micronuclei Induction and Nuclear Abnormalities in The Peripheral Erythrocytes of Clarias gariepinus (Burchell 1822) Exposed to Water Sample from Apodu Reservoir.2020; 01(01): 01 –07.
54. Furnus, G.N.A., Caffetti, J.D., García, E.M., Benítez, M.F., Pastori, M.C. and Fenocchio, A.S. et al. Baseline micronuclei and nuclear abnormalities frequencies in native fishes from the Paraná River Argentina Braz. J. Biol.,2014; 74 (1): 217-221.
55. Bhatnagar, A. Yadav, A. S. and Cheema ,N. Genotoxic Effects of Chlorpyrifos in Freshwater FishCirrhinus mrigala Using Micronucleus Assay Advances in BiologyVolume. 2016; 1-6.
56. Kumar, R., Nagpure, N. S., Kushwaha, B., Srivastava, S. K. andLakra, W. S.et al. Teleost fish Channa punctatus (Bloch) using themicronucleus test and comet assay. Arch. Environ. Contam. Toxicol.2010;58,123–130.
57. Mansour, S.A., Mossa , A.H., and Heikal T.M., Cytogenetic and Hormonal Alteration in Rats Exposed to Recommended "Safe Doses" of Spinosad and Malathion Insecticides International Journal of Agriculture & Biology.2008.
58. Ateeq, B. M. Abul farah, M. Niamat Ali, Waseem Ahmad Induction of micronuclei and erythrocyte alterations in the catfish Clarias batrachus by 2,4-dichlorophenoxyacetic acid and butachlor ..Mutation Research2002; 518: 135–144
59. Nwani, C.D., Sahebrao,N., Nagpure, Kumar,R., Kushwaha,B., Kumar, P.,Lakra,W.S.et al. Induction of micronuclei and nuclear lesions in Channa punctatus following exposure to carbosulfan, glyphosate and atrazine ISSN: 0148-0545 (print), 1525-6014 (electronic)Drug Chem Toxicol.2013; 1-8.
60. Anubani,S. and Mohan Kumar,M,N, Nuclear and Cytoplasmic abnormalities in the fish Catla catla(Hamilton)exposed to chemicals and ionizing radiation.Research Journal of Environmental Sciences5.2011;(12):867-877.
61. Monteiro,V.,Cavalcante,D.G.S.M.,Vilela,M.BF.A.,Sofia,S.H.,Ma rtinez,C.B.R.et al. In vivo and in vitro exposure for the evalation of the genotoxic effects of lead on the Neotropical fesh water fish Prochilodus lineatus.Aquatic Toxicology.2011;104:291-298
62. Diler,S.B.,Ergene,S Nuclear anomalies in the buccal cells of calcite factory workers.Genetics and Molecular Biology. 2010;33(2): 374-378.
63. Schlegel, R, Mac Gregor, J.T, Everson, R.B. Assessment of cytogenetic damage by quantitation of micronuclei in human peripheral blood erythrocytes. Cancer Res.1986; 46: 3717–3721.
64. He,B.,Gnawali,N.,Hinman,A.W,Mattingly,A.J.,Osimani,A.,Cimi ni,D.et al.Chromosome missegregated in to micronuclei contribute to chromosome instability by missegregating at the next division.Oncotarget,2019;10 (28): 2660-2674.
65. Schmid, W., The micronucleus test for cytogenetic analysis. InChemical mutagens, principles and methods for their detection, ed. A. Hollander, New York Plenum Press.1976; 4;31-53.
66. Yamamoto, K. I., Kikuchi, Y. A Comparison of Diameters of Micronuclei Induced by Clastogens and by Spindle Poisons .Mutat Res.1980; 71(1):127-31.
67. MacGregor, J. T., Heddle, J.A., Barry ,Margolin ,H.,Rame, C.,.Salamone, M. F., Tice,, R. DieterWild, R.et al. Guidelines for the conduct of micronucleus assays in mammalian bone marrow erythrocytes.Mutation Research/Genetic Toxicology.1987; 189 (2):103-112.
68. Lederberg,V.I.M.,Schmid , W.The micronucleus test methodological aspectsMutation Research/ Fundamental and Molecular Mechanisms of Mutagenesis.1973; 19 (1): 109-117.
69. MacGregor, J. T., Schlegel, R., Choy, W. N ., Wehr, C .M.et al.Micronuclei in Circulating Erythrocytes: A Rapid Screen for Chromosomal Damage During Routine Toxicity Testing in Mice.Dev Toxicol Environ Sc. 1983;11:555-8.

70. Lahdetie.J Micronucleated spermatids in the seminal fluid of smokers and nonsmokers Mutation Research/Genetic Toxicology.1983; 172 (3):255-263.
71. Tates ,A.D., Boogaard, P.J., Darroudi , F. A.,Natarajan, T., Caubo, M.E.,van Sittert, N.J.et al. Biological effect monitoring in industrial workers following incidental exposure to high concentrations of ethylene oxide Mutation Research/ Fundamental and Molecular Mechanisms of Mutagenesis. 1995; 329, (1): 63-77.
72. Siboulet, R., Grinfeld, P., Deparis, P., Jaylet, A., Micronuclei in red blood cells of the new Pleurodeles waltl Michah: induction with X-rays and chemicals. Mutat. Res.1984; 125: 275–281.
73. Jaylet ,A., Deparis, P., Ferrier, V., Grinfeld, S., Siboulet, R.et al. A new micronucleus test using peripheral blood erythrocytes of the newt Pleurodeles waltl to detect mutagens in fresh-water pollution.Mutat Res.1986; 164(4):245-57.
74. Biswas ,S.and Manna,G.K. The Hay-Bacillus Bacillus substills as genotoxic agent in treated fresh water tilapia. Perspective in Cytology and Genetics, (Eds. G.K.Manna and S.C.Roy, AICCG Publication, Kalyani University).1992; 7:945-952.
75. Carrasco, KR., Tilbury, KL. and Myers, M.S.,et al. Assessment of the piscine micronucleus test as an in situ biological indicator of chemical contaminant effects. Canadian Journal of Fisheries and Aquatic Sciences.1990; 47: 2123-2136.
76. Fagr, A., El-shehawi, A.M., and Seehy, M.A., Micronucleus Test in Fish Genome: A sensitive Monitor for Aquatic Pollution, African J. Biotechnol.2008; 7: 606-612.
77. Grisolia, C.K., Cordeiro, C.M.T., Variability in micronucleus induction with different mutagens applied to several species of fish, Genet. Mol. Biol.2000; 23 :235–239
78. Utani ,K., Kohno, Y., Okamota, A., Shimizu, N.et al. (2010) Emergence of micronuclei and their effects on the fate of cells under replication stress. PLoS One Article (PDF Available) in PLoS ONE.2010; 5(4):10089 .
79. Crasta ,K., Ganem, N. J., Dagher, R., Lantermann, A. B., Ivanova, E. V., Pan, Y., Nezi, L., Protopopov, A., Chowdhury, D., Pellman, D . 2012 Jan.DNA Breaks and Chromosome Pulverization From Errors in Mitosis Nature . 2012;482(7383):53-8.
80. Celik,T.A., and Aslanturk,O.S.(2010)evaluation of Cytotoxicity and Genotoxicity of Inula Viscosa leaf extracts with Allium test.Journ.of Biomedicine and Biotechnology.2010; 2010 : 1-8.
81. Tasneem,S.,Yasmeen,R.(2018)Induction of micronuclei and erythrocytic abnormalities in peripheral blood of fish Cyprinus carpio on exposure to Karanjin.Iranian Journaal of Toxicology2018; 12(2): 37-43.
82. Okpokwasili, GC Contributions of Industrial Pollution and Environmental Degradation on the Emergence of Communicable and Non-Communicable Diseases. A paper presented at the 5th Annual National Conference of Society for Occupational Safety and Environmental Health (SOSEH), November. 2009.