



Genotoxic Effects of Rice-Agrochemicals on *Channa punctatus* (Bloch) and *Cyprinus carpio* (Linnaeus) Using Micronucleus Assay and Alkaline Single Cell Gel Electrophoresis

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ABSTRACT

Rice-cum-fish culture is a cost-effective practice for marginal farmers but the major constraint is the indiscriminate use of agrochemicals. Present work was designed to assess the genotoxic effects of rice agrochemicals in *Channa punctatus* (Experiment 1 CP1 to CP3) and *Cyprinus carpio* (Experiment 2 CC1 to CC3); using micronucleus, chromosome aberration, and single cell gel electrophoresis/Comet assay. Two experiments with three treatments (CP1/CC1: without pesticide; CP2/CC2: recommended doses; CP3/CC3: farmers' dose) were maintained in triplicates. The presence of tail DNA and micronuclei depicted significant DNA damage ($P < 0.05$) in all the treated fish. The mean percent frequency of MN showed significant ($P < 0.05$) differences with respect to the initial. The chromosomal aberrations and mean frequencies of tail DNA (%) were significantly abundant in CP3 and CC3 indicating high a genotoxic effect. Keeping in view the low genotoxic effects, treatment of CP2 and CC2 with recommended doses of pesticides may be disseminated to farmers.

INTRODUCTION

Rice is crucial to worldwide food security serving as the principal ingredient in the everyday diets of around three billion individuals. Rice is the main food crop on the planet with upgraded varieties developed globally in a wide range of biological conditions and water systems (Tsuruta et al. 2011). Coordinated paddy cum fish cultivation can assume a significant part in contributing food, pay, and sustenance to the farmers and tackling their economic problems. However, the major constraint of this aspect is the indiscriminate and extensive use of pesticides. Farmers have become progressively dependent on exorbitant pesticides that may ultimately subvert the efficiency of agro-biological systems and influence the environment. Pesticide deposits deteriorate aquatic waters and are harmful to non-target life forms, thus making their way through the food chain undermining the environmental equilibrium and biodiversity.

Farmers are quite unconcerned about the environmental drawbacks of pesticides. The agrochemicals used in rice fields may be toxic to stocked fish tending to build up residues in fish tissue due to stability in the environment causing genotoxicity and oxidative stress. Genotoxicity is the ability of the agrochemicals (xenobiotics) to impair genetic information contained in the cell. Micronuclei are

shaped during the telophase of mitosis or meiosis while reconstructing the nuclear envelope around the daughter cell's chromosomes (Udroiu 2006). Genotoxic effects of pesticides (chlorpyrifos, imidacloprid, pretilachlor, cartap hydrochloride, lambdacyhalothrin) applied in rice fields can be evaluated through different biomarkers like evaluation of structural modifications in chromosomes, sister chromatid exchanges, micronucleus frequency, DNA adducts and breaks (Bombail et al. 2001). Among these biomarkers, comet, micronucleus, and chromosome aberration assays are simple, reliable, and less time-consuming. Chromosomal aberrations are the abnormalities of chromosomes such as end-to-end joining, stickiness and clumping, attenuation, chromatid break, acentric fragment, pycnosis, despiralization of chromosomes, erosion of chromatid material; occur during the cell division due to physical, chemical or physiological factors. Comet assay is independent of chromosome number and does not require pre-treatment with chemicals like mitotic inhibitors. Therefore, it is the most frequent and recommended method to detect DNA damage in organisms including fishes. Owing to the importance of integrated fish culture, the present attempt was executed to assess the genotoxic effect of pesticide exposure on *Channa punctatus* and *Cyprinus carpio* in paddy-cum-fish culture.

MATERIALS AND METHODS

The suitable quality confirmation strategies for the preparation of samples, handling, and protection were done as per US EPA protocols without causing any damage to the test organism. All chemicals utilized were of scientific grade from Himedia, India.

Experimental Design, Seedling Transplantation, Stocking and Harvesting

Pusa Basmati 1121, a rice assortment of the 120-day term was utilized for the present study. Twenty days old rice seedlings were transplanted in rows, roughly 25-30 cm apart. Fish refugees were filled with water and stocking (1 fish 3 m²) of *C. punctatus* (15.50±0.40 g) and *C. carpio* (13±0.47 g) was done on the 25th day after transplanting the seedlings in respective experiments (Table 1). The experimental design and the use of agrochemicals were similar to Bhatnagar et al. (2021). Fish harvesting was done after a culture period of 85 days, 10 days before paddy harvesting. All treatment ponds were fertilized using urea and zinc sulfate monohydrate while pesticides and insecticides were sprayed uniquely in CP1-CP3 and CC1-CC3. Agrochemical exposure to treatment CP2 and CC2 was done with recommended doses, whereas in CP3 and CC3 dosage was according to farmers (they used these agrochemicals two to three times randomly to control paddy pests). To elucidate the genotoxic effect of paddy

agrochemicals on fish in paddy fields micronucleus, chromosome aberration, and comet assays have been evaluated in the present studies.

Evaluation of Genotoxicity

The cell viability and count of the peripheral blood were checked utilizing a trypan blue dye exclusion test and hemocytometer to ensure enough number of live cells preceding the comet and micronuclei assay. The samples with 90% or more viability and a cell count of a minimum of 10⁶ cells mL⁻¹ proceeded for the tests.

Micronuclei test (Fenech et al. 2004)

A thin smear of collected blood samples was made on methanol-treated glass slides and air-dried overnight in sterilized conditions at room temperature. Fixed slides were stained with 2% Giemsa after air drying and were observed under Olympus CX-41 trinocular microscope at 40/100X magnification. Image acquisition of representative peculiarities just as control cells was finished with the assistance of Olympus Digital Camera (C-7070 Wide Zoom) for future records and study. Micronuclei frequency was determined as follows:

$$\% \text{ Micronuclei (MN)} = \frac{\text{Number of cells containing MN}}{\text{Total number of cells counted}} \times 100$$

Table 1: Experimental design and agrochemicals (fertilizers, pesticides, insecticides, and weedicide) used in paddy fields.

Experimental design for the first experiment				
CP1	CP2		CP3	
Paddy field with <i>Channa punctatus</i> , No pesticide	Paddy field with <i>Channa punctatus</i> , Recommended dose		Paddy field with <i>Channa punctatus</i> , Farmers' dose	
Experimental design for the Second experiment				
CC1	CC2		CC3	
Paddy field with <i>Cyprinus carpio</i> , No pesticide	Paddy field with <i>Cyprinus carpio</i> , Recommended dose		Paddy field with <i>Cyprinus carpio</i> , Farmers' dose	
S. No.	Agrochemicals (Fertilizers and Pesticides)			Recommended doses/Acre
	Commercial Name	Chemical Composition	Nature	
1.	Eifit 50 EC	Pretilachlor 50% EC	Herbicide	500 g
2.	Urea	46% Nitrogen	Fertilizer	40 kg
3.	Zinc sulfate monohydrate	Zinc 33% and Sulphur 15%	Fertilizer	10 kg
4.	Clorguard 20 EC	Chlorpyriphos 20% EC	Insecticide	1 Litre
5.	Padan	Cartap Hydrochloride 4% EC	Pesticide	5 kg
6.	Bravo TM 5000	Lambdacyhalothrin 5%EC	Insectide	25 kg
7.	Pulsor®	Thifluzamide	Insecticide	250 mL
8.	Parrymida	Imidacloprid 17.8 %	Insecticide	150 mL

Chromosome Aberration Assay (Galloway 2000)

At the termination of the experimental period, five fish were randomly chosen from each treatment, and processed for chromosomal aberration assay for evaluating the effect of pesticide on fish chromosomes. Kidney tissue was extracted after injecting the test fish with 0.05% Colchicine. The tissue suspension was prepared in saline solution and fixed in chilled Carnoy's fixative. The cell suspension was centrifuged, fixed repetitively, and finally, the cells of the pellet were suspended in a small amount of fixative. One / or two drops of this suspension were then dropped from a height of about 2 feet on a clean, grease-free slide, held in a slightly slanting position. The air-dried slides were stained with 4% Giemsa and scanned thoroughly under Olympus CX 41 trinocular microscope for well-spread metaphase plates. The selected plates were photographed by using Olympus C-7070 wide zoom camera at a magnification of 1000X. The frequency of chromosomal aberrations was scored. A standard control procedure was employed to determine the various types of chromosomal aberrations.

Single-Cell Gel Electrophoresis (SCGE)/Comet Assay (Tice et al. 2000)

0.2 mL blood was diluted with 0.5 ml PBS and coated gently on 0.5 mL of histopaque (Sigma) density gradients. Slides were prepared using 10,000 cells in 20 μ l PBS mixed with 80 μ l of 0.5% low melting point agarose (LMPA).

The DMSO (Dimethyl sulfoxide) was added to the lysing solution to prevent radical-induced DNA damage. Before electrophoresis, the slides were incubated in alkaline (pH>13) electrophoretic buffer for 30 minutes. Electrophoresis of microgels was done at 18V (0.7-1.0 Vcm^{-1}) for 20 minutes and the current was maintained at 300 mA (milliamperes) by raising or lowering the buffer level in the tank. This process was carried out in very dim light and at 4°C. Slides were then dehydrated in absolute methanol for 5-10 min.

After the completion of neutralization, slides were kept on an aluminum tray and allowed to dry completely in an incubator at 50°C for 30 min. The staining solution (75 μ l Ethidium bromide) was poured in 4-5 small, equally spaced droplets over the slides in such a way that it completely and uniformly covered the slides. This entire technique was performed under faint light to keep away from the photolysis and artefactual DNA harm. For comet visualization, two slides per sample and 50 comets per slide were scored. LUCIA Comet Assay™ image analysis software installed in Olympus Trinocular fluorescent research microscope CX-41 (having specific filters) attached with CCD camera at 10X objective was used in the present study. Visual scoring of comet cells (at least 100) was done by categorizing them into 4 classes and giving them scores of 1-4. For visualization of DNA damage, analysis was performed using the above-said image analysis system. It computed the integrated intensity profile for each cell, estimated the comet cell components, head, and tail, and evaluated the percentage of DNA in the comet head and tail, tail length, tail moment and tail inertia, etc.

Statistical Analysis

Analysis of variance (ANOVA) followed by Duncan's multiple range test (Duncan 1955) for all the experiments was used to determine the significant variation between the different treatments. Statistical significance was settled at a probability value of $P < 0.05$. All statistics were performed using SPSS Version 18.0 for Windows. LUCIA Comet Assay software was used for visual scoring of the comet cells.

RESULTS

Induction of Micronucleus (MN) or Micronuclei Frequency

The results of micronucleus (MN) analysis in erythrocyte of *C. punctatus* and *C. carpio* reared in different treatments are summarized in Table 2 and Fig.1. There was

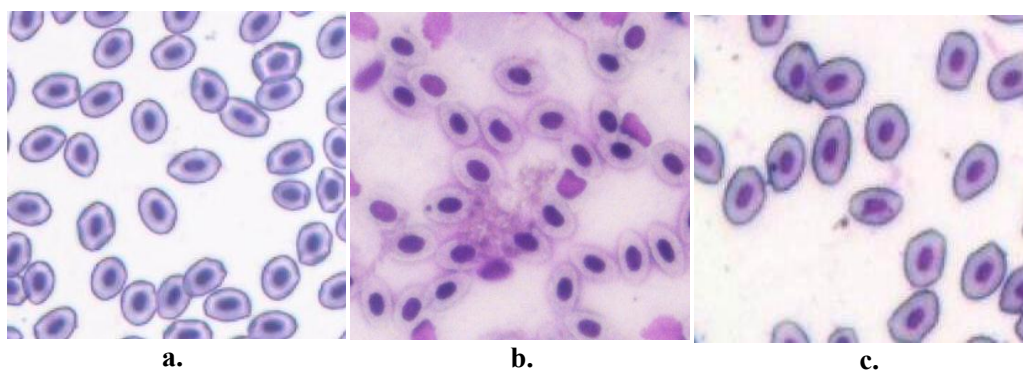


Fig. 1: Micronuclei in blood cells of fish stocked in treatments CP1-3 and CC1-3 in paddy fields on exposure to agrochemicals (a. Control, b. and c. micronuclei due to pesticide exposure).

significant induction of MN in the erythrocyte of fish due to exposure to different doses of agrochemicals applied in treatments CP1-CP3 and CC1-CC3. Maximum doses of pesticides used in treatment CP3 induced MN frequency of 2.6% in blood erythrocytes of fish which was significantly higher ($P < 0.05$) as compared to MN frequency of 0.94% in treatment CP2 in which recommended doses of pesticides were used and minimum frequency was observed in treatment CP1 where no agrochemicals were used. The mean percent frequency of MN in CC1 (0.436 ± 0.098), CC2 (1.23 ± 0.072), and CC3 (2.46 ± 0.027) showed significant differences at a 0.05 level with respect to the control group. The result further depicted that the frequency of micronuclei increased with an increase in the dosage of pesticides.

Chromosome Aberration

Somatic metaphase depicted that the diploid number of chromosomes in *C. punctatus* and *C. carpio* was 32 ($2n=32$) and 100 ($2n=100$), respectively. The aberrations in the diploid chromosome are stickiness, clumping, end-to-end joining (non-homologous association), chromosome gap, and precocious separation (Fig. 2). Results depicted that the chromosomal aberrations were higher in CP3 and CC3 where the fish were stocked in paddy fields with paddy agrochemicals as used by farmers.

Pycnosis (P) was characterized by differential staining of chromosomal portions due to more condensation in some regions due to the failure of other regions to condense properly. Pycnosis was observed in CP3 and CC3. If the centromere of chromatids of chromosomes got separated precociously then the count of chromosomes was equal to 4X.

Precocious separation (PS) was not accompanied by spindle apparatus and was observed in CP2, CP3, CC2, and CC3. The centromeric region of a chromosome got elongated and did not stain properly forming a centromeric gap. Chromatid break and gap (CG) were observed in CP2, CP3, CC2, and CC3. In a few metaphase plates of treated specimens, some chromosomes were extraordinarily small, their arms seemed thicker and smaller. This may be due to hypercoiling or hyper-condensation, giving a stubby appearance to chromosomes. Such stubbed arms (SA) were observed in CP3, CC2, and CC3. A single or two chromosomal breaks yielded an acentric fragment (AF) which was observed in CP2, CP3, and CC3. Chromatin material was eroded due to pesticide application. Erosion of chromatin material (EC) was encountered only in CP3 and CC3. Stickiness among a few chromosomal ends or clumping of all the chromosomes was observed as a result of severe effects in certain cells due to DNA depolymerization. Stickiness and clumping (SC) were encountered in all the treatments. A disturbance in the condensation of chromosomes at some sites resulted in the thinning of chromatids as attenuation (A) and was observed in CP2, CP3, CC2, and CC3. End-to-end joining was found in all the treatments whereas uncoiling the metaphasic chromosomes as Despiralization (DS) was observed only in CP3 and CC3.

DNA Damage (Comet Assay)

DNA damage was measured as head area, head DNA (%), tail area, tail length, tail moment, % tail DNA, olive moment, integral intensity, and head radius (Table 3). Fig. 3 indicates an increase in the size of the comet tail from Type 0 (undamaged

Table 2: Frequency of micronuclei in blood cells of *Channa punctatus* and *Cyprinus carpio* at different treatments in paddy cum fish culture fields.

Treatments	Initial	CP1/CC1 (No pesticide)	CP2/CC2 (Recommended dose)	CP3/CC3 (Farmers' dose)
Number of examined blood cells	1000	1000	1000	1000
<i>Channa punctatus</i>				
MN (Mean \pm S.E.)	3.33 ± 0.694^C	5.33 ± 0.720^C	9.43 ± 0.981^B	26.00 ± 1.240^A
% MN frequency (Mean \pm S.E.)	0.33 ± 0.069^C	0.53 ± 0.072^C	0.94 ± 0.098^B	2.6 ± 0.124^A
<i>Cyprinus carpio</i>				
MN (Mean \pm S.E.)	4.33 ± 0.347^C	4.66 ± 0.981^C	12.33 ± 0.720^B	24.66 ± 0.027^A
% MN frequency (Mean \pm S.E.)	0.43 ± 0.034^C	0.46 ± 0.0981^C	1.23 ± 0.072^B	2.46 ± 0.027^A

All values are Mean \pm S.E. of the mean.

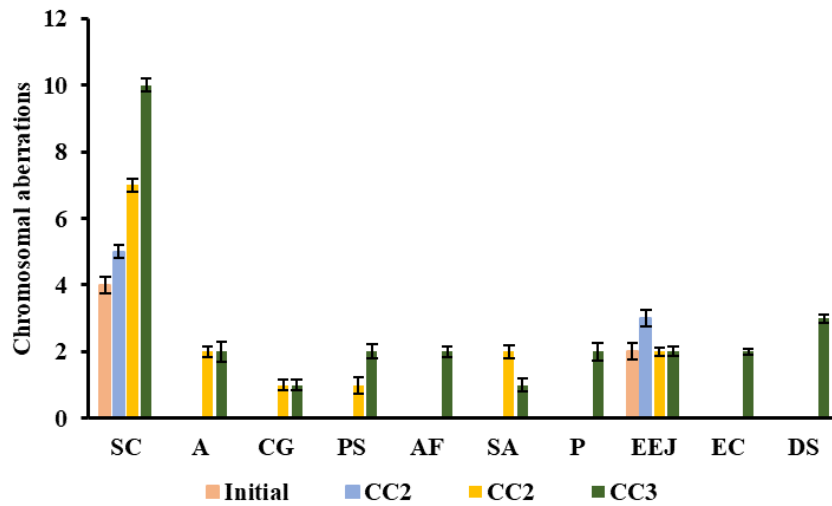
Means with different letters in the same columns are significantly ($P < 0.05$) different. (Duncan's Multiple Range test)

nuclei) to Type 4 (damaged nuclei) comet. A general analysis of comet type showed that frequency of comet type '0' was high in fish of CP1/CC1(control), frequency of comet Type 1 and 2 were high in CP2/CC2; whereas Type 3 and 4 comet were high in CP3/CC3 where agrochemicals were sprayed three times during experimental period indicating that size of comet increased with increase in the dose of agrochemicals. The values when compared with the initial control depicted more damage in treatment groups. Results revealed that fish reared in CP3/CC3 have maximum DNA

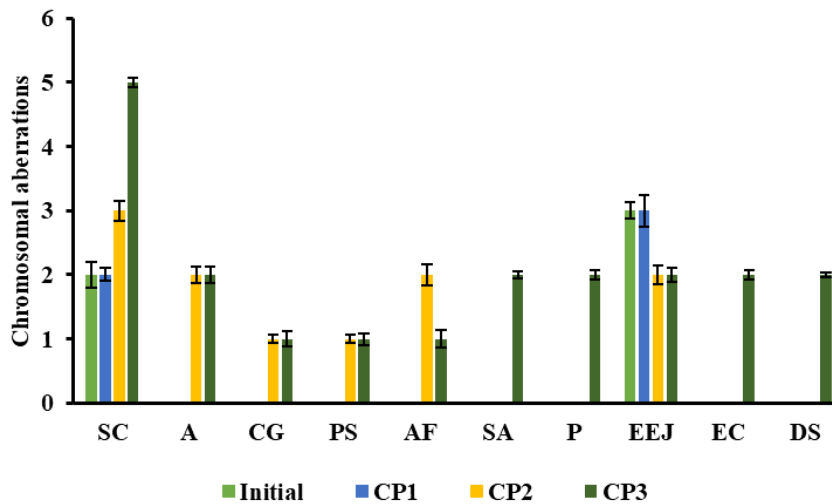
damage as compared to fish stocked in treatment CP2/CC2 where recommended doses of agrochemicals were used.

DISCUSSION

In the present study, various agrochemicals were used to combat the pest in the paddy fields, and the same piece of land was utilized for the integrated culture of *Channa punctatus* and *Cyprinus carpio*. Although the agrochemicals within recommended dose enhance paddy production (Bhatnagar



A



B

Fig. 2: Chromosomal aberration in the kidney cells per 125 metaphases in A) *Channa punctatus* and B) *Cyprinus carpio* stocked in paddy fields after 85 days (SC = Stickiness, A = Attenuated Chromosome, CG= Chromatid break, and Gap, PS = Precocious separation, AF = Acentric Fragments, SA= Stubbed arms, P= Pycnosis, EEJ = End to End joining, EC= Erosion of chromatid materials, DS= Despiralization of chromosomes.

Table 3: Assessment of genetic damage in erythrocytes of *Channa punctatus* and *Cyprinus carpio* reared in paddy fields after 85 days of different treatments.

Parameters	Treatments (Mean±S.E.)			
	Initial/Control	CP1/CC1 (No pesticide)	CP2/CC2 (Recommended dose)	CP3/CC3 (Farmers' dose)
<i>Channa punctatus</i>				
Head Area (μm^2)	1461.56±35.18 ^B	2289.58±633.38 ^B	2801.52±725.18 ^A	781.58±164.08 ^C
Head DNA(%)	95.46±0.65 ^{AB}	97.18±0.32 ^A	91.70±1.76 ^{AB}	84.42±2.76 ^B
Tail Area(μm^2)	141.63±24.62 ^C	250.08±91.71 ^B	287.18±104.43 ^A	261.82±41.85 ^{AB}
Tail length(μm)	2.00±0.71 ^B	2.66±0.86 ^{AB}	2.94±1.12 ^{AB}	3.47±0.92 ^A
Tail moment	0.21±0.009 ^{AB}	0.10±0.04 ^B	0.91±0.33 ^{AB}	1.11±0.34 ^A
Tail DNA(%)	4.53±0.21 ^B	2.82±0.32 ^C	8.30±0.76 ^{AB}	15.57±0.79 ^A
Olive moment	0.54±0.12 ^{AB}	0.42±0.13 ^B	1.36±0.37 ^{AB}	1.78±0.39 ^A
<i>Cyprinus carpio</i>				
Head Area (μm^2)	578.47±51.33 ^B	1047.72±79.44 ^{AB}	927.42±206.42 ^{AB}	1859.31± 499.19 ^A
Head DNA (%)	95.44±0.72 ^A	97.40±0.51 ^A	88.35±3.58 ^B	83.64±1.38 ^C
Tail Area(μm^2)	58.32±10.22 ^C	122.28±19.67 ^B	218.04±38.51 ^{AB}	346.51±45.56 ^A
Tail length (μm)	1.01±0.22 ^B	2.77±0.64 ^{AB}	2.34±1.06 ^{AB}	5.42±1.30 ^A
Tail moment	0.33±0.20 ^B	0.15±0.05 ^{AB}	0.77±0.37 ^{AB}	2.06±0.45 ^A
Tail DNA (%)	2.59±0.516 ^B	4.55±0.89 ^{AB}	11.64±3.58 ^{AB}	16.35±1.38 ^A
Olive moment	0.397±0.11 ^B	0.43±0.07 ^{AB}	1.35±0.42 ^{AB}	2.58±0.28 ^A

All values are Mean ± S.E. of the mean.

Means with different letters in the same rows are significantly ($P < 0.05$) different.

(Duncan's Multiple Range test)

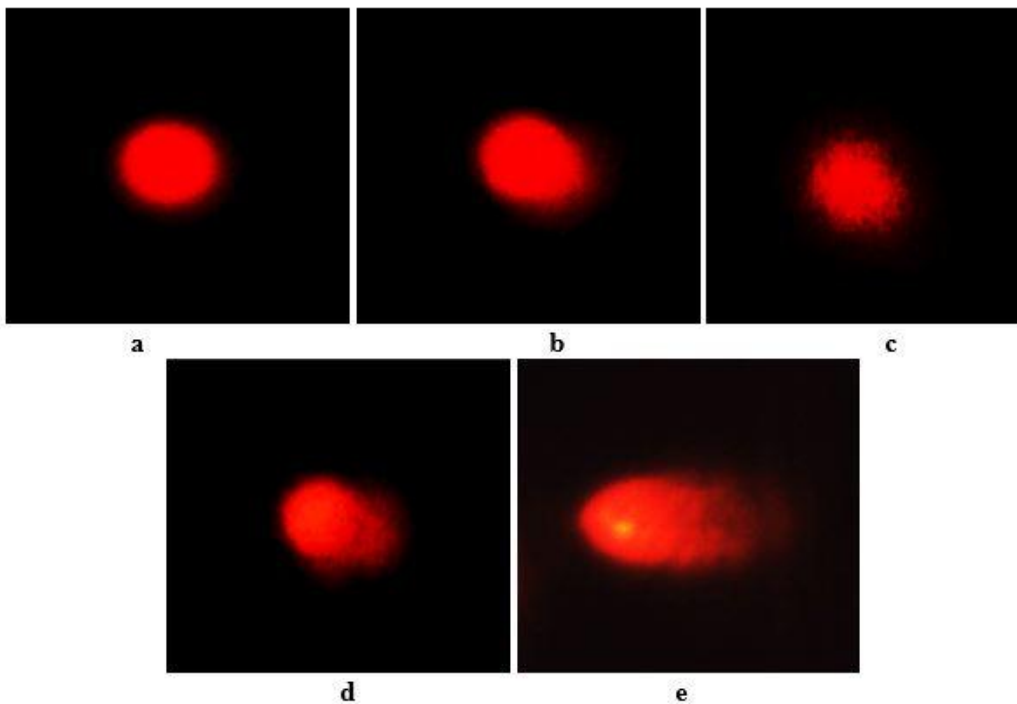


Fig. 3: Blood cells showing various types of comets (100X) a. Type 0 comet, b. Type 1 comet, c. Type 2 comet, d. Type 3 comet, e. Type 4 comet

et al. 2021) yet their presence causes a genotoxic effect on the organisms. In the present study, *C. Carpio* and *C. punctatus* were employed for analyzing the genotoxic effect in terms of micronucleus, chromosomal aberration, and comet assay in fish tissue due to the agrochemicals present in the paddy fields. Xenobiotics initiate DNA damage by causing strand breaks or by implication through the formation of reactive oxygen species (ROS) which at that point harms the DNA by shaping adducts and lesions (Marnett 2000). DNA repair mechanism, which attempts to mend the damaged section, can do so to a specific limit, beyond which DNA harm can persevere, as injuries or mis-repaired DNA, prompting mutagenesis and carcinogenesis (Moustacchi 2000). The dependable genotoxicity endpoints which are utilized for screening the genotoxicity of such xenobiotics are the micronuclei (MN) test, chromosomal aberrations, and the single-cell gel electrophoresis (comet assay). The MN test is essentially used to check the clastogenicity of a specific compound, while the comet test is utilized to check the impact of the compound on the respectability of DNA (Bolognesi & Cirillo 2014). The acquired aftereffects of the current investigation may likewise be valuable to alleviate contaminant impacts at a whole lot initial stage.

The outcome of the micronucleus assay uncovered that recurrence of micronuclei was fundamentally higher in fish erythrocyte stocked in the rice fields for 85 days in CP3 and CC3, where the amount of the agrochemicals used by the farmers was three times more than the recommended dose (CP2 and CC2) affirming the impact of agrochemical on genetic material. Micronuclei are particles comprising acentric sections of chromosomes or whole chromosomes which fall behind at the anaphase phase of cell division. After telophase, these parts may not be retained in the nuclei of daughter cells and structure single or various micronuclei in the cytoplasm. Consequently, micronuclei contain either chromosome part or the entire chromosome. MN recurrence in freshwater species was shown to be a delicate biomarker to distinguish genotoxic damage incited by pesticides (Clasen et al. 2018) and herbicides (Nwani et al. 2013) permitting to recognition of contaminant concentration gradients. The pertinence of occasional effect in the enlistment of chromosomal effect in the acceptance of chromosomal damage was additionally distinguished (Ergene et al. 2007). The outcomes have announced that these synthetic substances repress cell division by impeding protein synthesis and in this way forming micronuclei (Çavaş & Ergene-Gözükara 2005). The results of the present study are supported by Bhatnagar et al. (2016) where the presence of CPF (Chlorpyrifos) increased the micronuclei frequency in a time- and dose-dependent manner. Erythrocytes with two nuclei are formed during aberrant cell division as a result of cytokinesis block (Çavaş

& Ergene-Gözükara 2005). The formation of eight-shaped erythrocytes, considered to be remnants of the mitotic spindle, is also used as a cytotoxicity marker because it reflects a failure in erythropoiesis (Baršienė et al. 2014). The results obtained in from this study data suggest that the presence of agrochemicals affected fish erythrocytes more significantly ($P < 0.05$) than the control.

Chromosome aberrations such as centromeric gap, attenuation, pycnosis, stickiness and clumping, erosion of chromatid material, and end-to-end joining in chromosomes were recorded maximum in CP3 and CC3 in comparison to CP2, CC2, CP1, and CC1. Different types of chemicals have been reported to be responsible for the production of various types of aberrations in the structure or number of chromosomes. By studying the comparative karyology after exposing the organisms to any chemical or physical agent, the clastogenic properties can be detected. In fish cytogenetic techniques have been applied widely as the most robust and consistent assay for genotoxicity assessment. The present study depicted that somatic metaphase has revealed that the diploid number of chromosomes in *C. carpio* was 100 ($2n=100$). The aberrations in diploid chromosome number in *C. carpio* have been found to be stickiness, clumping, end-to-end joining (non-homologous association), chromatid gap, precocious separation, etc. frequency of each type of aberration were higher in CC3 where the fish were stocked in rice fields with rice agrochemicals as used by farmers. For *C. punctatus*, somatic metaphase depicted that the diploid number of chromosomes is 32 ($2n=32$). The aberrations in diploid chromosome number in *C. punctatus* are stickiness, clumping, end-to-end joining (non-homologous association), chromosome gap, precocious separation, etc. The results depicted that the chromosomal aberrations were higher in CP3 in *C. punctatus* also. These results are in agreement with Rishi and Grewal (1995).

Organophosphates are ubiquitous environmental contaminants owing to their extended utilization in the fields. However, these are neurotoxic inhibiting acetylcholinesterase activity with subsequent disruption of nervous functions, thus interfering with various metabolic and physiological activities (Jokanović 2018). Imidacloprid is a neurotoxic chemical, which is utilized successfully against sucking insect pests of rice and diverse crops around the world. In any case, by its methodical nature, imidacloprid moves effectively between plant tissue and from the roots to the soil underneath and water in the field. The average amount of pesticides applied by the farmers is nearly twice or thrice as high as the upper dosage recommended by the distributing companies. There is a decline in the number of freshwater fish with the use of these chemicals. Furthermore, farmers shower their harvests in the wake of seeing bugs or weeds in the field notwithstand-

ing preventive splashing, especially in the more remote areas. The greater part is utilizing high fertilizer rates and applying an enormous concentration than required.

Structural and numerical modifications in chromosomes result from abnormalities in DNA duplication during the 'S' phase. The physiological changes that appeared in the fish are not just a reaction to low pesticide levels yet, in addition, give a comprehension of contaminations in organic terms and show a model for vertebrate toxicity. The present study's observations are similar to the studies by Saxena and Chaudhari (2010), Yadav et al. (2010), and Ahmed et al. (2015). All these studies are concerned about genotoxic effects in fishes when exposed to specific chemicals/pesticides/herbicides at specific doses under laboratory conditions. The present investigation revealed that chromosomal abnormalities were higher in CP2, CP3, and CC2, CC3 as compared to CP1/CC1. The number of aberrations was high in CP3 and CC3 where farmers used the agrochemicals 2-3 times the recommended dose. The numerical values of aberrations were comparatively less in CP2 and CC2 only once recommended doses of pesticides were used and were lowered in CP1 and CC1 where no pesticide treatment was given supporting the fact that these agrochemicals can cause chromosome aberrations.

The SCGE or Comet assay, recognize DNA strand breaks and alkali labile locales by estimating the movement of DNA from immobilized nuclear DNA and helpful procedure for environmental contamination bio-monitoring. It is a fast and delicate technique to quantify DNA lesions. Kumaravel and Jha (2006) revealed that parts of broken DNA just as loosened up DNA move towards the anode delivering the tail of the comet.

The fragmented DNA tends to move unreservedly during the electrophoresis, whereas the relaxed DNA loops are hauled out of the nuclear head. The tail length determines the distance relocated by DNA with the smallest one moving the farthest, the tail length is predominately directed (Kumaravel & Jha 2006) by the size of the DNA fragments generated during the alkaline unwinding step of the Comet Assay. Kilemade et al. (2004) revealed that % tail DNA is a mainstream and reasonable boundary that estimates the level of DNA that has moved from the head. Pandey et al. (2018) confirmed that profenofos (PFF) have genotoxic, mutagenic effects and are exceptionally harmful to *C. punctatus*. PFF causes biomagnification in the tissues of the test organisms and is neurotoxic for the hindrance of cerebrum and gill AChE in the fish (Rao et al. 2003). In the present studies, comet assay was performed after 85 days only and no such time-dependent response could be quantified. However, in CP2 and CC2 the spray of agrochemicals at recommended doses was done only once at lower and recommended doses

whereas in CP3 and CC3 the spray was done thrice (as suggested by the farmers) supporting the results that there might be a time-dependent decrease in DNA damage due to DNA repair as revealed by the size of the comet in CP1 and CC1. Stocking fish in rice fields reduces pest infestation and thus if not eliminates the need for the application of agrochemicals, reduces the quantity supporting the results of the present study.

The presence of micronuclei and tail DNA (%) is profoundly associated with one another and may happen in a portion subordinate and additionally time-subordinate way. The MN test and comet can, in this manner, be dependably used to evaluate the genotoxicity of agrochemicals in paddy cum fish culture. The current investigation recommends restricting the utilization of agrochemicals in rural practices to keep away from the possible defilement of nearby water bodies. Agrochemicals applied in rice fields may cause adverse effects that are specific to the toxicant and damage the organism without causing any observable changes. Keeping in view the low genotoxic effects in CP2 and CC2 (with recommended doses of pesticides) may be disseminated to farmers for economic benefits with paddy cum fish culture.

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