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COMPARATIVE EFFECT OF PROFENOFOS AND CARBOFURAN ON MICRONUCLEUS FORMATION IN CHANNA PUNCTATUS (BLOCH)

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ABSTRACT

Profenofos and Carbofuran are pesticides used for agricultural and household practices to reduce the impact of pests in agricultural and household practices. The effect of these pesticides ultimately changes disturbs the ecology of aquatic ecosystem and harshly disturb the physiological conditions of different fresh water fish. *Channa punctatus*Bloch. is a model organism for the studies for the effect of pesticides on various genotoxic parameters such as formation of micronucleus. In the present study it was revealed that the percentage of micronucleus were **0.27, 1.47, 2.54** and **3.60** in control, lower, middle and higher doses of Profenofos while the percentage of micronucleus were found to be **0.27, 1.66, 2.96** and **4.13** in control, lower, middle and higher doses of Carbofuran respectively. Thus, Carbofuran was found more toxic in relation to the formation of micronucleus for an experimental time frame of 96 hours in artificial conditions in relation to Profenofos.

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INTRODUCTION

India is an agriculture based country and thus the extensive agricultural practices for Kharifand Rabi crops pesticides are used to maximize the productivity. The two most common pesticides used in Indian agriculture system includes Profenofos and Carbofuran (Nayak and Solanki, 2021; Patidar and Shrivastava, 2024). The residue of these extensively used pesticides ultimately reaches to the different water bodies through runoff water due to irrigation and heavy rainfall. The fresh water ecosystem gets heavily polluted near the agricultural fields (Dutta and Upadhyay,2024).The genotoxic effect of Profenofos and Carbofuran was evaluated on a Fresh water fish *Channa punctat*us Bloch. commonly known as "Garai". *Canna punctatus* is a fish of brackish and fresh water system commonly used for the ecological studies and for the study of the effect of different pesticides (Srivastavand Kumar, 2015). Micronucleus (MN) formation occurs due to genotoxic stress leading to chromosome fragments or entire chromosomes not integrating into the daughter nucleus during cell division (Fenech*et al.,* 2011; Luzhna*et al.*, 2013). Numerous researchers have demonstrated the genotoxic potential of different pesticides over erythrocyte nuclear abnormalities, which are thought to be signs of cell destruction. De Flora *et al.* (1993) used the erythrocyte micronucleus assay to track the mutagenic behavior and potential of pesticides. These genotoxins cause micronuclei to develop, which means that their chromosomes are broken or damaged. During the process of cell division, tiny chromosomal fragments that are not integrated into the main nucleus separate into tiny nuclei known as micronuclei.

It is crucial to understand that the induction and creation of micronuclei can only be seen in cells that are exposed to genotoxic substances when they are dividing (Krupina et al. 2021).Using the micronucleus assay, genotoxicity in fish has been studied using *Channa punctatus* as a model fish for PFF (Profenofos) pesticides. The results show that the micronucleus test is a valid and trustworthy method for assessing the possible genotoxicity and mutagenicity of various xenobiotic substances, and that Profenofos is possibly harmful for aquatic organisms.

MATERIALS AND METHODS

Four (04) groups of acclimated *Channa punctatus* (Bloch) were given two different pesticide treatments: Profenofos and Carbofuran. Each group has 10 adult fish,*Channa punctatus* (Bloch), measuring 18.0±5.0 cm and weighing 70±5.0 g, were collected from a local market in Bhagalpur. They were housed in muddy water aquarium for 10-15 days to acclimatize for laboratory conditions and provide a balanced diet (Pandey*et al.,* 2011). The model fish (*Channa punctatus*)received all treatments in accordance with the regular dosage procedure outlined in the treatment protocol. Smears of peripheral blood samples were used to directly make the slides. A1-Shabti and Metcalfe (1995) proposed a slide preparation methodology.

Treatment protocol

Preparation of Slides: To prevent the cells from bursting, a single drop of suspension was applied to the spotless, grease-free slides that

had previously been placed on a moist towel. A uniformly distributed smear of the suspension was then created. After air drying, the smeared slides were fixed in pure methanol for ten minutes. Slides were once more dried, cooled and stored for staining after being fixed.

micronucleus formation in the Lower dose (P_1), Middle dose (P2) and Higher dose (P_3) experimental groups for a exposure period of 96 hours (Table 3.0 and Figure 1.0). Higher dose exposure showed maximum micronucleus formation (109) while lower dose comprises only 45 micronucleus out of 3000 cell observed.

Table 1. Experimental design for Profenofos pesticide treatments

Group	Treatment Protocol	Symbol	Dose	LC_{50}
1	Control	C	No Treatment of Profenofos	-
2	Lower dose of Profenofos	P_1	0.27 μg/L ($\approx \frac{1}{10}$ of LC_{50})	2.68μg/L (Pandey*et al*., 2011)
3	Middle dose of Profenofos	P_2	0.54 μg/L ($\approx \frac{1}{5}$ of LC_{50})	
4	Higher dose of Profenofos	P_3	1.34 μg/L ($\approx \frac{1}{2}$ of LC_{50})	

Table 2. Experimental design for Carbofuran pesticide treatments

Group	Treatment Protocol	Symbol	Dose	LC_{50}
1	Control	C	No Treatment of Carbofuran	-
2	Lower dose of Carbofuran	CF_1	0.09 mg/L ($\approx \frac{1}{10}$ of LC_{50})	0.9 mg/L (Tiwari*et al*., 2016)
3	Middle dose of Carbofuran	CF_2	0.18 mg/L ($\approx \frac{1}{2}$ of LC_{50})	
4	Higher dose of Carbofuran	CF_3	0.45 mg/L ($\approx \frac{1}{2}$ of LC_{50})	

Table 3.Effect of Profenofos on Micronucleus formation in control and treatedwith three different doses of Profenofos

Group	Experimental Protocol	Symbol	No. of Cells observed	No. of Micronuclei	%±S.E
1	Control	C	3000	08	0.27±0.094
2	Lower Dose	P_1	3045	45	1.47±0.218*
3	Middle Dose	P_2	3140	80	2.54±0.280*
4	Higher Dose	P_3	3023	109	3.60±0.338*

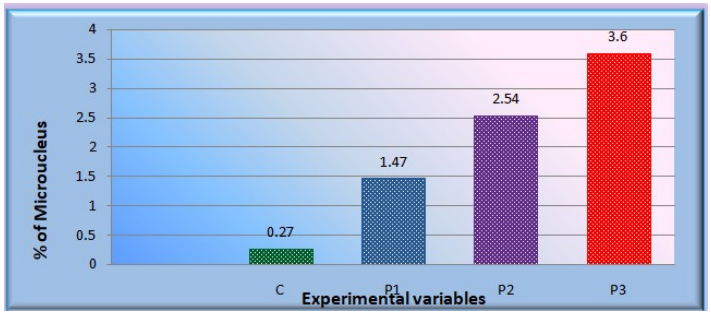

Figure 1. Showing Micronucleus formation in control and threedifferent doses of Profenofos treatment groups

Table 4. Effect of Carbofuran on Micronucleus formation in control and treated with three different doses of Carbofuran

Group	Experimental Protocol	Symbol	No.ofCell observed	No. of Micronuclei	%±S.E
1	Control	C	3000	08	0.27±0.094
2	LowerDose	CF_1	3130	52	1.66±0.228*
3	Middle Dose	CF_2	3100	92	2.96±0.304*
4	HigherDose	CF_3	3170	131	4.13±0.353*

(* = significant at p< 0.05)

Staining: The smeared slide was stained with 6% Giemsa (Pandey*et al.*, 2014). After being cleaned with tap water, the stained slides were allowed to air dry.

Screening of the slide: For each treatment group, about 3000 cells were examined in order to calculate the quantity and proportion of micronuclei.

Statistical Analysis: The statistical analysis was carried out using the t-test at P<0.05 for its significance and the data are expressed as %±S.E (Standard Error).

RESULTS

For the control, lower, middle and higher doses of Profenofos, the percentage of micronuclei was 0.27, 1.47, 2.54, and 3.60, respectively. The percentage of micronuclei is considerably higher at the various doses compared to the control (Table3.0 and Figure 1.0).The percentages of micronuclei in three distinct doses of Profenofos are much higher than the control. It was revealed that the exposure of Profenofos showed a linear progression for the

The experiment was repeated for five time (n=5) for the valid statistical calculations.

Effect of Carbofuran: Three distinct sub-lethal doses of Carbofuran insecticides are administered to the second experiment group. In the control, lower, intermediate, and higher doses of Carbofuran, the percentage of micronuclei was 0.27, 1.66, 2.96 and 4.13, respectively.The percentage of micronuclei is considerably higher than the control at the various doses. The percentages of micronuclei in three different doses of Carbofuran are considerably higher than the control. Therefore, micronucleus production was triggered by three distinct dosages of Carbofuran (Table 4.0 and Figure 2.0).It was revealed that the Carbofuran causes the formation of micronucleus in Lower, Middle and Higher doses when compared to the control group. The progression of micronucleus formation was found similar to Profenofos but the effect is more pronounced as evident from the table (Table 4.0 and Figure 2.0) the Lower dose (CF_1) causes the 52 micronucleus, Middle dose(CF_2) produces 92 while Higher dose (CF_3) resulted in the formation of 131 micronucleus in about 3000 cells observed. The experiment was repeated for five time (n=5) for the valid statistical calculations.

Comparative study: The comparative study of Profenofos and Carbofuran for their genotoxic potential on fresh water fish *Channa punctatus* Bloch. reveals that the Carbofuran was proved to be more potent genotoxic compound in terms of Micronucleus formation as a result of promoting dis-appropriate nuclear divisions, producing a tiny nucleus known as micronucleus. All the three doses concerned in this present piece of work revealed that Carbofuran had higher number of micronucleus at Lower, Middle and Higher doses respectively (Table 5.0 and Figure 3.0). It was revealed that for lower dose Carbofuran produces an additional 07 micronucleus, middle dose produces additional 12 micronucleus while the higher dose comprises an excess of 22 micronucleus for same experimental conditions.

nuclei. Therefore, Profenofos (PFF), a widely used organophosphate insecticide, is highly toxic to fish and induces significant genotoxic effects. Its genotoxic behaviour is primarily characterized by DNA strand breaks and nuclear abnormalities in various tissues, even at sub-lethal concentrations. Carbofuran is a potent carbamate pesticide that induces significant genotoxicity in various fish species, typically through the induction of DNA fragmentation, micronuclei (MN), and other nuclear abnormalities. Its effects are often dose and time dependent, as shown by various biomarkers like the Comet assay and micronucleus test. The genotoxic behaviour as evident from the present piece of work can be correlated with the earlier works of Hamed*et al*., (2014) on African catfish *Clarias gariepinus,* Khalid *et*

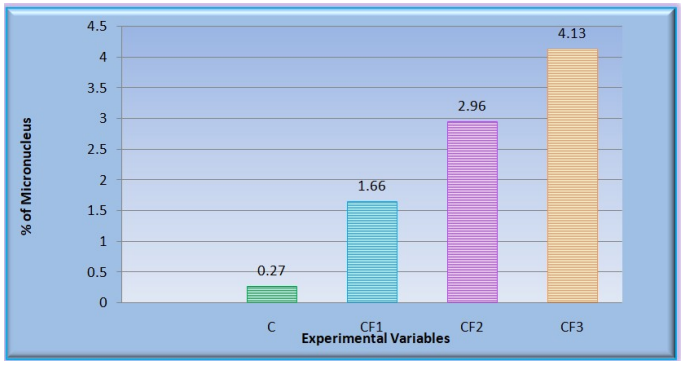

Figure2. Showing Micronucleus formation in control and three different doses of Carbofuran treatment groups

Table 5. Comparative study for the Effect of Profenofos and Carbofuran on Micronucleus Formation

Group	Experimental Protocol	Symbol	No.ofCell observed	No. of Micronuclei	%±S.E
1	Control	C	3000	08	0.27±0.094
2	LowerDose	P_1	3045	45	1.47±0.218*
		CF_1	3130	52	1.66±0.228*
3	Middle Dose	P_2	3140	80	2.54±0.280*
		CF_2	3100	92	2.96±0.304*
4	Higher Dose	P_3	3023	109	3.60±0.338*
		CF_3	3170	131	4.13±0.353*

(* = significant at p< 0.05)

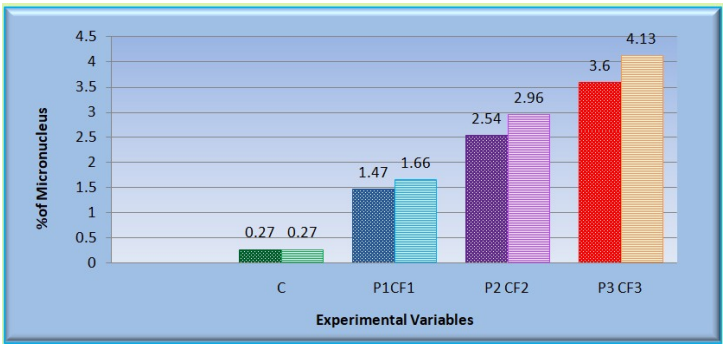

Figure 3. Showing Comparative Micronucleus formation in control and three Different doses of Profenofos and Carbofuran treatment groups

The data were statistically verified at a significance level p<0.05 and observed in the form of %± S.E. (Standard Error). The results were all significant and considerable for the assessment of result and for concluding the findings of the present piece of work.

CONCLUSION

`Both Profenofos and Carbofuran are found to be potent genotoxic compound capable of producing altered mitotic divisions resulting in the formation of micronucleus. The Profenofos was known to be genotoxic in marine fish, *Theraponjarbua* (Therapon, 2012). Similar findings were observed by Pandey *et al*., (2018) in *Channa punctatus* Bloch. and by Pouil and Pepey (2025) in Nile tilapia (*Oreochromis niloticus*). The cause of micronucleus formation may lies in exposure leads to various nuclear abnormalities including the induction of micronuclei (MN), lobed nuclei, notched nuclei, and vacuolated on Rohu fish (*Labeo rohita),* Kumar and Singh (2025) on Snake head fish (*Channa punctatus* Bloch.). The comparative study reveals that Carbofuran was more genotoxic in comparison to Profenofos the findings was found similar to Hamed *et al*., (2014) in African catfish (*Clarias gariepinus*), Kumar and Singh (2025); Nwani *et al*., (2010) *in Channa punctatus*, Ullah and Zorriehzahra (2015) in *Cirrhinus mrigala*. A comparative analysis across multiple studies indicates that Carbofuran (a carbamate) often exhibits higher genotoxicity and cellular toxicity compared to Profenofos (an organophosphate) at equivalent sub-lethal exposure levels in several fish species. This is frequently attributed to Carbofuran potent ability to induce immediate chromosomal damage and severe oxidative stress, which leads to higher frequencies of micronucleiand DNA strand breaks.

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Conflict of Interest: There is no conflict of interest between the authors.

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