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A Preliminary Genotoxic study of MNT assay in the Erythrocytes of the fish *Cyprinus carpio* exposed to Deltamethrin technical grade and 11% EC

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Abstract

A preliminary study of the genotoxic effect by Micronucleus assay (MNT) is attempted in the erythrocytes of the blood of the fish *Cyprinus carpio* exposed to lethal and sublethal concentration of deltamethrin and technical grade and 11% EC for a period of 4 days and 10 days respectively. It serves as a biomarker study that can be the indices of the toxic action of the toxicants both deltamethrin technical grade as well as 11% EC. The fish showed certain changes in the nucleus in the frequency of their occurrence which is in the order to technical grade lethal > technical grade sub-lethal > lethal 11% EC > sub-lethal 11% EC. The exposure of the MNT assay showed the alterations which are observed compared to the control as the small micronucleus, blebbed micronucleus, binucleated cell and notched nucleus. The changes with other earlier studies are going to be discussed with the available literature.

Keywords: MNT assay, *Cyprinus carpio*, Deltamethrin, Synthetic pyrethroid, 11% EC, Technical grade, lethal, sublethal concentrations.

INTRODUCTION

Pollution due to pesticides is alarming. The agricultural land is in highest risk of pesticides which was cautioned for consideration and taking certain measures (Mac Namara 2021, Tang *et al.* 2021), Sebastian and Schultz (2014) opined in the similar lines and cautioned especially as a threat to aquatic biodiversity. They suggested that the current measures of regulation to conserve and preserve the organisms that needs to be revised and the application of the pesticides on the crops is the worst ways and such operations have to be thoroughly revised so that the global environmental impacts of the pesticides as mentioned above which has to be controlled. Anamika Srivastava *et al.* (2016), Pallavi Srivastava *et al.* (2016), Indira Devi *et al.* (2017) and Agarwal *et al.* (2010) also, with reference to Indian scenario, the status of the pesticide pollution in the sub-continent which in their opinion that was mentioned as also alarming.

In spite of these cautions and operations of spraying and their transportation into the aquatic system happened, they are causing certain effects in the organisms, also the fish, the important aquatic organisms more in number and that can be a study organisms and in fact several studies of such effects to the non-target organism are reported, earlier.

In fact, the fish as a study, some biomarkers are selected by Sana Ullah *et al.* (2019a) and Anvila Kaviraj and Abhik Gupta (2014). One such indices of the toxic action of the pesticide chemical is Genotoxicity, changes in the nature of the structure of the chromosomes, which is termed as the cladistic effects as alterations. Among them one test micronucleus test (MN) provides a clue for that and for a pesticide as the toxicant such studies are attempted and reported.

Claudia and Hayashi (2011) reported the spontaneous MN. frequencies in erythrocytes (MN/1000 cells) in the different species of fish which is presented as table 1 and the list of the species and the chemical pesticides as toxicants to it is also present as table 2, (Sabazaz Ahmad *et al.* 2016). The genotoxic effects of studies were also reported in the review articles of Ullah and Zorriezatara (2015); Prusty *et al.* (2015) Hasibur Rahman *et al.* (2014), Murthy *et al.* (2013) and Ahrar Khan (2012), for the synthetic pyrethroids as well as for other pesticides also.

According to Sabzar *et al.* (2016) due to the contamination of the aquatic system, the toxicants that remain on residues have genotoxic potential. As the effect is on DNA, result 'enduring' and ardent consequences. They also mentioned the genotoxic studied of MN test provide the necessary information of genotoxic effect which are of cladistic nature. The organophosphates are no exceptions along with synthetic pyrethroids that are reported. Hence, in the present study, such genotoxic tests by Micronucleus test (MN) is attempted in the fish *Cyprinus carpio* using, Deltamethrin technical grade and 11% EC in both lethal and sublethal concentrations.

Table.1. Spontaneous MN frequencies in erythrocytes (MN/1000 cells) from different fish species under laboratory condition and in the field.(Courtesy: Chaudia Bolangnesi and Makoto Hayashi, Mutagensis. **26(1)**: 205-211 (2011).)

Species	MN per 1000 cells
Carassius auratus	0.18, 0.0 0.26 2.26 3.17
Carassius auratus gibelio	4.5-6.6 13; 10; 5.2
Carassius sp.	1.8
Channa punctatus	0.02 0.028-0.048 0.72-4.11
Cheirodon interruptus interruptus	0.0-1.0
Clarias batrachus	0.47-3.95
Clarias lazera	0.31
Cyprinus carpio	0.02 0.03 0.3-05 0.52 0.9 1.2 3.5 6.2
Esax lucius	5.3
Genyanemas lineatus	0.8
Ictalurus nebulosus	0.14
Misgurnus angullicaudatus	1.6
Odonotobutis oscura obscura	2.3
Oncorhynclus mykiss	0.12 0.181 0.33 1 1.12 2.5
Oreochormis niloticus	0.006 0.21 0.8 1.72 2.02 15.8-17.2
Paralamax clathratus	0.8
Perca fluviatilis	10-9
Pholis gunnellus	1
Phoxinus phaxinus	0.3-0.7
Poecilia latipinna	0-0.4
Pseudopleutonectes americanus	0-1.30
Rutilus rutilus	13-3
Salmo trutta	0.4-2.3
Salmo truta fario	2.8-0.75
Umbra limi	0.14
Umbra pygmaea	0.0

2 List of Researchers experimented on Genotoxicity by MNT to the different fish

S.No.	Fish	Chemical(s)/Pollutants
1	***Oreochromis mosambicus***	Aldrin, Cadmium chloride and x-rays
2	***Cyprinus carpio and Tincathinca***	Aflatoxin B1, arochlor 1254, benzidine, benzo(a)pyrene and 20-methylechloanthrene
3	***Heteropneustes fossilis***	Mitocycin C and paper mill effluent: allylformate
4	***Esoxlucius***	Radiocesium
5	***Oncorhynchus mykiss***	*In situ* to heavily polluted tributary of the River Po (Northern Italy)
6	***Carassiusauratus gibelio***	Selenium, mercury and methyl-mercury
7	***Salmotrutta fario***	PCB77
8	***Channa punctatus***	Malathion
9	***Oncorhynchus mykiss***	A textile industry effluent
10	***Cheirodoninterruptus interruptus***	Pyrethroidλ-cyhalothrin
11	***Astyanaxbi maculatus***	Cyclophosphamide, vinblastine sulfate
12	***Channa punctatus***	Malathion
13	***Oncorhynchus mykiss***	Colchicine, mitomycin, cyclophophamide, acrylamide, methyl-methanesulfonate and N-ethyl-N-nitrosourea
14	***Clarius batrachus***	2,4-dichlorophenoxyacetic acid and butachlor
15	***Heteropneustes fossils***	Pentachlorophenol
16	***Channa punctatus***	Pentachlorophenol and 2,4-dichlorophenoxyacetic acid
17	***Anguilla anguilla, Phoxinus phoxinus and Salmo trutta***	Metals, hydrocarbons, pesticides
18	***Cyprinus carpio***	Disinfectants (sodium hypochlorite, peracetic acid and chloride dioxide)
19	***Cyprinu scarpio, Carassius gibelio, Corydoras paleatus***	Cadmium chloride and cooper sulphate
20	***Oreochromis niloticus*** **and** ***Tilapia rendalli***	Domestic sewage
21	***Scophthalmus maximus***	Dialkyl phthalate, bisphenol-A, tetrabromodiphenyl ether
22	***Oncorhynchus mykiss***	Mixture of heavy metals
23	***Clarias gariepinus, Oreochromis niloticus*** **and** ***Oreochromis aureus***	Heavy metals
24	***Channa punctatus***	Chlorpyrifos
25	***Canna punctatus***	Malathion
26	***Cnesterodon decemmaculatus***	Aficida (insecticide)
27	***Carassiuscar assius***	Agricultural runoff
28	***Apteronotus bonapartii***	Benzene
29	***Labeo rohita***	λ-cyhalothrin
30	***Carassius carassius***	Endosulfan

Source: Sabzaz Ahmad Dar Abdul Rahman Yousuf and Masood-Ul-Hasan Balkhi (2016). An introduction about genotoxicology methods as tools for monitoring Aquatic ecosystem: present status and future prospectus. *Fisheries and Aquaculture Journal*.**7:** 158, doi 10.41.72/2150-3508. 1000158.

MATERIALS AND METHODS

The freshwater common carp, *Cyprinus carpio* which is an edible and economically important fish was selected with a size range of about 3 to 5 cm and 4.5 grams of weight, irrespective of their sex, have been chosen as the test organisms for present investigation. The healthy and active fish were obtained from local fish farm. The fish were acclimatized to the laboratory conditions in large plastic water tanks for three weeks at a room temperature of 28±1^{o}C. Water was renewed every day with 12-12 h dark and light cycle. During the period of acclimatization, the fish were fed (*ad libitum*) with groundnut oil cake and rice bran. The feeding was stopped one day prior to the actual toxicity test. All the precautions laid by committee on toxicity tests to aquatic organisms (APHA 1998, 2005 & 2012) were followed and such acclimatized fish only were used for experimentation. If mortality exceeded 5% in any batch of fish during acclimatization, the entire batch of that fish were discarded.

The fish were exposed to 96h LC_{50} concentration 2.0 µg/L and 0.8 µg/L as lethal concentration and sublethal as ($1/10^{th}$ of 96 hours LC_{50} values)0.2 µg/L and 0.08 µg/L of both technical grade and 11% EC of deltamethrin for 10 days.

Sample of the Blood

Fish were euthanized by an overdose of MS-222 and then weighed and measured. Blood was sampled by caudal severance from the disease-free test fish during the early hours of the day and stabilized with 50 IU sodium heparin (anticoagulant)/ml blood.

Micronucleus (MN) assay: The micronucleus test was performed according to Heddle (1973) and Schmid (1975) and nuclear abnormalities were evaluated according to Carrasco et al (1990) with a slight modification. At each sampling a drop of blood was immediately smeared on a clean slide. On drying, the smears were fixed in methanol for 10 minutes, left to air dry at room temperature and finally stained with 6% Giemsa solution in Sorenson buffer (pH 6.9) for 20 minutes. After drying the slides were rinsed with distilled water to remove extra stain. A total of about 4,000 erythrocytes were examined for each specimen per concentration (1,000 cells per slide) under the light (Olympus) using 100x oil immersion lens binocular microscope. The characteristics used for the identification of the micronucleus were circular or oval bodies having no connection with the main nucleus, smaller than one-third of the main nucleus and showing the same staining and focusing pattern as the main nucleus. Micronucleus frequency was calculated from the formula:

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

RESULTS :

The results of micronuclei for 1000 blood cells of *Cyprinus carpio* is given in table-3 and image of stained micronucleus showing effect is given in plate-1, fig.1-8. A control of 1000 erythrocyte blood cells showed no changes where as in lethal as well as in sub- lethals of both the toxicants i.e., technical and 11% EC exposure showed the changes and the image has given indication of the toxic action as micronucleus of the clasto-genic nature both in lethal as well as in sub-lethal concentrations. The technical grade as well as 11% EC of deltamethrin in lethal and sub-lethal concentrations of the exposure had the effect in the micronucleus test as the appearance as a small blebbed structure, sub-nucleated cell and notched nucleus (Plate 1 fig 1-8). The occurrence of the frequencies of MN is in the order as lethal technical grade followed by sub-lethal technical grade followed by lethal 11% EC and followed by sub-lethal 11% EC.

Table-3. Percentage of micronuclei cells in blood of *Cyprinus carpio* exposed to lethal and sublethal concentrations of 96h Technical grade and 11% EC of Deltamethrin.

	Lethal				Sublethal			
Control/ Exposing	**TG**	**%**	**11% EC**	**%**	**TG**	**%**	**11% EC**	**%**
1000 as control	10	0	0	0	0	0	0	0
1000 as exposed	7	0.7	4	0.4	3	0.3	2	0.2

PLATE-1

GENOTOXIC EFFCTS - MN ASSAY Cyprinus carpio exposed to Lethal and Sub Lethal Concentration of Deltamethrin Techinical grade and 11% EC

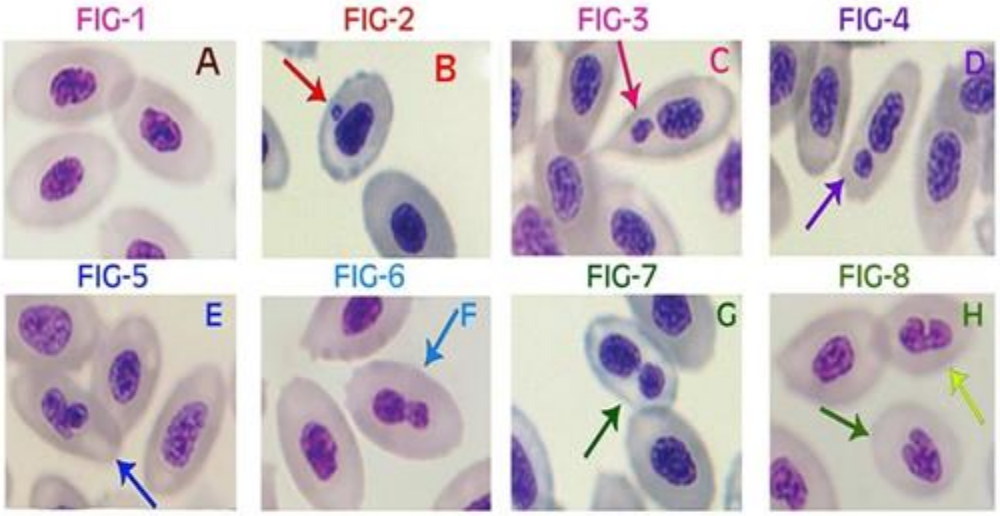

FIG-1→CONTROL-NORMAL-ERYTHROCYTES.
FIG-2→SMALL MICRONUCLEUS FORMATION.
FIG-3→BLEBBED NUCLEUS.
FIG-4→BLEBBED NUCLEUS.
FIG-5→BLEBBED NUCLEUS.
FIG-6→BINUCLEATED CELL.
FIG-7→NOTCHED NUCLEUS.
FIG-8→NOTCHED NUCLEUS.

DISCUSSION

Nnamdi *et al.* (2020) while studying on the fish African catfish *Clarias glariepinus*, the toxicity and effects of the ten (10) different pesticides that belong to the different classes, and one among them was Deltamethrin studied for micronucleus test they reported the genotoxic effect. Ranking as the 5th one in its toxic action Deltamethrin as a toxicant, they reported that the effect of the toxicant and a band shaped Nuclei was observed in the blood cells as in the present study. Organophosphates and the synthetic pyrethroids along with carbamates share a common effect, as AChE inhibitors. The action of the blood due to the impairement of the cholinergic nerve transmission, delay, haemolysis as the main cause of such effect as a sort of quick replacement of the production of blood cells. The osmatic imbalance that prevailed in the plasma of blood is also responsible of such changes. (Singh and Srivastava 2010). Anaemia the, decrease in the Ht values were also specifically, the resultant causes of the genotoxic effect as opined by Riaz-uL Huq *et al.* (2018).An hypoxia condition was also mentioned by Pakanit and Kinchareon (2011) as the causefor such effect. The affirmation of the genotoxic effect by all the ten pesticides as reported and for the first time

in the present experiment study, both technical grade and 11% EC in lethal and sub-lethal concentration resulted such aspects and the frequencies of its occurrence is varied.

In the review article by Sana Ullah *et al.* (2019b), certain of the genotoxic effects were mentioned, that Sana Ullah *et al.* (2019b) in the fish *Hypophthalmichthus maltrix* reported due to exposure of Deltamethrin which resulted DNA damage and such nature in the peripheral blood cells. Viera and dos Reis Marhnez (2018) in the fish *P. Lineatus* due to cyalothrin DNA was damages, Lieu *et al.* (2018), using the Deltamethrin in the fish *Danio rerio*. Ullah (2015) in the fish *Labeo rohita* using cypermethrin, Polletta *et al.* (2013) in the fish *Prochilodus lineatus* using the same toxicant, Ansari *et al.* (2011) also using the same one in the fish *Channa punctata*; Kan *et al.* (2012) in the fish *Oreochromis niloticus* using Deltamethrin as the toxicant, λ-cyhalothrin in the fish *Gambusia affinis* by Gokalap Muranli and Guner (2011) who all reported the genotoxic effects of the synthetic pyrethroids. All the above studies were of the opinion that the production of reactive oxygen species (ROS), made to have the DNA genetic material which was broken by OH^- and H_2O_2, the resultant parameters of such happennings. Even in the present study in the fish *Cyprinus carpio* due to Deltamethrin toxic action the pyrethroid, effect of the RBC cell as in DNA which was a genotoxic nature. Sana Ullah *et al.* (2016) reported in the fish *Labeo rohita* using Malathion an organophosphate that produced genotoxic action. The cytotoxic action of the toxicant caused the damage in very structure of DNA which they provided as the valid reason of such effect which is true even in the present study.

Hemalatha *et al.* (2019) studied and reported on the genotoxic effect of Quinalphos in the fresh water fish *Cyprinus carpio* (the present studied fish). As the percent frequency of the damage was time dependent and concentrate dependent, here in the present study lethal and sub-lethal concentrations of both the toxicants (technical grade and 11% EC) as such vary. The reasons of such clastogenic effect they mentioned were, the reactions between the toxicant molecules and DNA (Nucleotides) molecules via ROS, or the interaction of the metabolites and genetic material and also by the inhibition of DNA repair enzymes, which, all, as a multi-lateral mechanism in them actions resulted the such genotoxic effect which is even true in the present study. The observations as per the table 3, indicate that TG lethal had more than to sub-lethal which is proves that it is concentration dependent as opined by the authors and is true even in the present study. The lethal concentrations have 4 days exposure and sub-lethal have 8 days exposure and the concentrations and duration of the exposure is true even in the present study. They also opined that such abnormalities in the DNA damage lead to an effect finally on the gene amplification and complicate furthe the problem. This was also reiterated by Ansari *et al.* (2009) and Nwami *et al.* (2009).

Faiza and Javed (2018) studied and reported that when a pesticide mixture (endosulfan and chlorpyrifos) exposed to the fish *Oreochromis niloticus*, had an impact on DNA and that resulted the genotoxic effect. The exposure was for a period of 70 days of $1/3^{rd}$ of lethal toxic values that resulted the toxic action the product of hydrogen peroxide, hydroxyl radical and superoxide anions, cumulatively produced the DNA damage. Single strand breaks, double strand breaks, DNA-DNA-DNA-protein cross links or the inhibition of enzymes involved in DNA repair all were possible to have such an adverse effect. Iyiola *et al.* (2018) studied and reported of genotoxic nature of the effects of the fish *Clarias gariepinus* exposed to chlorpyrifos, in liver and gill tissue. They reported by a comet analysis and the abnormalities in the chromosomes.

Anitha Bhatnagar *et al.* (2016) reported in the fish *Cirrhinus mrigala* due to exposure to chlorpyrifos as a toxicant by MN assay only in $1/20^{th}$, $1/10^{th}$ and $1/5^{th}$ of LC_{50} values of 96 hrs (0.44 mg/L^{-1}), for 2, 4, 12, 21, 28 and 35 days. They reported that the results after the exposure for a duration of 14 days only the effect was maximum. Fish due to promote some of the defensive mechanisms to reduce the toxic action by the toxicants in the body to stabilize, all, that the micronuclei has appearance initially that is after showed a recovery to have a state of the normalcy. It has inducted the genotoxic effect initially and later stabilized and that was the explanation given by them.

Renu Chandhari and Kamal Kumar Saxena (2016) in the fish *Channa punctatus* using bioallethrin, a synthetic pyrethroid as a toxicant reported that at three different sub-lethal concentrations in the erythrocytes had a genotoxic effect which they confirmed by the MN test only. Because of the lipophilicity, the toxicant can be absorbed as in the present study also, that caused the mutagenic toxic action resulting micronuclei formation and other resultant changes only.

Rodriguez *et al.* (2015) in the fish *Oreochromis niloticus* using Imida cloprid an organophosphate reported by the MN test the genotoxic effect and they too opined the ROS mechanism onlyfor the effect.

Mohammad Ismail (2018) reported on the Genotoxic effect of chlorpyrifos exposure in the fresh water fish *Labeo rohita* by the MN test and opined as of similar lines of the above reports (Nnamadi *et al.* 2020 & Sana Ullah (2019 a,b & 2016).

Naqui *et al.* (2016) reported in the fish *Oreochromis niloticus*, the genotoxic effects due to pesticides by the MN test. The pesticides that were exposed as toxicants include chlorpyrifos 40% EC, Malathion 57% exposed EC, Cypermethrin 10% EC and lambda cyhalothrin 2.5% EC and an herbicide buctyrol 60% EC and the genotoxicity was in the order as cypermethrin > chlorpyrifos > Malathion > lambda cyhalothrin and buctril. Here in the present study the technical grade > lethal > technical grade sublethal > 11% EC of lethal > 11% EC of sub-lethal of Deltamethrin, in that order the effect was resulted.

Pallavi Srivastava *et al.* (2016) reported in their review article of pesticides toxicity in fishes resulted the genotoxic effects in different fish and presented a diagrammatic way about the chromosomal abnormalities that can occur. They are of opinion that three types of genotoxic actions such as carcinogens, mutagens or birth defect causing agents (teratogenic). The present toxin as genotoxin which is of the mutagenic nature.

Jaya Prakash and Shettu (2013) reported in the fish *Channa punctatus* exposed to Deltamethrin for a period of 15, 30 and 45 days in two concentrations of 0.075 mg/L and 0.15 mg/L. The changes that occurred in the morphological shape of the RBC which are due to shrinkage of hemolysis result in aberrations of the genetic chemical substance DNA.

Kamal and Hasheem (2012) reported for Deltamethrin in the fish *Clarias gariepinus*, the biochemical changes of the blood got effected and the antioxidant defense role finally passed the way to have the certain genetic effects of DNA in RBC.

Pandey *et al.* (2011) reported in the fish *Channa punctatus*,due to the profenofos exposure only, induced the DNA damage that is shown by using single gel electrophoresis study. When the fish exposed to three sub-lethal concentrations 0.58 ppb, 1.16 ppb and 1.74 ppb based on 96 hr LC_{50} value. The study is of by comet analysis a different methodology but the end result of the confirming the toxic action resulted the DNA damage. The gill electrophoresis study, the strands in alkaline medium were significantly showed variations wherein in the present study the MN test showed a physical change as a dot of fragmented chromosal part that contains chemically DNA.

Jaqueli *et al.* (2011), reported the genetic toxicity effect by MN test in the fish *Cyprinus carpio, Hypostomus punctatus, Rhamdia quelen* and *Oreochromis niloticus* (A study that was conducted in the Sata Calarina state of Brazil). They reported the MN frequency range in the fish that studied varied from 6.21 and 13.78 because the waters waters contain the pesticides which were responsible for the genotoxic effect.

Claudia and Hayashi (2011) reported as review article of Micronucleus assay in aquatic annuals and the few reports by different authors (which is presented as table.1). Genotoxic action of the pesticide of the toxic stress had an impact on the DNA damage.

Nwani *et al.* (2010), using carbo sulfan an insecticide effect on the fish *Channa punctatus*, reported that Micronuclie test and also the observation of DNA strands by alkaline single gel Electrophoresis. The fish were exposed to three concentrations $1/4^{th}$, $½^{nd}$ and $3/4^{th}$ of LC_{50} values for a period of 4 days 96h and the sampling was done for every 24 h. The method which is different from the present one showed genotoxic effect and ROs mechanism as opined by other researchers was responsible for such cause as in the present study.

David Ali *et al.* (2008), made the genotoxicity study in the fish *Channa punctata* due to chlorpyrifos toxic action by the MN test that resulted only the damage of the DNA. Thus the genotoxic effect as expressed by different authors of the present study which by the MN test only a preliminary one and that can be used as a biomarker type for assessing the toxic action of the pesticides and also the indices of the pesticide pollution.

CONCLUSION

As one of the biomarker study, the MN test provide a basic information for the structural changes that have resulted in the erythrocytes (nucleated) nucleus. The study also provide a clue of chromosomal effects which contain DNA only. If any changes that are resulted in its chemical nature leads to mutagenic affects which can be called as of the clastogenic nature. It requires, further, to study the molecular ways and means to study the actual mechanism involved between the toxicant and the DNA, the chemical constituent of the Nucleus.

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