Genotoxicity of the Synthetic Pyrethroid Insecticide Bifenthrin Technical in Zebra Fish Danio rerio Gill Tissue Assessed by the Alkaline Comet Assay


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Abstract- The genotoxic potential of the synthetic pyrethroid bifenthrin in fish was assessed using the alkaline comet assay. A sublethal concentration was selected for evaluation of genotoxicity in gill tissue of Danio rerio. DNA damage was observed in the treated group and it increased with subsequent exposure with respect to the control, suggesting that bifenthrin interacts with DNA of fish and is capable of causing DNA damage, in the form of single strand breaks. It was found that % DNA damage reduced in the reversal group. The present study reveals that bifenthrin is genotoxic in fish at the concentration tested; causing single strand breaks and also affects the repair systems in the gill tissue, warranting caution in usage and exploratory studies. Our results also highlight that comet assay is a useful biomarker for studying chemical-induced genetic damages in aquatic organisms.

Keywords: Comet assay; Bifenthrin technical; Genotoxicity; Danio rerio; Gill tissue.

I. INTRODUCTION

Bifenthrin (2-methyl-1, 1-biphenyl-3-yl)-methyl-3-(2-chloro-3, 3, 3-trifluoro-1-propenyl)2, 2 dimethyl cyclopropanecarboxylate) is a pyrethroid insecticide that affects the nervous system. Bifenthrin is registered as a Restricted Use Pesticide (RUP) and highly toxic to aquatic organisms and also a potent ATPase inhibitor. Aquatic vertebrates are more sensitive to ATPase inhibitors than terrestrial vertebrates due to their high dependence on ATP synthesis in the gills to maintain osmotic balance. Bifenthrin is very highly toxic to fish, crustaceans and aquatic animals. The LC50 after a 96-hour exposure is 0.00015 mg/l for rainbow trout, 0.00035 mg/l for bluegill, and 0.0016 mg/l for Daphnia. However, not much information is known on genotoxicity of bifenthrin in other fish species. Available data on the mutagenic effects of bifenthrin is inconclusive [14] gene mutation studies show positivity, while other tests on bacteria and mammalian cells were negative (EPA’s Pesticide Fact Sheet Database).

Comet assay include the relative ease of application to any tissue of interest, the detection of multiple classes of DNA damage and the generation of data at the level of the single cell. Among the various versions of the assay, the alkaline method enables detection of the broadest spectrum of DNA damage. It can detect double- and single-strand breaks, alkali-labile sites that are expressed as single-strand breaks and single-strand breaks associated with incomplete excision repair [15], [17]. The present study was conducted to assess the genotoxicity of bifenthrin in zebra fish using alkaline comet assay.

II. MATERIALS AND METHODS

Hatchery bred Danio rerio procured from a commercial fish farm (Manimangalam, Padappai) were quarantined for one month in cement tanks of the laboratory. Fish were acclimatized in glass aquaria for 10 days to the laboratory condition prior to the exposure and fed with commercial fish flake feed. The weight and length was recorded and the mean value was found to be 0.521 g and 2.6 cm, respectively. The feed was withdrawn 24h prior to exposure. The physico-chemical parameters of the water used was recorded and found to be pH (7.3-7.6), dissolved oxygen (7.4-7.8 mg/l), hardness (185 mg/l) (APHA, 1975) and temperature (27 ± 1°C). Bifenthrin technical (purity 95%) was obtained from a commercial source. Low melting agarose, normal melting agarose and Tris base were purchased form Sigma-Aldrich (USA). All the other chemicals used for the comet were purchased from Merck (India).

A. Acute toxicity exposure and dose fixing

A static acute toxicity exposure was conducted for a period of 4 days with ten fish and the 96th LC50 was determined as 31.80 µg/L using NCSS 2000 statistical package. 1/10th of the LC50 was used for the chronic exposure of 3 weeks followed by a 10 day recovery. Danio rerio was exposed to Bifenthrin technical spiked medium for a period of 4 weeks. Every week 2 fish from each group was collected for assessing the DNA damage by In vivo comet assay in gill tissue. The exposed fish were dissected and the gill tissue was collected, weighed and placed in ice cold Phosphate buffered saline (PBS). The gill tissue was homogenized with a micro pestle and allowed to settle. The supernatant which contained single cells was used for comet assay.

B. Comet assay

Comet assay was performed using the method of Singh et al [26] with slight modifications. For the basal layer, 1%
Normal melting agarose in PBS was prepared; slides were dipped in the agarose solution and air dried. About 20 µl of the cell suspension was mixed with 80 µl Low Melting agarose (0.7% in PBS) and added to the basal agarose layer, covered with cover glass and allowed to solidify. This was followed by the addition of the third layer of agarose (100 µl 0.5% low melting agarose) over slipped and allowed to solidify. After removal of cover slips, the slides were immersed in 50 mL cold lysing solution and slides were kept in dark at 4°C for 1 hour in refrigerator. To avoid any additional DNA damage, the procedure was performed under dim light. The slides were removed from the lysing solution and placed on a horizontal electrophoresis tank for DNA unwinding for 20 minutes in the electrophoresis buffer (300 mM NaOH, 1 mM Na₂ EDTA, pH >13). The cells were exposed to alkali for DNA unwinding. This was followed by electrophoresis at 18 V (0.7-1.0V/cm) 300 mA for 10 minutes at 4°C. After electrophoresis, the gel slides were washed in neutralizing buffer (0.4 M Tris-HCl, pH 7.5) for 15 minutes and then stained with 75 µl of ethidium bromide (2µg/mL) and screened for comets using a Carl Zeiss Axiostar fluorescence microscope (Carl Zeiss, Germany) at 400X magnification. The migrated cells resemble the comets with a head region containing undamaged DNA and a tail containing broken DNA. The amount of DNA able to migrate and the distance of migration indicate the number of strand breaks present in that cell and the extent of DNA migration indicates the level of DNA breakage in the cell. Cells with increased DNA damage display an increased migration of chromosomal DNA from the nucleus towards the anode. The cells are scored visually based on their tail intensities and categorized (Fig. 2) as 0 (Undamaged), 1 (mild), 2 (moderate), 3 (severe) and 4 (extensive). [20] About 50 comet images were visually scored at random for each fish covering a total of 100 cells per group [27]. The percentage of damage is calculated and is compared between the control and treated groups.

C. Statistics

TOXSTAT 3.5 Version (WEST, Inc. and Dave Gulley, Univ. of Wyoming; 2003, Central Avenue, Cheyenne, WY82001, USA) was used for statistical evaluation. Statistical comparison of DNA damage in the controls and treatment group was performed using the Dunnett’s test.

III. RESULTS

The genotoxic effect of bifenthrin in fish gill tissue was evaluated using the alkaline comet assay. The arbitrary unit (AU) was used to express the extent of DNA damage and was calculated as follows:

\[ AU = \sum n_i \times i \]

Where \( n_i \) is number of cells in damage degree \( i \) (0, 1, 2, 3, 4) [24]. DNA damage percentage was found to be high and statistically significant in the treated group with respect to the control. The solvent control also showed considerable damage. However, the damage in the treated group was exceedingly higher than that recorded in the untreated and solvent control groups. The results are expressed in the graph. (Fig.1); the damage started during the first weeks of exposure and increased in the subsequent weeks. However, in the reversal group the graph declined suggesting an ongoing repair process in the treated cells following the exposure and subsequent recovery period. The number of cells in each degree of DNA damage and DNA damage scores in control, solvent control and treated groups as arbitrary units (AU) has been presented in Table 1.

IV. DISCUSSION

The genotoxic effects of environmental pollutants can be monitored using a broad range of in vitro and in vivo biomarker assays. Comet assay is superseding other assays due to its sensitivity in detecting DNA damage, robustness, feasibility of application to any eukaryotic cell and is economical [2], [4], [1], [7], [8], [9], [10], [11]. Velisek et al studied the acute toxicity, haematological, biochemical and histological effect of Talstar EC 10 (100 g/l bifenthrin) on rainbow trout [28]. Jin et al had studied the developmental toxicity in larval stages in zebra fish and reported its sublethal toxicity [18]. The effect of the pyrethroid bifenthrin on the steroid hormone levels and gonadal development in the rainbow trout under hyper saline conditions were reported by Forsgren et al [25]. Cui et al [12] stated the need for a detailed ecological risk assessment for synthetic pyrethroids since their increased and broad use contaminates surface waters and sediment biosorption. Adsorbed pyrethroid released in the water may cause acute or chronic toxicity and induce cytotoxicity, genotoxicity (DNA strand breaks, gene mutation and apoptosis. A considerable data package on ecotoxicological effects of bifenthrin was presented in FAO [23] and aquatic organisms like Daphnia magna, mysid shrimp and several fish species were stated to be very sensitive to low levels of the compound [12].

DNA damage in fish is used more often as a biomarker of the effects of pollutant exposure. DNA adducts have been studied in fish exposed to xenobiotics, as investigations of genotoxic compounds are deemed highly relevant [6]. Mitchelmore and Chipman recommended that DNA strand breaks, particularly as measured by the comet assay, act as a biomarker of genotoxicity in fish and other aquatic species [21]. Under standard conditions, the comet assay detects the amount of cells with DNA single strand breaks. These are consequences of incomplete excision repair sites [22]. DNA strand breakage is a sensitive marker of genotoxic damage as they are potential pre- mutagenic lesions [19].

Accumulation of DNA damage may occur either through an increase in the number of DNA-damaging events or a decrease in DNA repair. Inhibition of repair of an induced DNA damage is measured by the comet assay due to the persistence of DNA lesions, which leads to strand breakage or an accumulation of DNA strand breaks related to an ongoing DNA repair. There are reports that

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state the inhibitory effects of various chemicals and physical agents that inhibit the DNA repair systems [16, 17]. The increase in the length of migration observed in the treated of bifenthrin could be reflecting DNA single strand breaks as a consequence of incomplete excision repair sites. The genotoxic effects of bifenthrin reported can be interpreted as a probable inhibitory effect on the DNA repair system, since the damage percentage reduced during the recovery phase i.e., in the absence of the chemical. Although the DNA repair mechanisms in fish are not as efficient as those in mammals [13], [29], they do exist.

The mechanism of action of synthetic pyrethroid on DNA is still obscure. The results indicate that bifenthrin causes DNA damage in fish. The effect is suspected to be unique chemical interactions causing strand breaks and the suppression of DNA repair systems and associated processes; however, with the present knowledge, it is difficult to comment on the exact mechanism. In conclusion, the results indicate that bifenthrin is certainly genotoxic to fish at the tested concentration, irrespective of the molecular interactions that precede the event of DNA damage.

Biomarkers are used as early warning pollution monitoring tools to signal the onset of sublethal deleterious effects at the physiological, molecular, cellular or subcellular level therefore being able to provide evidence for changes at higher levels of the biological organization [5]. Our results highlight that comet assay is a useful biomarker for studying chemical-induced genetic damages in aquatic organisms.

V. CONCLUSION

Chemical pesticides have been employed extensively for various advantages they offer in terms of productivity, rapidity in crop yield and protection from infestation by pests despite their potential hazards to biotic components of the ecosystem. The present study also highlights such an effect of a chemical insecticide on fish, one of the major components of the ecological web and recommends cautious use of the same to protect the ecosystem.

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REFERENCES


[29] Velsiek J, Svobodova Z, Piackova V Effects of acute exposure to bifenthrin on some haematological, biochemical and histopathological parameters of rainbow trout (Oncorhynchus mykiss) Veterinarni Medicina. 54, 2009 (3): 131–137.
Table 1  
NUMBER OF CELLS IN EACH DEGREE OF DNA DAMAGE AND THE DNA DAMAGE SCORES (IN ARBITRARY UNIT, AU) IN CONTROL AND TREATED GROUPS

<table>
<thead>
<tr>
<th>Wk</th>
<th>Sample</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>DD</th>
<th>AU</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>94 ± 1.4</td>
<td>3.5 ± 3.5</td>
<td>2.50 ± 2.1</td>
<td>0.00 ± 0.0</td>
<td>0.00 ± 0.0</td>
<td>6</td>
<td>8.5</td>
</tr>
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<td></td>
<td>Sol. con</td>
<td>85 ± 2.83</td>
<td>15 ± 2.83</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>73 ± 2.83</td>
<td>14 ± 8.49</td>
<td>9.50 ± 0.71</td>
<td>1.00 ± 1.41</td>
<td>0.00 ± 0.00</td>
<td>24.5</td>
<td>36</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>94.5 ± 3.54</td>
<td>5 ± 2.83</td>
<td>0.50 ± 0.71</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>5.5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Sol. con</td>
<td>83.5 ± 2.12</td>
<td>15.5 ± 3.54</td>
<td>1.00 ± 1.41</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>16.5</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>67.5 ± 0.71</td>
<td>20. ± 1.41</td>
<td>8.50 ± 2.12</td>
<td>4.00 ± 2.83</td>
<td>0.00 ± 0.00</td>
<td>32.5</td>
<td>49</td>
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<tr>
<td>3</td>
<td>Control</td>
<td>93.5 ± 2.12</td>
<td>6 ± 2.83</td>
<td>0.50 ± 0.71</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>6.5</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Sol. con</td>
<td>85.5 ± 2.12</td>
<td>11 ± 4.24</td>
<td>2.50 ± 0.71</td>
<td>1.00 ± 1.41</td>
<td>0.00 ± 0.00</td>
<td>14.5</td>
<td>19</td>
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<tr>
<td></td>
<td>Treated</td>
<td>60.5 ± 2.12</td>
<td>16.5 ± 0.71</td>
<td>20.0 ± 1.41</td>
<td>3.00 ± 1.41</td>
<td>0.00 ± 0.00</td>
<td>39.5</td>
<td>65.5</td>
</tr>
<tr>
<td>R</td>
<td>Control</td>
<td>93.5 ± 2.12</td>
<td>6.5 ± 2.12</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>Sol. con</td>
<td>84.5 ± 2.12</td>
<td>14.5 ± 0.71</td>
<td>1.00 ± 1.41</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>15.5</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>68.5 ± 4.95</td>
<td>29 ± 1.41</td>
<td>2.50 ± 3.54</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>31.5</td>
<td>34</td>
</tr>
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Fig. 1: Graph showing the trend of DNA damage during exposure and subsequent recovery