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DNA integrity as molecular biomarker of genotoxic effect of Endosulfan in *Oreochromis mossambicus* (Peters)

Rani.A.P.A[1], Kumaraguru. A.K[2]

[1](*Assistant Professor, PG & Research Department of Zoology, Lady Doak College, Madurai – 625002, Tamil Nadu, India.*
[2](*Senior Professor & Head, Department of Environmental Studies, Madurai Kamaraj University, Madurai – 625 021, Tamil Nadu, India. On Lien: Vice Chancellor, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India. Ph: 091-0462-2337231*

ABSTRACT *: Ecotoxicity is the science of studying the effects of pesticides and other contaminants that get into the natural environment and affect the non-target wild plants and animals and its impacts on individuals, populations, natural communities and ecosystems. Environmental biomarkers revolve around 3R's – repeatability, reliability and relevance and in many cases repeatability and reliability is not always equal to relevance. Environmental biomarkers should be sensitive enough, easily and rapidly assayed, respond in a dose-dependent manner, valid for the species concerned and evaluated for possible influence by other endogenous and exogenous factors. In the present study molecular biomarker 'DNA integrity' has been studied as dose dependent and time dependent responses of Oreochromis mossambicus to exposure of endosulfan. The fishes were exposed to three different sub-lethal concentrations of endosulfan for different time intervals of 7 days from 14th day up to 28 days. The DNA isolated from fish fin of control (plain and acetone) and exposed fishes by standard protocol was subjected to spectrophotometric analysis, gel mobility shift analysis. The observed results of these studies performed confirm that endosulfan does produce effect on DNA molecule and alter the DNA stability or integrity. Alteration in DNA gains significance in that any change or mutation in DNA would rather interfere with replication, transcription and translation process thereby produces mutants and code for abnormal proteins with altered functional capacity.*

Keywords: *Environmental biomarker, DNA integrity, endosulfan, Gel electrophoresis, Oreochromis mossambicus, Spectrophotometric analysis*

I. INTRODUCTION

Pesticides are important chemical substances which are of prime importance since they alter the global climate of soil and water bodies. Endosulfan is one such acaricade pesticide which has lots of side effects even though they are good at controlling the pest of plants. Endosulfan, first introduced into United States in 1954 by Farbwerke Hoechst A.G. under the registered trademark, "Thiodan ®" (Maier-Bode, 1968), is a persistent organic pesticide (POP chemical) and has the tendency to bioaccumulate and persists in the environment for a long period (Jaffrey *et. al.,* 1990). One of the most important commercial non-targeted animals affected is fish. One such fish is *Oreochromis mossambicus* which is a good protein supplement to fight against protein malnutrition. Agricultural run-off has caused ecotoxicity of the aquatic environment and that leads to death of targeted and non-targeted organisms (Joseph and Raj, 2011). The genotoxicity of endosulfan is still under debate as there are reports which are either positive or negative. Hence the present study is aimed at adding information on the genotoxic capacity of endosulfan using *Oreochromis mossambicus* as test animal, by performing analysis of DNA.

II. MATERIALS AND METHODS

Commercial endosulfan produced by Rallis India Ltd. was purchased and used for the experimental purpose. Endosulfan is a volatile organochlorine pesticide used as a broad spectrum pesticide to control insect pests' whitefly, aphids and others. Endosulfan was dissolved in acetone solvent and then applied into the experimental tanks. *Oreochromis mossambicus* (Tilapia) was used as test animal. The Tilapia fish (*Oreochromis mossambicus*) of weight about 35g was obtained and acclimatized to our laboratory conditions in a 150L capacity FRP (Fiber reinforced plastic) tanks. The endosulfan- exposed Tilapia was processed to obtain DNA and do further characterization of the damage caused to DNA.

III. EXPERIMENTAL PROCEDURE

Based on the calculated LC_{50} (5 µg/ L), 3 different sub-lethal concentrations (0.5 µg/ L, 0.05 µg/ L, 0.005 µg/ L) were used for the present study to evaluate its genotoxic effects on the erythrocytes of tilapia fish. Both plain and acetone control was maintained. Our experimental design consisted of a total of 30 *O. mossambicus* (averaging 30 ± 4 g in weight and 12 ± 4 cm in length), divided into 5 tanks of six fishes in each tank. Sampling was done at an interval of 7 days from 14thday to and 28th day and studied further.

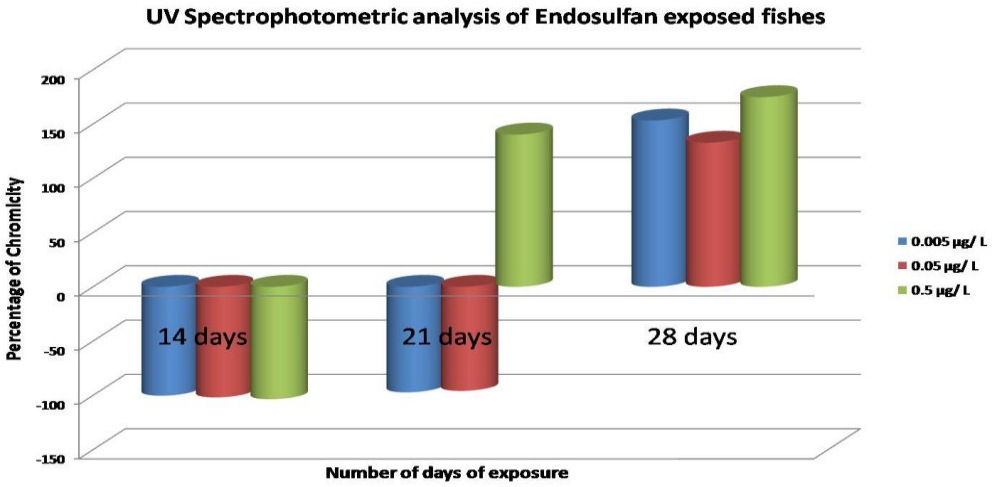
DNA isolation was done using fish tail as sample as per the procedure given by Amrita Virtual lab [4]. The fin was cut and made into paste with lysing solution and incubated at 55°C O/N and centrifuged. The supernatant was transferred into fresh tube and subjected to Phenol:Chloroform:Iso-amyl alcohol treatment followed by Chloroform:Iso-amyl alcohol treatment. The DNA present in the supernatant was precipitated using absolute ethanol and washed with 70% ethanol. The washed DNA pellet was dried and stored by dissolving in Tris-EDTA buffer. The harmful effect of endosulfan on DNA was assessed through spectrophotometric analysis, gel mobility shift assay of the isolated DNA.

The isolated DNA was scanned using Thermo Scientific UV-Vis Spectrophotometer from 230 nm to 290 nm. Percentage of chromicity, which is an indicator of DNA stabilization or damage, for 3 different sub-lethal concentrations were calculated. Presence of red shift or blue shift was also checked.

DNA isolated from control and endosulfan-exposed fishes were run on 1% agarose gel (Hi Media) using 1X Tris-EDTA-Boric acid (TEB) buffer. The run gel was stained with ethidium bromide and scored for the presence of any alteration or shift in the pattern of bands and mobility of DNA bands using Gel Doc system.

IV. RESULTS

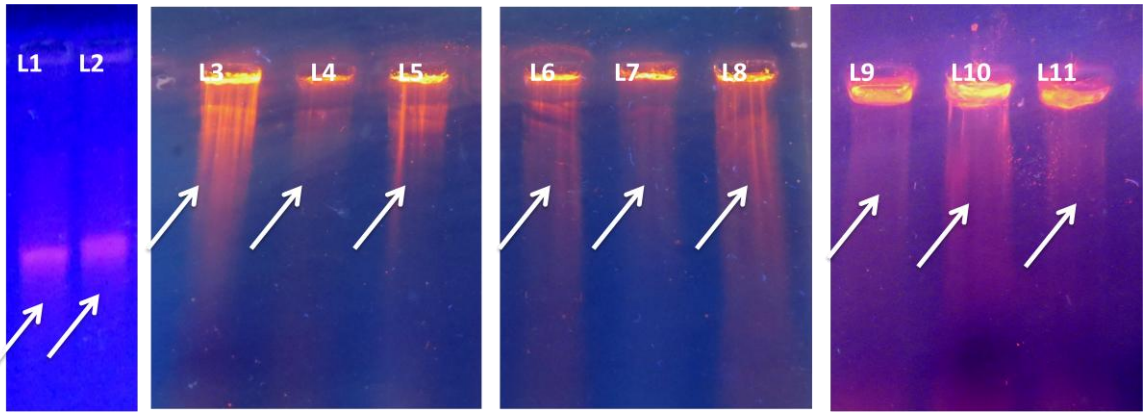
The result of the spectrophotometric analysis as percentage of chromicity (with reference to the control DNA) observed is presented as Graph. 1.



Graph 1: Percentage of Chromicity induced by Endosulfan in endosulfan-exposed fishes for different concentrations for different days of exposure

From the graph we observe that hypochromicity is induced for all concentrations on 14th day. For 21st day, hyperchromicity is observed for the maximum dose and hypochromicity for the other two concentrations. As the number of days of exposure increases, all three doses exhibit hyperchromicity. There is no significant blue or red shift in the readings obtained.

Fig. 1(a), 1(b) and 1(c) and 1(d) represent the gel patterns obtained for control DNA and endosulfan-exposed DNA at 3 different sub-lethal concentrations respectively.



1 (a) 1 (b) 1 (c) 1 (d)

Figure 1: Gel electrophoresis of control, acetone control and endosufan-exposed isolated DNA; 1 (a) – L1-Plain control; L2-Acetone Control; 1 (b) – 14 days of exposure, L3-L5; 1 (c) – 21 days of exposure, L6-L8; 1 (d) – 28 days of exposure, L9-L11; L3,L6,L9 – 0.005µg/L dose; L4,L7,L10 – 0.05µg/L dose; L5,L8,L11 – 0.5µg/L dose of endosulfan

In control sample, intact DNA is observed which is seen as a single clear band (Fig. 1a) and which moves away from the well. In the case of endosulfan-exposed DNA (Fig. 1b-1d, L3-L11), DNA band is shifted towards the well. As the number of days of exposure increases from 14th -28th day, level of DNA damage increases. During 14th day of exposure, we find clear strand breaks which are evident from the Fig. 1(b). On 21st day we find that in L7 and L8 (Fig. 1 (c)), smearing of DNA bands starts and by 28th day smearing is observed in all the lanes (Fig. 1 (d), L9-L11). With reference to the information on dose-dependent effect, we find that the lower concentration induces strand break (L3, L6) and highest dose induces smearing of DNA bands (L5, L8). Smearing of DNA implies that more strand breaks are introduced.

V. DISCUSSIONS

Spectrophotometry and gel electrophoresis could be used for testing the effect of pesticides on DNA (Ahmadi, 2011). DNA is a more stable molecule and is the genetic information carrier. Absorbance spectrophotometry is also a valuable tool for analysis of alterations in the secondary structure of DNA, RNA, and related molecules such as poly (ADP-ribose) (Minaga and Kun, 1983). This technique takes advantage of the well-known hyperchromicity of DNA and RNA, resulting from intrinsic differences in absorbance among nucleotides and single-stranded and double-stranded species. In addition, absorbance measurements at 220 nm and 280 nm are frequently employed to detect and quantify proteins, while other wavelengths within this range are used to analyze structural changes induced within DNA and RNA upon binding of biomolecules or exogenous chemicals. In the present study the increase in percentage of hyperchromicity by 28th day indicates that structural changes have been introduced in DNA due to the binding off endosulfan. Any alteration in DNA can induce carcinogenesis or other clinical conditions. The alkylation of DNA is the first step in the initiation of chemically induced carcinogenesis and cancer (Prakash and et al., 1998). DNA adducts, if not repaired before the onset of DNA replication or misreported, are capable of inducing gene mutations and putatively initiate the conversion of exposed natural cells to irreversibly altered pre-neoplastic states. Endosulfan does not introduce strand separation but alter the conformation of DNA due to the non-covalent binding of endosulfan. Endosulfan attacks at alkali-labile sites, thereby inducing strand breaks which are evident from the presence of more than one band and DNA smearing. Accumulation of the changes induced followed by the reduction in the rate of repair and modification of the affected DNA takes place in response to the increase in dose and time of exposure. This expresses itself as more DNA strand break and smear.

VI. CONCLUSION

DNA damage has occurred which correlates with the dose and time of exposure. The present study indicates that endosulfan acts on genetic information carrier and so it can be considered as genotoxic. Pollutant induced alteration of structure of DNA is of prime importance owing to the interference in major cellular processes like replication, transcription and translation process. This would in-turn lead to the production of mutants and abnormal proteins with altered function. Thus Tilapia can be used as environmental biomonitoring system and DNA molecular marker could be an effective method of assessing the damage of DNA.

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