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RESEARCHARTICLE

EVALUATION OF TRIAZOPHOS INDUCED GENOTOXICITY IN A FISH, *ANABAS TESTUDINEUS*

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ABSTRACT

Introduction: Presence of different potent genotoxic substance in the aquatic environment has led to the development and adaptations of modern and reliable techniques for quick monitoring. Random Amplified Polymorphic DNA (RAPD-PCR) is a remarkable technology that has immense application in clinical medicine, forensic science, pathogen detection, genotoxicants detection etc.

Aim and Objectives: To assess the genotoxic effect of triazophos using RAPD. To assess the apoptosis induced by the triazophos using DNA ladder method. Recovery of triazophos exposed Anabas test udineus using fresh medium.

Materials and Methods: Test species - Fresh water fingerlings of Anabas testudineus of length 16cm±1cm and weight 72±1g were procured from the fish farm at kolathur, Tamilnadu, Chennai, South India. The collected fishes were acclimated to laboratory conditions in dechlorinated tap water for 15 days. The fish specimens were fed with commercial fish a feed during acclimation. The physiochemical characteristics of the water used throughout the experiments.

Results: In the present study the RAPD was performed on gill tissues Anabas testudineus from control and fishes exposed to triazophos for 3 days and followed by 3, 6 days of exposed to fresh medium confirmed remarkable recovery on remutial. Three different 10 primers were used to study the genotoxic effect of the pesticide. The triazophos exposure shows variation and polymorphism in band patterns from that of the control samples. Minimum variation in polymorphism was observed in primer 3, gill samples. Maximum variation in DNA band polymorphism was observed in primer 1 gill sample. DNA band intensity variation was also observed in the treated fish compared to control samples.

Discussion: In the present study the RAPD analysis on the fish exposed to triazophos for 3 days of followed by 3, 6 days of recovery in water. Three different 10 primers were used to study the genotoxic effect of the pesticide. The triazophos exposure shows variation and polymorphism in band patterns from that of the control samples. Minimum variation in polymorphism was observed in primer 3, gill samples. Maximum variation in DNA band polymorphism was observed in primer 1 gill sample.

Summary and Conclusion: The pesticide triazophos is found to be more toxic to the fish Anabas testudineus. The acute toxicity studies in Anabas testudineusat 96 hrs of LC_{50} value for triazophosis was0.270 ppm. The histological investigations in Anabas testudineus exposed to Triazophos were found to be highly toxic and the histological alterations were increasing with increase in concentration and duration. The triazopohs exposure shows variation and polymorphism in band patterns from that of the control samples

INTRODUCTION

Presence of different potent genotoxic substance in the aquatic environment has led to the development and adaptations of modern and reliable techniques for quick monitoring. Random Amplified Polymorphic DNA (RAPD-PCR) is a remarkable technology that has potential immense in diverse areas of research that includes clinical medicine, forensic science, pathogen detection, genotoxicant detection etc. Besides the technique is cost effective and less labour intensive than other similar kinds of molecular techniques like arbitrarily primed-PCR(AP-PCR). Moreover this technique does not require any previous knowledge of the species genome sequence. It also avoids the use of radioisotopes. In the field of ecotoxicology, most RAPD studies describe the RAPD changes such as different in band intensity as well as gain or loss of stable RAPD bands (Atienzar and Jha, 2006).In the recent past, a number of attempts have been made to use the knowledge of molecular biology to reveal genetic damage. The randomly amplified polymorphic DNA (RAPD) analysis method developed by Williams *et al*. (1990) and Walsh and McClelland (1990) is one such method and it is simple,

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sensitive and effective in detecting genetic damages (Rong and Yin., 2004).

AIM AND OBJECTIVES

To assess the genotoxic effect of triazophos using RAPD analysis.To assess the apoptosis induced by the triazophos using DNA ladder method. *R*ecover of triazophos from exposed *Anabastestudineus* using fresh water medium.

MATERIALS AND METHODS

Test species - Fresh water fingerlings of *Anabas testudineus* of length 16cm±1cm and weight 72±1g were procured from the fish farm at kolathur, Chennai, Tamilnadu, South India. The collected fish sample were acclimated to laboratory conditions in dechlorinated tap water for 15 days. The fishes were fed with commercial fish feed during acclimation. Water quality - during the period of acclimatization and experimentation used wasmaintained asclear, dechlorinated ground water pumped from a deep well within the college campus. The physiochemical parameter of the water was assessed throughout the experiments as under.

Total dissolved Solids mg/l	: 1598
PH	: 7.06
Alkalinity pH (as $CaCO_3$) mg/l	: 0
Alkalinity Total (as $CaCO_3$) mg/l	: 228
Total Hardness (as $CaCO_3$) mg/l	: 520
Calcium (as Ca) mg/l	:124
Magnesium (as mg) mg/l	: 50
Iron (as Fe) mg/l	: 0.08
Free Ammonia (as NH_3) mg/l	: 0.31
Nitrite (as NO_2) mg/l	: 0.01
Nitrate (as NO_3)mg/l	: 3
Chloride (as Cl) mg/l	: 549

RAPD ANALYSIS (Material and Methods)

DNA isolation kit (Pure Fast® Bacterial Genomic DNA purification kit), PCR Master Mix, Agarose gel electrophoresis consumables and Primers purchased from HELINI Biomolecules, Vettuvangani, Chennai, and South India.2X Master Mix: It contains 2U of Taq DNA polymerase, 10X Taq reaction buffer, 2mM MgCl2,1µl of 10mm dNTPs mix and PCR additives.Agarose gel electrophoresis: Agarose, 50X TAE buffer, 6X gel loading buffer and Ethidium bromide are purchased from HELINI Biomolecules, Vettuvangani, Chennai Tamilnadu, Southindia.

RAPD PRIMERS

RAPD analysis primer 1 - 5'-GGTGCGGGAA-3'
RAPD analysis primer 2 - 5'-GTTTCGCTCC-3'
RAPD analysis primer 3 - 5'-GTAGACCCGT-3'

PROCEDURE

1. 20mg of tissue sample was taken in fresh 1.5ml tube.
2. 400µl of Lysis buffer and 40µl of Proteinase K [10mg/ml] is added and homogenized well using micro pestle.
3. Incubated in water bath at 70°C for 10 min.
4. Transferred whole lysate into Pure Fast spin column and centrifuged at 10000rpm for 1min.
5. Discarded the flow throughout and added 500µl of Wash Buffer and Centrifuge at 10000rpm 1min.
6. Discarded the flow throughout and added 500µl of Wash Buffer-2 and centrifuged at
10000rpm for 1min. and the process was repeated concurrently.
7. Discarded the flow through and Centrifuged using column for additional 2 minute to removeany residual ethanol.
8. Eluted DNA by adding 100µl of Elution Buffer and Centrifuged for 1min.
9. Quality and Quantity of extracted DNA is checked by loading in 1% agarose gel and 10µl of extracted DNA is used for PCR amplification.

PCR Procedure:

[25µl of Master Mix contains: 10X Taq buffer, 2mM Mgcl2, 0.4mM dNTPs mix, and 2U*Proofreading* Taq DNA polymerase]

1. Reactions set up as follows;

Components	Quantity
In PCR vial Master mix	25µl
RAPD primer [10pmoles/ul]	1µl
Genomic DNA	**1µl**
Water, nuclease free	23µl
Total volume	50µl

2. Mixed gently and spin down briefly

Place into PCR machine and program it as follows;

Initial Denaturation: 94°C for 3 min

Denaturation: 94°C for 1 min
Annealing: 37°C for 1min
Extension: 72°C for 2min
} 35 cycles

Final extension: 72° C for 5 min

Loading

1. Prepared 1.8% agarose gel. [1.8gm of agarose in 100ml of 1x TAE buffer]
2. Mixed 8µl 6X Gel loading dye to each PCR vial and loaded 35µl of PCR sample.
3. Run electrophoresis at 50V till the dye reached three fourth distances and observed the bands in UV Tran's illuminator.

DNA Ladder Assay- Agarose gel electrophoresis

1. Prepared 1.8% agarose. (1.8gm agarose in 100ml of 1X TAE buffer and melted usingmicro oven)
2. When the agarose gel temperature was around 60ºC, added 5µl of Ethidium bromide.
3. Poured warm agarose solution slowly into the gel platform.

4. Kept the gel set undisturbed till the agarose solidifies.
5. Poured 1XTAE buffer into submarine gel tank.
6. Carefully placed the gel platform into tank. Maintained the tank buffer level 0.5cm above than the gel.
7. PCR Samples was loaded after mixed with gel loading dye along with 10µl HELINI 100bp DNA Ladder and 1kb DNA Ladder.
8. Run electrophoresis at 50V till the dye reached three fourth distance of the gel.
9. Gel viewed in UV Tran's illuminator and observed the bands pattern.

RESULTS

RAPD ANALYSIS

In the present study the RAPD analysis was made in thegill tissues of fish *Anabas testudineus*from control and fishes exposed to triazophos for 3 days and followed by 3, 6 days of recovery in water. Three different 10 primers were used to study the genotoxic and effect of the pesticide.

The triazophos exposure showed variation and polymorphism in band patterns from that of the control samples. Minimum variation in polymorphism was observed in primer, gill samples (Figure 3&4). Maximum variation in DNA band polymorphism was observed in primer 1 gill sample. DNA band intensity variation also observed in the treated fishes compared to control samples (Figure 1 & 2).

Table:1 primer sequence and amplification product characterstic Y=Yes, N= No

Primer	Sequence (5→3)	Polymorphic
RAPD analysis primer 1	5'-GGTGCGGGAA-3'	Yes
RAPD analysis primer 2	5'-GTTTCGCTCC-3'	No
RAPD analysis primer 3	5'-GTAGACCCGT-3'	Yes

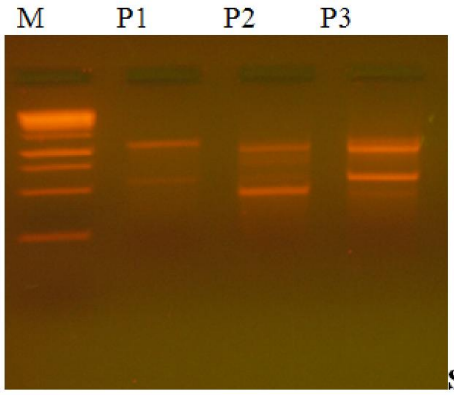

Figure 1. Control of the RAPD Analysis RAPD(P1)

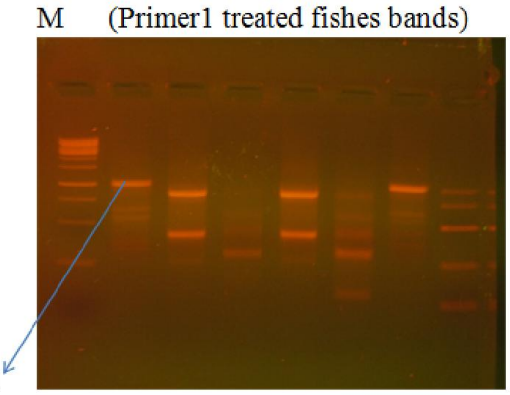

Figure 2. Experimental Analysis of the

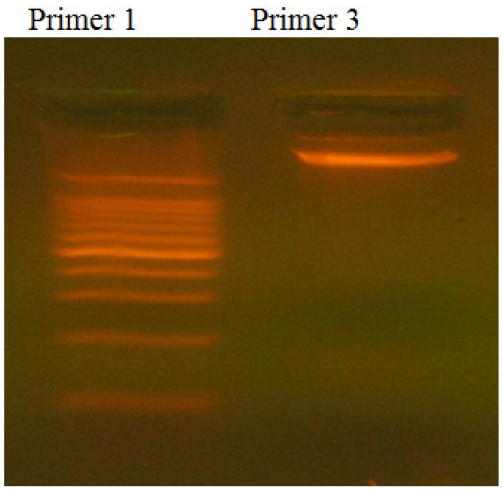

Figure 3. Control of the Genomic DNA (1)

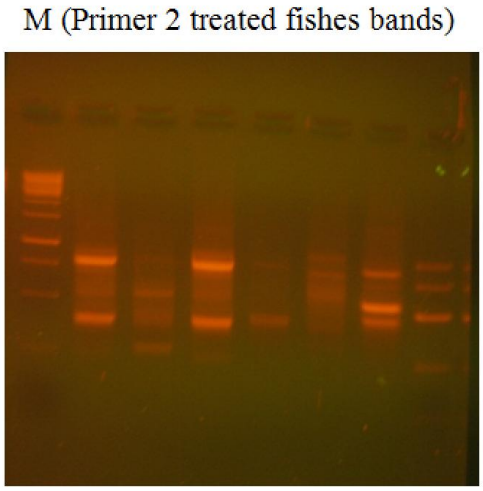

Figure 4. Experimental Analysis of the RAPD (P2)

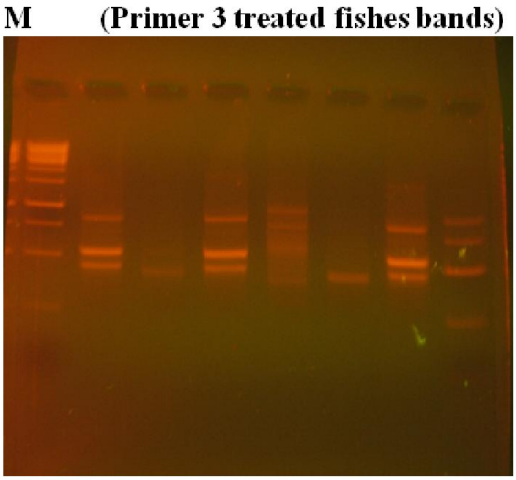

Figure 5. Experimental Analysis of the RAPD (P3)

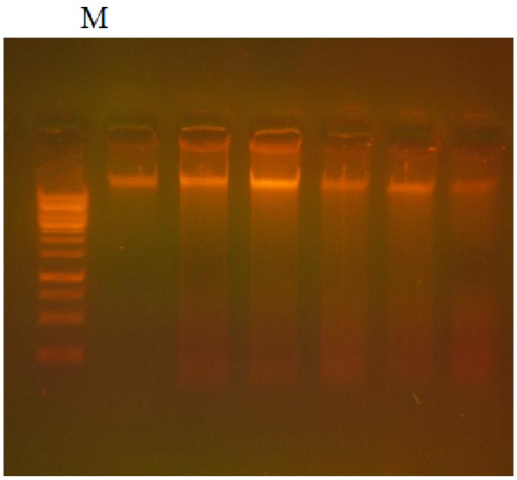

Figure 6. Experimental Analysis of the Genomic DNA (2)

DNA LADDER ASSAY

The control DNA isolated from the fishes showed intact and in high intensity in single band. The treated and recovery treatment fish revealed reduced intensity of the DNA in the gel, as well as it was determined that the lower molecular weight DNA in the gels. So it was confirmed that the apoptotic effect of the pesticide triazophos on genomic DNA of *Anabastestudineus*

DISCUSSION

In the present study the RAPD analysis confirmed treated gill tissue of *Anabas testudineus*exposed to triazophos for 3 days and followed by 3, 6 days of recovery in fresh medium of water. Gene indicated the genotoxic effect of triazophos. The triazophos exposure confirmed variation and polymorphism in band patterns from that of the control samples. Minimum variation in polymorphism was observed in primer 3, gill samples. Maximum variation in DNA band polymorphism was observed in primer 1 gill sampleand hence RAPD-PCR is an important and useful method in the field of ecotoxicology studies (De Wolf *et al.,* 2004). RAPD-PCR band analysis process takes into account band loss and/or gain as well as alterations in band intensities between exposed and nonexposed specimens (Atienzar and Jha 2006), which might have arisen due to DNA damage, mutations or structural rearrangements induced by genotoxic agents, affecting the primer sites and/or interrpriming distances (Atienzar and Jha., 2006). However, RAPD assay does not provide information on the nature and extent of these genotoxic induced DNA alterations, however it is often used in a quantitative way (Atienzar and Jha., 2006). Becirril*et al.,* 1999 and Castano and Berricil 2004 adopted RAPD-PCR technique to assess the effects of mitomycin C and benzo [a] pyrene on an established fish cell line RTG-2 respectively. Both qualitative and quantitative analysis showed an increase in the DNA template instability in the chemicals exposed cells in a time and concentration – dependent manner. They explained that since the chemical is directly actingangenotoxicants, so they bear the potentiality to attack the hot spots present in the DNA sequence directly and hence the number of new and stable bands are formed with the increase of the time and dose at the number of new stable bands obtained in the RAPD – PCR technique. Experiment showed both appearance of new bands and disappearance of existing bands in pesticide treated animals compared to control. The appearance of new bands or the disappearance of bands in all the three primers indicate a cleat ability of the pesticides to induce DNA damage in fish species.

Summary and Conclusion

The pesticide triazophos is found to be more toxic to the fish *Anabas testudineus*. The acute toxicity studies in *Anabas testudineus*at 96 hrs LC_{50} value for triazophosis was0.270 ppm. The histological investigations in *Anabas testudineus* exposed to Triazophos were found to be highly toxic and the histological alterations were increasing with increase in concentration and duration. The triazopohs exposure shows variation and polymorphism in band patterns from that of the control samples.The recovery studies of triazophos exposed to *Anabas testudineus* shows damages in tissues and recovery pattern was less when compared to Triazophos treated fishes.

REFERENCES

Atienzar, F. A. and Jha, A. N., 2006. The random amplified polymorphic DNA (RAPD) assay and related techniques alied to genotoxicity and carcinogenesis studies: A critical review. *Mutation Research,* 613: 76 – 102. doi:10.1016/j.mrrev.2006.06.001

Bacerril, C., Ferrero, M., Sanzx, F., Castano, A. 1999. Detection of mitomycin C induced genetic damage in fish cells by use of RAPD. Mutagenesis 14: 449 – 456.

Castano, A. and Becerril, C. 2004. In *vitro* assessment of DNA damage after short- and long-term exposure to benzo (a) pyrene using RAPD and the RTG-2 fish cell line. *Mutation Research,* 552: 141 – 151. doi:10.1016/j.mrfmmm.2004.06.010

De Wolf, H., Blust, R. and Backeljau, T. 2004. The population genetic structure of *Littorinalittorea* (Mollusca: Gastropoda) along a pollution gradient in the

Scheldt estuary (the Netherlands) using RAPD analysis. *Science of the total Environment,* 325: 59 – 69. Doi: 10.1016/j. Scitotenv. 2003.11.004.

Rong, Z. and Yin, H. 2004.A method for genotoxicity detection using random amplified polymorphism DNA with *Daniorerio*. Ecotoxicology and Environment safety 58: 96-103.

Walsh, J, and McClell and, M. 1990. Genomic fingerprinting using arbitrary primed PCR and a matrix of pairwise combination of primers. *Nucleic acids Research* 18:7213-18.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18: 6531-6535.

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