

*Dedicated to my Family*

# Declaration

I declare that the thesis entitled “**Genotoxic effects of fungicide(s) in the fish *Pethia conchonius* (Hamilton, 1822) from river Teesta of northern region of West Bengal**” has been prepared by me under the guidance of **Professor Min Bahadur, Department of Zoology, University of North Bengal**. No part of this thesis has previously formed the basis for the award of any degree or fellowship.

*Arpita Ray*  
**Arpita Ray**

**Department of Zoology  
University of North Bengal  
Raja Rammohunpur, Siliguri  
District: Darjeeling, West Bengal-734013  
India  
Date: 20<sup>th</sup> August, 2024**



## CERTIFICATE

This is to certify that **Ms. Arpita Ray** has prepared the thesis entitled “**Genotoxic effects of fungicide(s) in the fish *Pethia conchonius* (Hamilton, 1822) from river Teesta of northern region of West Bengal**” for the award of Ph.D. degree of the University of North Bengal, under my guidance. She has carried out the work at the Department of Zoology, University of North Bengal. The content of the thesis has not been submitted elsewhere for any degree.

Supervisor

Prof. Min Bahadur

Department of Zoology

University of North Bengal

Date: 20<sup>th</sup> August, 2024

Dr. Min Bahadur

Professor

Department of Zoology

University of North Bengal

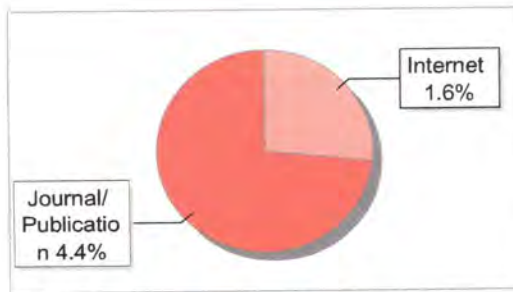
Siliguri-734013, West Bengal

### Submission Information

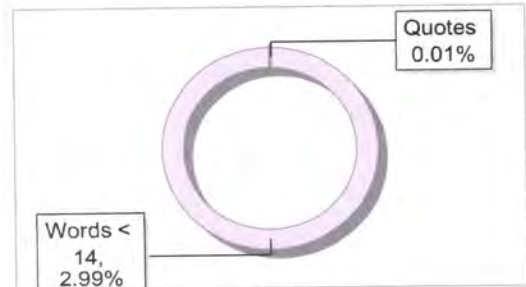
|                          |   |
|--------------------------|---|
| Author Name              | Arpita Ray  |
| Title                    | Genotoxic effects of fungicide(s) in the fish Pethia conchoniuss (Hamilton, 1822) from river Teesta of northern region of West Bengal |
| Paper/Submission ID      | 2225857   |
| Submitted by             | nbuplg@nbu.ac.in  |
| Submission Date          | 2024-08-13 11:19:02   |
| Total Pages, Total Words | 107, 34579  |
| Document type            | Thesis  |

### Result Information

Similarity **6 %**



Sources Type



Report Content

### Exclude Information

|                             |              |
|-----------------------------|--------------|
| Quotes                      | Excluded     |
| References/Bibliography     | Excluded     |
| Source: Excluded < 14 Words | Excluded     |
| Excluded Source             | <b>2 %</b>   |
| Excluded Phrases            | Not Excluded |

### Database Selection

|                        |         |
|------------------------|---------|
| Language               | English |
| Student Papers         | Yes     |
| Journals & publishers  | Yes     |
| Internet or Web        | Yes     |
| Institution Repository | Yes     |



*Arpita Ray* 20/08/24  
Signature of the Candidate

*Dr. Min Bahadur* 20.08.2024  
Signature of the Supervisor

**Dr. Min Bahadur**  
Professor  
Department of Zoology  
University of North Bengal  
Siliguri-734013, West Bengal

# Acknowledgment

I take this opportunity to express my deep sense of gratitude and indebtedness to those who helped me in carrying out this investigation.

My family have been a constant source of inspiration throughout my doctoral study, without their support and encouragement it was quite impossible to finish such hard work.

I am immensely indebted and sincerely grateful to my honourable supervisor Prof. Min Bahadur, Genetics and Molecular Biology Laboratory, Department of Zoology, University of North Bengal. His invaluable suggestions, guidance and supervision throughout my work help me to solve problems and to take correct decisions during hard times of my research work.

I am thankful to Prof. Dhiraj Saha, Head, Department of Zoology, University of North Bengal for ensuring the necessary facilities in the Department, particularly the instrumentation facility. I express my thanks to my respected teachers Prof. Soumen Bhattacharya, Dr. Tilak Saha (Associate Professor), Dr. Sourav Mukherjee (Assistant Professor), Dr. Arpan Kumar Maiti (Assistant Professor), Dr. Ritwik Mondal, Dr. Subhra Prakash Hui for their valuable guidance during my work.

I would like to acknowledge Dr. Debi Sharma, Dr. Partha Pratim Chowdhury, Food Safety Referral Laboratory, ICAR-IIHR, Bangalore for their untiring help for LC-MS/MS analysis.

I would like to sincerely acknowledge Council of Scientific and Industrial Research-Human Resource Development Group for providing financial support as a fellowship (CSIR-HRDG Vide File No. 09/285(0095)/2019-EMR-I).

I am grateful to Dr. Bappaditya Ghosh, Dr. Mahua Rudra and Mr. Goutam Debnath for their guidance and unconditional cooperation as my elder sister and elder brother, respectively.

I am also thankful to Dr. Susmita Dutta, Mrs Swati Singh, Mrs Trisita Mazumdar, Dr. Saugata Ghosh, Mr. Debojit Dutta for their untiring help and support throughout my work.

Special thanks will go to my colleagues Mr. Debojit Dutta, Mr. Sourav Sarkar, Mr. Debabrata Modak, Mr. Rejuan Islam and Mr. Subhajit Das.

I would like to thank Mr. Suman Biswas, Ms. Esha Bhattacharya, Mr. Joydip Nag and Ms. Aritri Sarkar for their immense help.

I would like to thank Mr. Ratan Sarkar (Fisherman), Badal Sarkar (Fisherman), Mr. Debojit dutta, Ms. Esha Bhattacharya, Mr. Suman Biswas, Mr. Joydip Nag for cooperating me during sample collection.

I would like to thank Mr. Praveen Kumar Mishra for his help in chemical supply.

I am thankful to Dr Sutanuka Chattaraj, Dr. Uttara Dey Bhowmick, Dr. Subhashis Paul, Mr. Tanmoy Dutta, Mr. Sudipto Kumar Roy, Dr. Minu Bharti, Dr. Priyanka Rai, Mr. Abhishek Subba, Mr. Manas Pratim Modak, Ms. Prerna Bhujel, Ms. Nilu Limboo, Mr. Abhirup Saha, Mr. Sagar Sarkar, Mr. Amlan Jyoti Ghosh, Mr. Supriyo Ghosh, Mr. Arijit Deb, Ms. Sashwati Ghosh, Ms. Farha Yasmin, Mr Sona Sutradhar, Mr. Arun Roy for extending their helping hands.

Thanks, are also due to the non-teaching staff Mr. Sudam Das, Mr. Samiran Debnath, Mr, Amit Kumar, and Mr. Tapan Das of the Dept. of Zoology for their assistance in departmental facilities.

I am also thankful to all administrative staff for their help during my Ph.D. work.

In the end, I thank all those who helped me or were associated with my work directly or indirectly.

*Arpita Ray*  
Arpita Ray  
Department of Zoology  
University of North Bengal  
Date: 20<sup>th</sup> August, 2024

## Preface

Agriculture is the back bone of India's economy, contributing around 17-18% to the GDP and employing about 50% of the workforce. Pesticides, including fungicides, are crucial for protecting crops from pests and diseases, enhancing productivity, minimizing crop losses, and maintaining produce quality. However, fungicides can run off into nearby water bodies, posing significant risks to aquatic ecosystems. This runoff leads to changes in the physicochemical properties of water, affects aquatic organisms, and causes biodiversity loss. Fungicides bioaccumulate in the aquatic organisms, affect trophic level of the entire food chain including birds and mammals. These hazardous chemicals can damage aquatic plants essential for ecosystem.

The Teesta River, a major river in West Bengal, originates from the Himalayas and flows through Kalimpong, Darjeeling, Jalpaiguri, and Cooch Behar in North Bengal. It is reported to be contaminated with several fungicides due to agricultural activities along its banks. To address the lack of information on the detrimental effects of fungicides on the aquatic organisms of Teesta, this study focuses on the resident fish *Pethia conchonius*. The research evaluates the genotoxic effects of the fungicides azoxystrobin and difenoconazole on *Pethia conchonius*.

The investigations stated in this thesis were conducted in the Genetics & Molecular Biology Laboratory, Department of Zoology, University of North Bengal. The thesis is comprised of five chapters. **Chapter 1** has an **Introduction**, a **Review of literature**, and **Objectives**. **Chapter 2** contains the **Materials and Methods** section of the entire study. **Chapter 3** presents the **Results** of the sampling, analysis of fungicide residue, acute toxicity bioassay, assessment of DNA and nuclear damages, study of biochemical changes, and study of gene expression in various tissues, and the others. **Chapter 4** includes a detailed **Discussion** of the findings obtained in the present investigation, leading to a **conclusion**. The **Research articles and papers presented during different conferences**, an **Appendix** are included in **Chapter 5**. In addition, the chapter also contains a **Bibliography** and **Index**.

## Abbreviations

|  |   |
|--|---|
| 4-HNE: 4-Hydroxynonenal                                | DMSO: Dimethyl sulfoxide  |
| AChE: Acetylcholinesterase                             | DNA: Deoxyribonucleic acid  |
| AOAC: Association of Official Analytical Collaboration | dNTP: Deoxynucleotide triphosphate  |
| apaf: apoptotic protease activating factor-1           | DPX: Distyrene Plasticizer Xylene   |
| APHA: American Public Health Association               | DT <sub>50</sub> : Dissipation time (Half-life)                           |
| AZX: Azoxystrobin                                      | DTNB: 5,5'-dithiobis-(2-nitrobenzoic acid)                                |
| Bax: bcl2 associated X protein                         | DZ: Difenconazole   |
| BChE: Butyrylcholinesterase                            | E2: 17 $\beta$ -estradiol   |
| Bcl2: b-cell lymphoma/leukemia-2                       | EBDC: Ethylenebisdithiocarbamate  |
| BL: Blebbed  | EC <sub>50</sub> : half maximal effective concentration                   |
| BLAST: Basic Local Alignment Search Tool               | EDTA: Ethylenediamine tetraacetic acid                                    |
| BSA: Bovine Serum Albumin                              | ENA: Erythrocytic nuclear abnormalities                                   |
| CAS: Chemical Abstracts Service                        | EPA: Environmental Protection Agency                                      |
| casp: Caspase  | EROD: 7-ethoxy-resorufin-O-deethylase                                     |
| CAT: Catalase  | ETU: Ethylenethiourea   |
| CBZ: Carbendazim                                       | FAO: Food and Agriculture Organization                                    |
| CDNB: 1-Chloro-2,4-dinitrobenzene                      | FAOSTAT: Food and Agriculture Organization Corporate Statistical Database |
| CHE: Cholinesterase                                    | FICCI: Federation of Indian Chambers of Commerce & Industry               |
| CYP: Cytochrome p40                                    |   |
| DMI: Demethylation inhibitor                           | g: gram   |

|  |  |
|--|--|
| G6PD: Glucose-6-phosphate dehydrogenase                  | LOOH: Lipid Hydroperoxide  |
| GDP: Gross Domestic Product                              | LPO: Lipid peroxide  |
| GOI: Government of India                                 | MAPK: Mitogen-activated protein kinase                           |
| GPx: Glutathione peroxidase                              | MDA: Malondialdehyde   |
| GR: Glutathione Reductase                                | Mdm2: Murine double minute 2                                     |
| GSH: Glutathione   | mg: milligram  |
| GSI: gonadosmotic index                                  | MMP: Mitochondrial Membrane Potential                            |
| GSSG: Glutathione disulfide                              | MN: Micronucleus   |
| GST: Glutathione S-transferase                           | NA: Nuclear Abnormality  |
| H <sub>2</sub> O <sub>2</sub> : Hydrogen peroxide        | NAC: N-acetylcysteine  |
| hsd: Hydroxysteroid dehydrogenases                       | NADH: Nicotinamide adenine dinucleotide (NAD) + hydrogen (H)     |
| IBM: International Business Machines Corporation         | NBT: Nitro blue Tetrazolium                                      |
| IGFBP: Insulin-like growth factor binding protein        | NCBI: National Center for Biotechnology Information              |
| ILO: International Labor Organization                    | ng: nanogram   |
| IUCN: International Union for Conservation of Nature     | NO: Notched  |
| IUPAC: International Union of Pure and Applied Chemistry | NRC: National Research Council                                   |
| L: liter   | OECD: The Organization for Economic Co-operation and Development |
| LC <sub>50</sub> : Median Lethal concentration           | OP: Organophosphate  |
| LCMS: Liquid Chromatography Mass Spectrometry            | OTM: Olive Tail Moment   |
| LDH: Lactate dehydrogenase                               | <i>p</i> : Probability value                                     |

PBS: Phosphate Buffered Saline

*PcDM*: *Pethia conchoni* specimens from site III (Domohoni)

*PcGB*: *Pethia conchoni* specimens from site II (Gajoldoba)

PCR: Polymerase Chain Reaction

*PcSV*: *Pethia conchoni* specimens from site I (Sevok)

PMS: Phenazine methosulfate

POD: Peroxidase

PUFAs: Polyunsaturated Fatty Acids

PYR: Pyraclostrobin

RNA: Ribonucleic acid

ROS: Reactive Oxygen Species

SCGE: Single Cell Gel Electrophoresis

SD: Standard Deviation

SE: Standard Error

SLC: Sub-lethal concentration

SOD: Superoxide Dismutase

SPSS: Statistical Package for the Social Sciences

TBARS: Thiobarbituric acid reactive substances

TBZ: Tebuconazole

TCA: Trichloroacetic acid

TNFR: Tumor necrosis factor receptors

USEPA: United States Environmental Protection Agency

USGS: United States Geological Survey

vtg: vitellogenin

WHO: World Health Organization

$\mu\text{g L}^{-1}$ : micrograms per liter

## List of Tables

|   |    |
|---|----|
| Table 1: Classification of fungicides and their mode of actions*.....   | 23 |
| Table 2: Occurrence of different groups of fungicides in the agricultural fields and water bodies around the world. ....                      | 27 |
| Table 3: Details of Azoxystrobin and Difenoconazole .....   | 55 |
| Table 4. List of primers, Accession No., References. ....   | 68 |
| Table 5: Concentration of detected fungicides in different seasons in different sites of Teesta. ..   | 74 |
| Table 6: Concentration of fungicides detected in the muscle of <i>Pethia conchoni</i> collected from river Teesta. ....                       | 75 |
| Table 7: Percent (%) mortality of fish <i>Pethia conchoni</i> at different concentrations of azoxystrobin. ....                               | 77 |
| Table 8: Percent (%) mortality of fish <i>Pethia conchoni</i> at different concentrations of difenoconazole. ....                             | 77 |
| Table 9: Bioaccumulation of azoxystrobin and difenoconazole in fish muscles after 96h of exposure. ....                                       | 79 |
| Table 10: Behavioral changes in the control and the azoxystrobin-treated <i>Pethia conchoni</i> at different exposure time and dose. ....     | 80 |
| Table 11 Behavioral changes in the control and the azoxystrobin treated <i>Pethia conchoni</i> at different exposure time and dose. ....      | 82 |
| Table 12: Effect of the three sub-lethal concentrations of azoxystrobin on the brain AChE activity (Mean±SD) in <i>Pethia conchoni</i> . .... | 89 |
| Table 13: Effect of the three sub-lethal concentrations of difenoconazole on brain AChE activity (Mean±SD) in <i>Pethia conchoni</i> . ....   | 91 |

|  |     |
|--|-----|
| Table 14: Effect of three sub-lethal concentrations of azoxystrobin on the CAT activity in brain tissue of <i>Pethia conchonius</i> at 96h of exposure. SLC= sub-lethal concentration.....                 | 92  |
| Table 15: Effect of three sub-lethal concentrations of difenoconazole on CAT activity in the gills and liver of <i>Pethia conchonius</i> at 96h of exposure (Mean±SD). SLC= sub-lethal concentration. .... | 93  |
| Table 16: Effect of three sub-lethal concentrations (SLC) of azoxystrobin on the SOD activity in gills and liver of <i>Pethia conchonius</i> at 96h exposure (Mean±SD). ....                               | 95  |
| Table 17: Effect of the three sub-lethal concentrations of difenoconazole on SOD activity in the gills and liver (Mean±SD) of <i>Pethia conchonius</i> at 96h of exposure. ....                            | 97  |
| Table 18: Effect of the three sub-lethal concentrations of azoxystrobin on the GST activity in the gills and liver of <i>Pethia conchonius</i> at 96h exposure (Mean±SD). ....                             | 98  |
| Table 19: Effect of three sub-lethal concentrations of difenoconazole on GST activity in the gills and liver of <i>Pethia conchonius</i> at 96h of exposure (Mean±SD).....                                 | 100 |
| Table 20: Effect of three sub-lethal concentrations of azoxystrobin on MDA level in the gill and liver tissues of <i>Pethia conchonius</i> at 96h of exposure (Mean±SD). ....                              | 101 |
| Table 21: Effect of three sub-lethal concentrations of difenoconazole on the MDA level in gills and liver of <i>Pethia conchonius</i> at 96h of exposure (Mean±SD).....                                    | 103 |
| Table 22: Frequency of different nuclear abnormalities in erythrocytes of <i>Pethia conchonius</i> exposed to different concentrations of azoxystrobin (Mean±SE).....                                      | 106 |
| Table 23: Frequency of different nuclear abnormalities in erythrocytes of <i>Pethia conchonius</i> exposed to different concentrations of difenoconazole (Mean±SE). ....                                   | 109 |
| Table 24: The %Head DNA, %Tail DNA, Tail Length and OTM in both control and azoxystrobin exposed <i>Pethia conchonius</i> for different sub-lethal concentrations and duration (Mean±SE). ....             | 113 |

|   |     |
|---|-----|
| Table 25: The %Head DNA, %Tail DNA, LTail and OTM in both control and difenoconazole exposed <i>Pethia conchonius</i> groups SLC I, II and III (Mean±SE). .....   | 117 |
| Table 26: Relative fold-change in the <i>cat</i> gene expression in gill and liver tissues of azoxystrobin-exposed <i>Pethia conchonius</i> for different doses and exposure durations (Mean±SE). ..... | 120 |
| Table 27: Relative fold change in <i>cat</i> mRNA in gills and liver tissues of difenoconazole-exposed <i>Pethia conchonius</i> for different doses and exposure durations (Mean±SE). .....             | 121 |
| Table 28: Relative fold change of <i>bcl2</i> mRNA in gill and liver tissues of azoxystrobin-exposed <i>Pethia conchonius</i> for different doses and exposure durations (Mean±SE). .....               | 123 |
| Table 29: Relative change of <i>bcl2</i> expression in gill and liver tissues of <i>Pethia conchonius</i> exposed to difenoconazole for different doses and exposure durations (Mean±SE). .....         | 125 |
| Table 30: Experimental fish populations collected from different sites of river Teesta showing location and designation of population. ....   | 127 |
| Table 31: Percent (%) head DNA, tail DNA, and tail length (L tail) in gill tissues of Upstream and Downstream specimens of <i>Pethia conchonius</i> in the river Teesta (Mean ± SE).....                | 127 |
| Table 32: Frequency of MN, NO and BL in the erythrocytes of the Upstream and Downstream fish specimens (population) of <i>Pethia conchonius</i> (Mean ± SE).....  | 128 |
| Table 33: CAT, GST, SOD activities and MDA level in the liver and gill tissues and AChE activity in brain tissue of PcSV, PcGB and PcDM collected from different sites of river Teesta (Mean±SD).....   | 131 |
| Table 34: The <i>cat</i> and <i>bcl2</i> mRNA transcripts in the gill and liver tissues of PcSV, PcGB and PcDM collected from different sites of river Teesta (Mean±SD). .....                          | 133 |

## List of Figures

|   |    |
|---|----|
| Figure 1: Schematic diagram of micronucleus (MN) and other nuclear abnormalities (NA) formation (Canedo et al., 2021) .....   | 36 |
| Figure 2: Map of the study area .....   | 50 |
| Figure 3: Photographs of the fish collection .....  | 51 |
| Figure 4: Photographs of fishes from river Teesta (a) <i>Puntius sarana</i> , (b) <i>Xenontodon cancella</i> , (c) <i>Rasbora daniconius</i> , (d) <i>Pethia conchoni</i> .....   | 52 |
| Figure 5: Photograph of the experimental fish <i>Pethia conchoni</i> .....  | 53 |
| Figure 6: Photographs of the survey and water collection.....   | 54 |
| Figure 7: Photograph of the experimental setup in the laboratory. ....  | 57 |
| Figure 8: Report of the fish sample identification. ....  | 73 |
| Figure 9: Fungicides detected in different seasons in different sites of Teesta.....  | 75 |
| Figure 10: Regression curve of probit kill of <i>Pethia conchoni</i> and log of concentration of Azoxystrobin.....  | 78 |
| Figure 11: Regression curve of probit kill of <i>Pethia conchoni</i> and log of concentration of difenoconazole. ....   | 78 |
| Figure 12: Behavioral changes in the control and azoxystrobin-exposed <i>Pethia conchoni</i> : (a) normal swimming in control; (b) downward swimming; (c) loss of equilibrium; (d) bottom aggregation in azoxystrobin-exposed fish.....   | 81 |
| Figure 13: Behavioral changes in the control and azoxystrobin-exposed <i>Pethia conchoni</i> : (a) normal swimming in control; (b) loss of equilibrium; (c) bottom aggregation; (d) downward swimming in difenoconazole-exposed fish..... | 82 |

Figure 14: Normal gill histoarchitecture with compact primary and secondary gill lamellae of *Pethia conchonius*. ‘PI’= primary gill lamellae, SI= secondary gill lamellae. Bar=50µm (H-E staining, magnification 40X).....83

Figure 15: Histological alteration in the azoxystrobin-exposed *Pethia conchonius*: SLCI (a-d) , SLCII (e-h) and SLCIII (i-l). Hy=epithelial hyperplasia, El=epithelial lifting, Fu=fusion of secondary lamellae, De=degeneration of secondary lamellae, Cu=curling of secondary lamellae, Th=thinning of secondary lamellae, La=lacunae formation, Bar=50µm. (H-E staining, magnification 40X). .....84

Figure 16: Histological alteration in the difenoconazole-exposed *Pethia conchonius*: SLCI (a-d) , SLCII (e-h) and SLCIII (i-l). Hy=epithelial hyperplasia, El=epithelial lifting, Fu=fusion of secondary lamellae, De=degeneration of secondary lamellae, Cu=curling of secondary lamellae, Th=thinning of secondary lamellae, An=aneurism. Bar=50µm. (H-E staining, magnification 40X). .....85

Figure 17: Normal architecture of Optic tectum in control group. ....86

Figure 18: Alteration in brain histology of *Pethia conchonius* exposed to difenoconazole (a-c) and azoxystrobin (d-f). .....87

Figure 19: Effects of the three sub-lethal concentrations of azoxystrobin on brain AChE activity in *Pethia conchonius*. .....90

Figure 20: Effects of the three sub-lethal concentrations of difenoconazole on brain AChE activity in *Pethia conchonius*, SLC= sub-lethal concentration.....91

Figure 21: Effects of azoxystrobin and difenoconazole on CAT activity in gills and liver of *Pethia conchonius*; effects of azoxystrobin on gill (a), liver (b) and difenoconazole on gill (c) liver (d) SLC= sub-lethal concentration.....94

Figure 22: Effects of azoxystrobin and difenoconazole on SOD activity in gills and liver of *Pethia conchonius*; effects of azoxystrobin on gills (a), liver (b) and difenoconazole on gills (c) liver (d). .....96

Figure 23: Effects of the three sub-lethal concentrations of azoxystrobin on GST activity (a) gill (b) liver and difenoconazole (a) gill (b) liver of *Pethia conchoni*.....99

Figure 24: Effects of the three sub-lethal concentrations of azoxystrobin (a) gills (b) liver and difenoconazole (a) gills (b) liver on MDA level in *Pethia conchoni*. ..... 102

Figure 25: Photomicrographs showing normal cell in control (a), blebbed nuclei (b), notched nuclei (c), binucleated cell (d), micronucleated cell (e) and karyorrehctic nucleus (f) in RBCs of AZX exposed *Pethia conchoni*..... 105

Figure 26: Percent nuclear damage in the *Pethia conchoni* erythrocytes (a) Micronuclei, (b) Notched nuclei, (c) Blebbed nuclei of control and azoxystrobin-exposed specimens for different duration and concentrations. .... 107

Figure 27: Photomicrographs showing normal cell in control (a), micronuclei (b), blebbed nuclei (c), notched nuclei (d) in RBCs of difenoconazole exposed *Pethia conchoni*. ..... 109

Figure 28: Percent nuclear damage in the *Pethia conchoni* erythrocytes (a) Micronuclei, (b) Notched nuclei, (c) Blebbed nuclei of control and difenoconazole exposed specimens for different duration and concentrations..... 110

Figure 29: Photomicrographs showing gill cells of *Pethia conchoni*. Normal cells in control group (a, e, i, m) and comet in SLC I (b, f, j, n), SLC II (c, g, k, o) and SLC III (d, h, l, p). ..... 112

Figure 30: DNA damage in gill cells of *Pethia conchoni*: % head DNA (a), % tail DNA (b), tail length (c) and OTM (d) of control and exposed specimens in response to duration and concentrations of azoxystrobin. .... 114

Figure 31: Photomicrographs showing gill cells of *Pethia conchoni*. Normal cells in control group (a, b, c, d) and comet in SLC I (e, f, g, h), SLC II (I, j, k, l) and SLC III (m, n, o, p). ..... 116

Figure 32: DNA damage in gill cells of *Pethia conchoni*: % head DNA (a), % tail DNA (b), tail length (c) and OTM (d) of control and exposed specimens in response to duration and concentrations of difenoconazole..... 118

|   |     |
|---|-----|
| Figure 33: Amplification curve of <i>cat</i> , <i>bcl2</i> and <i>beta</i> actin gene. ....   | 119 |
| Figure 34: Melting curve of <i>cat</i> , <i>bcl2</i> and beta actin gene. ....  | 119 |
| Figure 35: Effects of the three sub-lethal concentrations of azoxystrobin (a) gill (b) liver and difenoconazole (a) gill (b) liver on <i>cat</i> gene expression in <i>Pethia conchoni</i> us. ....   | 122 |
| Figure 36: Effects of the three sub-lethal concentrations of azoxystrobin (a) gill (b) liver and difenoconazole (a) gill (b) liver on <i>bcl2</i> gene expression level in <i>Pethia conchoni</i> us. ....                                  | 126 |
| Figure 37: Percent (%) head DNA, tail DNA, and tail length (L tail) in gill tissues of Upstream and Downstream specimens of <i>Pethia conchoni</i> us in the river Teesta. ....   | 128 |
| Figure 38: Frequency of MN, NO & BL in the erythrocytes of the Upstream and Downstream fish specimens (population) of <i>Pethia conchoni</i> us. ....   | 129 |
| Figure 39: Brain AChE activity in <i>Pethia conchoni</i> us collected from different sites of river Teesta. ....  | 129 |
| Figure 40: CAT, GST, SOD activities and MDA level in the gills (a and b) and liver (c and d) a and AChE activity in brain tissue of <i>PcSV</i> , <i>PcGB</i> and <i>PcDM</i> collected from different sites of river Teesta (Mean±SD) .... | 132 |
| Figure 41: The <i>cat</i> and <i>bcl2</i> mRNA transcripts in the liver and gill tissues of <i>PcSV</i> , <i>PcGB</i> and <i>PcDM</i> collected from different sites of river Teesta (a) gill and (b) liver. ....                           | 134 |