Dedicated to my Parents

Declaration

I declare that the thesis entitled "**Molecular and biochemical assessment of agricultural pesticide(s)-related toxicity in *Pethia conchonius* (Hamilton, 1822), a resident fish of the river Teesta in the 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 formed the basis for the award of any degree or fellowship previously.

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CERTIFICATE

This is to certify that **Mr. Debojit Dutta** has prepared the thesis entitled "**Molecular and biochemical assessment of agricultural pesticide(s)-related toxicity in *Pethia conchonius* (Hamilton, 1822), a resident fish of the river Teesta in the northern region of West Bengal**" for the award of Ph.D. degree of the University of North Bengal, under my guidance. He 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.

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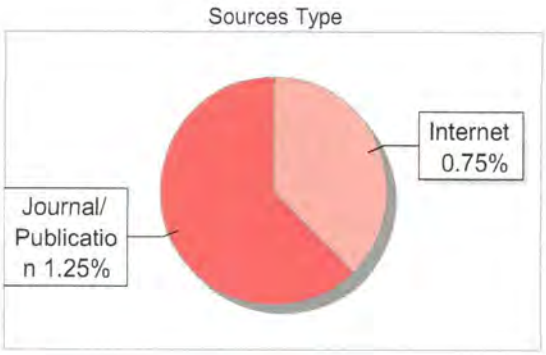

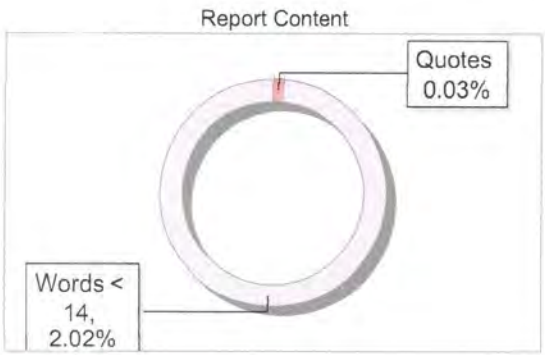

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Debajit Dutta

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Preface

The effects of agricultural pesticides on aquatic ecosystems have emerged as a major source of concern. The inappropriate use of pesticides in agricultural fields to control pest populations has adversely affected the aquatic bodies due to chemical runoff via air and water. Pesticide bioaccumulation endangers fish survival by altering the aquatic environment. The Teesta, which runs through the northern area of West Bengal, provides a lifeline for locals. Its tributaries are subjected to increasing human interference due to fast population increase, untreated sewage discharge, and the indiscriminate use of pesticides and fertilizers in agriculture, all of which have affected water chemistry. *Pethia conchonius*, often known as the Rosy Barb, is a resilient and ecologically valuable fish species in this complex ecosystem.

This study evaluates the **effects of agricultural pesticides, imidacloprid, and cypermethrin on *Pethia conchonius*, a fish species found in the River Teesta in the northern part of West Bengal**. The aim is to assess the genotoxicity caused by these insecticides. A significant quantity of pesticides from agricultural fields consistently contaminates river water through surface run-off, causing detrimental effects on non-target creatures, particularly fish commonly consumed by humans. The data generated on the effects of harmful pesticides, such as **imidacloprid and cypermethrin**, on fish in the Teesta River will raise awareness about the importance of minimizing chemical insecticides and refraining from consuming contaminated fish. This precaution is crucial as it can prevent serious health risks in near future. In this context, a research plan has been designed to investigate the genotoxic effects of these pesticides on the vertebrate system under both natural and laboratory conditions to evaluate the harmful consequences of synthetic insecticides. This study has been conducted in the Genetics & Molecular Biology Laboratory, Department of Zoology, University of North Bengal. The thesis is comprised of five chapters. **Introduction, Review of literature**, and **Objectives are included in Chapter 1**. **Chapter 2** encompasses the **Materials and Methods** used in the study. **Chapter 3** embodies the **Results** of the entire study, such as sampling, analysis of pesticide residue, acute toxicity bioassay, assessment of DNA and nuclear damage, biochemical changes, and the gene expression in various tissues. **Chapter 4** provides a detailed **Discussion** on the findings and a **conclusion**. **Chapter 5** includes **Appendix** consisting Publications, **list of Seminars and conferences** attended, and an elaborate **Bibliography** and **Index**.

Abbreviations

FAO: Food and Agriculture Organization

EPA: Environmental Protection Agency

GOI: Government of India

USD: United States Dollars

kg/ha: kilograms per hectare

WHO: World Health Organization

UTs: Union Territories

GDP: Gross Domestic Product

US: United States

mm: million

µg: microgram

µL: microliter

LD_{50}: Median Lethal Dose

LC_{50}: Median Lethal Concentration

ROS: Reactive Oxygen Species

ATPs: Adenosine Triphosphatases

AChE: Acetylcholinesterase

SCGE: Single-Cell Gel Electrophoresis (Comet Assay)

MN/MNi: Micronuclei

DNA: Deoxyribonucleic Acid

RNA: Ribonucleic Acid

ENVIS: Environmental Information System

EROD: 7-ethoxyresorufin-O-deethylase

GST: Glutathione-S-transferase

SOD: Superoxide Dismutase

CAT: Catalase

GSH: Glutathione

MDA: Malondialdehyde

L: liter

NGRBA: National Ganga River Basin Authority

HCH: Hexachlorocyclohexane

DDT: Dichlorodiphenyltrichloroethane

PCB: Polychlorinated Biphenyls

OCP: Organochlorine Pesticides

CPCB: Central Pollution Control Board

FID: Flame Ionization Detector

µg L^{-1}: micrograms per liter

ng: nanogram

g: gram

mg: milligram

IMI: Imidacloprid

CP: Cypermehrin

ACT: Acetochlor

TBZ: Tebuconazole

AO: Acridine-orange

ppm: Parts Per Million

pH: Potential of Hydrogen

NaAsO2: Sodium Arsenite

MMS: Methyl Methanesulfonate

PUFAs: Polyunsaturated Fatty Acids

LOOH: Lipid Hydroperoxide

LPO: Lipid Peroxidation

MDA: Malondialdehyde

ENAs: Erythrocytic Nuclear Abnormalities

DMP: Dimethyl Phthalate

TBARS: Thiobarbituric Acid Reactive Substances

ATPase: Adenosine Triphosphatase

nAChRs: Nicotinic Acetylcholine Receptors

ACh: Acetylcholine

THM: Thiamethoxam

AChE: Acetylcholinesterase

***GABA*:** γ-aminobutyric acid

***fosab*:** FOS homolog

***glsa*:** Glutaminase A

***grin1b*:** Glutamate Ionotropic Receptor 1b

gad1b: Glutamic Acid Decarboxylase

gad2: Glutamic Acid Decarboxylase 2

gabrg2: Gamma-aminobutyric Acid Receptor Gamma 2

***gabra1*:** Gamma-aminobutyric Acid Receptor Alpha 1

***slc6a1a*:** Solute Carrier Gene Family 6 Member 1a

***slc6a1b*:** Solute Carrier Gene Family 6 Member 1b

abat: Aminobutyrate Aminotransferase

RNS: Reactive Nitrogen Species

HO•: Hydroxyl radicals

O2•-: Superoxide anions

HOO-: Perhydroxy radicals

NO: Nitric oxide

DMP: Dimethyl Phthalate

GR: Glutathione Reductase

il-1β: Interleukin-1 beta

il-8: Interleukin-8

il-10: Interleukin-10

tnf-α: Tumor Necrosis Factor-alpha

ifn-γ: Interferon-gamma

HSP70: Heat Shock Protein 70

IGF-I: Insulin-like Growth Factor I

IGF-II: Insulin-like Growth Factor II

CYP450: Cytochrome P450

ZSI: Zoological Survey of India

PBS: Phosphate Buffered Saline

***Pc*SV:** *Pethia conchonius* specimens from site I (Sevok)

***Pc*GB:** *Pethia conchonius* specimens from site II (Gajoldoba)

***Pc*DM:** *Pethia conchonius* specimens from site III (Domohoni)

$KMnO_4$: Potassium permanganate

OECD: Organisation for Economic Co-operation and Development

LC-MS/MS: Liquid Chromatography-Tandem Mass Spectrometry

GC-MS/MS: Gas Chromatography-Tandem Mass Spectrometry

SD: Standard Deviation

AOAC: Association of Analytical Communities

TQ8040: Model of triple quadrupole mass spectrometer manufactured by Shimadzu Corp., Japan

HPLC: High-Performance Liquid Chromatography

RT-qPCR: Reverse Transcription quantitative Polymerase Chain Reaction

IUPAC: International Union of Pure and Applied Chemistry

CAS: Chemical Abstracts Service

APHA-AWWA-WPCF: American Public Health Association-American Water Works Association-Water Environment Federation

SPSS: Statistical Package for the Social Sciences

IBM: International Business Machines Corporation

***p*:** Probability value

Welch correction: A statistical method to adjust for unequal variances

SEM: Standard Error of the Mean

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List of Tables