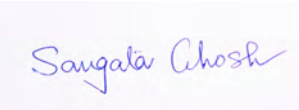
Dedicated to

all my respected teachers

DECLARATION

I declare that the thesis entitled 'A STUDY ON THE DEFENSE SYSTEM OF THE TEA PEST *Hyposidra talaca* (WALKER, 1860) (LEPIDOPTERA: GEOMETRIDAE) AGAINST CHEMICAL PESTICIDES AND ENTOMOPATHOGENS' has been prepared by me under the supervision of Professor (Dr.) 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. Saugata Ghosh has prepared the thesis entitled '**A STUDY ON THE DEFENSE SYSTEM OF THE TEA PEST *Hyposidra talaca* (Walker, 1860) (Lepidoptera: Geometridae) AGAINST CHEMICAL PESTICIDES AND ENTOMOPATHOGENS**' 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.

Supervisor

15/05/2023

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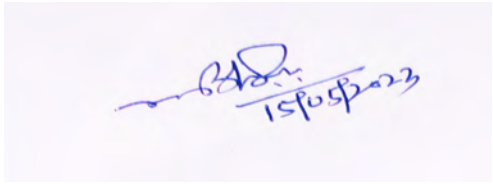

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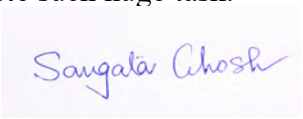

University of North Bengal — *Saugata Ghosh*

Raja Rammohunpur, Siliguri — 15th May, 2023

Preface

Insects, the most successful group of animals colonize almost all ecological niches and are responsible for massive crop-damage leading to huge loss in world economy and human livelihood. To manage the variety of insect pests, an array of chemical pesticides has long been used worldwide. To cope with these chemicals, as well as naturally occurring entomopathogens, insects have evolved an effective defense system. Indiscriminate use of the synthetic pesticides facilitates the development of higher tolerance or resistance against these chemicals in many insect pests leading to frequent failure in the pest control strategies. Pesticide resistance is a most serious problem throughout the world including India. In this situation, the in-depth knowledge about the defense strategy of various insect pests makes it possible to develop a target specific control management against the pests in a sustainable way.

The lepidopteran geometrid looper pest, *Hyposidra talaca* causes immense defoliation in the tea plantations of Darjeeling Terai region resulting in huge economic loss and so to manage this major tea pest, several chemical pesticides are regularly being used indiscriminately leading to development of the resistance of the pests against the pesticides as well as causing environmental and health problems. Therefore, the present study has been planned to investigate the cellular and humoral components of the defense system and the defense enzymes of *Hyposidra talaca* in the Darjeeling Terai region of West Bengal, India to assess the effects of the selected chemical pesticides and entomopathogens on *H. talaca.* This is a pioneer study which has focused on some aspects of the defense system of this geometrid tea pest for the first time. In this context, the knowledge of defense system of this pest along with the immune response against the regularly used chemical pesticides will help the plant growers for selective spraying of the pesticide instead of 'no threshold' spraying throughout the calendar year. Moreover, the information on the effect of the potential entomopathogens on the host's defense system and the subsequent immune response will help in the development of efficient bio-pesticides using those entomopathogens.

The **first part** of this thesis consists of an **introduction** of the Indian tea in global perspective, *Hyposidra talaca* as one of the major tea pests in Darjeeling Terai region of northern part of West Bengal, management strategy against *H. talaca* and the

defense system of insects in general. The **second part** is focused on the **review of literature**, from the multidimensional research areas on the insect defense system and immune responses to chemical pesticides and entomopathogens and different aspects of *H. talaca* and related species including, taxonomy, ecology, pest status, control strategies. The **third and fourth sections** contain the major **objectives** of this research work and detailed **materials and methods**, respectively for fulfilling the objectives of this study. An elaborate **results and discussions** have been included in the **fifth section** comprising development of *Hyposidra talaca*, haemocyte count in respect to diet regimes, chemical pesticides (emamectin benzoate and cypermethrin) and entomopathogens (*Bacillus thuringiensis* and nucleopolyhedrovirus) at different developmental stages (ages), detoxifying enzyme assays for general esterase, cytochrome P450 and glutathione S-transferase and electrophoretic analysis of haemolymph proteins/peptides. Finally, a **conclusion** has been added in the thesis mentioning future prospects of this study and a **summary** for quick understanding. An elaborate **bibliography** has been included with all the references cited in the text. There is also an **appendix** consisting of index, publications and list of seminars/symposia attended and presented the papers.

The outcome of this dissertation is expected to be helpful to the tea growers in optimizing the pest management strategy to control *Hyposidra talaca,* the major tea pest in the Darjeeling Terai plantations in the sub-Himalayan region of West Bengal, India without any adverse effect to the non-target populations and environment and opens up a multidisciplinary arena for future research.

SAUGATA GHOSH 15th May, 2023

ABBREVIATION

°C	Degree Centigrade
µl	Micro litre
µmol	Micro mole
20-E	20-hydroxyecdysone
AChE	Acetylcholine Esterase
ACP	Acid phosphatase
AD	Artificial diet
AH	Adipohaemocyte
ALP	Alkaline phosphatase
AMP	Anti-microbial peptide
ANOVA	Analysis of variance
ApoIII	Apolipophorin III
ApoLp	Apolipophorin
BOD	Biological oxygen demand
BSA	Bovine serum albumin
Bt	*Bacillus thuringiensis*
Btk	*Bacillus thuringiensis* var. *kurstaki*
BV	Budded Virion
C	Control
CDNB	1-chloro-2, 4-dinitrobenzene
CO	Coagulocyte
COVID 19	Corona Virus Disease 2019
CP	Cypermethrin
Cry	Crystal
CYP	Cytochrome P450
Cyt	Cytolytic
D	Day
DDT	Dichloro-diphenyl-trichloroethane
DHC	Differential haemocyte count
DPX	Distyrene, plasticizer and xylene
DOPA	3,4-dihydroxyphenyl alanine
DOPAC	3,4-dihydroxyphenyl acetic acid
EB	Emamectin benzoate
EC	Emulsifiable Concentrate
***Ecob*NPV**	*Ectropis obliqua* nucleopolyhedro virus
F	F-distribution value used in ANOVA
FBs	Fibrillar Bodies
GABA	γ-Aminobutyric Acid
GE	General Esterase
GI	Geographical indicator
GluCl	Glutamate-gated chloride channel
GR	Granulocyte
GSH	Reduced glutathione
GST	Glutathione S-Transferase
H	Test statistic for the Kruskal-Wallis test
hrs	Hours
***Hyta*NPV**	*Hyposidra talaca* nucleopolyhedrovirus
***Hz*NPV**	*Heliothis zea* single capsid nucleopolyhedrovirus
IRAC	Insecticide Resistance Action Committee
JH	Juvenile hormone
kDa	Kilo Dalton
Kg	Kilogram

L	Litre
LC_{50}	Mid-lethal concentration
L:D	Light: Dark
Lp	Lipophorin
LPS	Lipopolysaccharide
LTA	Lipoteichoic acid
max.	Mximum
min	Minute
ml	Mili litre
mM	Mili mole
MNPV	Multiple Nucleopolyhedrovirus
MOA	Mode of action
MW	Molecular weight
MW U	Value used in Mann-Whitney U statistics
n	Number of individuals
NADA	N-acetyl dopamine
NBAD	N-β-alanyl dopamine
NBU	North Bengal University
ND	Natural diet
nm	Nano meter
nmol	Nano mole
NPV	Nucleopolyhedrovirus
OB	Occlusion body
ODV	Occlusion-derived virion
OE	Oenocytoid
OP	Organophosphate
p	Probability that the null hypothesis is true
PAGE	Polyacrylamide gel electrophoresis
PAMP	Pathogen-associated molecular pattern
PBS	Phosphate buffered saline
PC	Podocyte
PL	Plasmatocyte
PO	Phenoloxidase
PPO	Pro-phenoloxidase
PR	Prohaemocyte
RH	Relative humidity
rpm	Rotation per minute
RNA	Ribonucleic acid
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SG	Water soluble granules
SP	Storage protein
SpliMNPV	*Spodoptera littoralis* multiple nucleopoly-hedrovirus
spp.	Species in plural
t	Value used in a t-test to determine whether to support or reject the null hypothesis
THC	Total haemocyte count
TMBZ	3,3', 5, 5' Tetra methyl benzidine
TRA	Tea research association
TV	Tocklai variant
UV/VIS	Ultra violet/ visible
var.	varietas (Latin)
VE	Vermicyte
VS	Virogenic Stroma

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