

## **Declaration**

I, **Tanushree Sarkar** hereby declare that the work embodied in my thesis entitled “**Studies on resistance of *Trichosanthes dioica* and their induction with chemical inducers against fungal pathogen**” has been carried out by me under the supervision of **Dr. Aniruddha Saha**, Professor, Department of Botany, University of North Bengal for the award of the Degree of Doctor of Philosophy in Botany. I also declare that, this thesis or any part thereof has not been submitted for any other degree/Diploma either to this or other university.



(Tanushree Sarkar)

Date: 22.8.2021

Place: Department of Botany,  
University of North Bengal  
Siliguri- 734 013



# Department of Botany

## UNIVERSITY OF NORTH BENGAL

Accredited by NAAC with Grade 'A'

ENLIGHTENMENT TO PERFECTION

**Professor Aniruddha  
Saha**

M.Sc. (Gold Med.)  
Ph.D.,  
FNRS

P.O. NBU, West Bengal, India  
(734013)

Contact: +91-8617361286  
Email: asahanbu@yahoo.co.in

---

### TO WHOM IT MAY CONCERN

This is to certify that the thesis entitled, “ **Studies on resistance of *Trichosanthes dioica* and their induction with chemical inducers against fungal pathogen**” submitted by Miss. Tanushree Sarkar for the award of the degree of Doctor of Philosophy in Botany is based on the results of experiments carried out by her. She has worked under my supervision at Department of Botany, University of North Bengal. I am forwarding her thesis for the Ph. D. degree (Science) in Botany of the University of North Bengal. She has fulfilled all requirements according to the rules of the University of North Bengal regarding the works embodied in her thesis. This thesis or any part thereof has not been submitted for any other degree/Diploma either to this or other University.

*Aniruddha Saha*

(Aniruddha Saha)

Supervisor

**Dr. Aniruddha Saha**  
Professor  
Department of Botany  
University of North Bengal

## **Acknowledgement**

*I have much pleasure in expressing my deepest sense of gratitude to my supervisor Dr. Aniruddha Saha, Professor, Department of Botany, University of North Bengal for his untiring guidance, help and involvement throughout the course of my academic endeavors. His incredible availability and pertinent comments largely improved the quality of the work. I never forget the numerous and passionate hours of discussion on and beyond my study topics with him and his confidence in me. I sincerely cannot express in words, my gratefulness to his tremendous support and able guidance throughout the course of this research work. I would like to thank Dr. Dipanwita Saha, Professor, Department of Biotechnology, University of North Bengal, for her inspiration, encouragement and guidance. I am grateful for her constructive suggestions which immensely helped me to sail through my academic endeavour successfully.*

*I would like to express my heartiest gratitude to Dr. M. Chowdhury, Head, Department of Botany, University of North Bengal, Prof. A. Sen, Prof. S.C. Roy, Dr. P. Mandal, Dr. J. B. Bhandari, Dr. S. Roy and Dr. P. Mathur, the faculties of our Department for their valuable advice and encouragement throughout the course of this work. My thanks also go for all the non-teaching staff of the Department of Botany for their various kinds of assistance.*

*I would like to thank Dr. Subrata Raha, Associate Professor, HOD Department of Botany, Sidho Kanho Birsha University for his valuable advice and support in my research work.*

*I am also grateful to my lab mates cum my siblings Dr. Shibu Das, Dr. Arnab Saha, Dr. Prosenjit Chakraborty, Mr. Arup Karmakar, Mr. Hrisikesh Mandal, Dr. Bikram Saha, Mrs. Piyali Sarkar, Mrs. Preeti Mangar, Ms. Smiriti Pradhan, Mr. Asit Ray, Ms. Suyojna Tamang, Mr. Biswajit Paul, Ms. Ankita Roy, Mrs. Ritabrita Saha, Mr. Kalyan Roy, Ms. Enakshi Sadhu, Mr. Subhrajyoti Saha and Mr. Niloy Roy for their continuous support in various aspect of my research work.*

*I am very thankful to my roommate cum sister, Dr. Minu Bharati for taking care of me and providing support whenever needed.*

*My grateful acknowledgement is also due to Mr. Priyankar Roy for his help and support.*

*I also want to extend my grateful acknowledgement to my friends Mr. Arghya Chatterjee, Ms. Moitri Bhadra, Dr. Payel Paul, Mr. Dipayan Das, Mr. Indranjit Singha, and Ms. Puja Shashankar) during the course of study for their constant support and encouragement.*

*I gratefully acknowledge the help and support that I received from Ms. Tuyelee Das, Ms. Ankita Biswas, Ms. Basanti Majumdar, Ms. Mitasha Karmakar, Ms. Moumita Chakraborty and Mr. Praveen Mandal.*

*I would like to acknowledge UGC-BSR (University Grants Commission – Basic Scientific Research) New Delhi for their financial support in the form of fellowship for pursuing this work otherwise which would not have been possible.*

*I would like to acknowledge Bioserve sequencing service and Mr. Sandipan Chakraborty for their co-operations.*

*I gratefully acknowledge Dr. Manish Sharma for taking care of me and his constant motivation.*

*Special thanks to Gurudev, Mrs. Shilpa Bhanot madam and all the member of my Art of Living family.*

*It would not be possible for me to accomplish my Ph. D work without the constant love, affection and encouragement of my beloved parents (Mr. Bhajan Sarkar and Mrs. Shila Sarkar) and Elder brother (Mr. Tanay Sarkar).*

*Dated: 22.8.2021*



*(Tanushree Sarkar)*

## Urkund Analysis Result

Analysed Document: Tanushree Sarkar\_Botany.pdf (D111128265)  
Submitted: 8/10/2021 7:53:00 AM  
Submitted By: nbuplg@nbu.ac.in  
Significance: 2 %

### Sources included in the report:

[https://www.in.gov/health/reports/mortality/2017/table05/tbl05\\_33.htm](https://www.in.gov/health/reports/mortality/2017/table05/tbl05_33.htm)  
<https://ir.nbu.ac.in/bitstream/123456789/1359/12/234963.pdf>  
[https://www.researchgate.net/publication/26575955\\_Comparative\\_study\\_on\\_the\\_induction\\_of\\_defense\\_related\\_enzymes\\_in\\_two\\_different\\_cultivars\\_of\\_chickpea\\_Cicer\\_arietinum\\_L\\_genotypes\\_by\\_salicylic\\_acid\\_spermine\\_and\\_Fusarium\\_oxysporum\\_f\\_sp\\_ciceri](https://www.researchgate.net/publication/26575955_Comparative_study_on_the_induction_of_defense_related_enzymes_in_two_different_cultivars_of_chickpea_Cicer_arietinum_L_genotypes_by_salicylic_acid_spermine_and_Fusarium_oxysporum_f_sp_ciceri)  
<https://ir.nbu.ac.in/bitstream/123456789/1353/13/223036.pdf>  
[https://www.researchgate.net/publication/225468523\\_Change\\_in\\_phenylalanine\\_ammonia\\_lyase\\_activity\\_and\\_isozyme\\_patterns\\_of\\_polyphenol\\_oxidase\\_and\\_peroxidase\\_by\\_salicylic\\_acid\\_leading\\_to\\_enhance\\_resistance\\_in\\_cowpea\\_against\\_Rhizoctonia\\_solani](https://www.researchgate.net/publication/225468523_Change_in_phenylalanine_ammonia_lyase_activity_and_isozyme_patterns_of_polyphenol_oxidase_and_peroxidase_by_salicylic_acid_leading_to_enhance_resistance_in_cowpea_against_Rhizoctonia_solani)  
[https://www.researchgate.net/publication/229569221\\_Effects\\_of\\_benzothiadiazole\\_and\\_acetylsalicylic\\_acid\\_on\\_-13-glucanase\\_activity\\_and\\_disease\\_resistance\\_in\\_potato](https://www.researchgate.net/publication/229569221_Effects_of_benzothiadiazole_and_acetylsalicylic_acid_on_-13-glucanase_activity_and_disease_resistance_in_potato)

### Instances where selected sources appear:

19



(Tanushree Sarkar)



(Aniruddha Saha)

Supervisor

**Dr. Aniruddha Saha**  
Professor  
Department of Botany  
University of North Bengal

# PREFACE

The history of agriculture began thousands of years ago with the civilization of the Indus Valley. In 2016, agriculture and allied sectors like horticulture, forestry, animal husbandry, and fisheries together accounted for 15.4% of the country's gross domestic product (GDP). In 2018, 50% of the Indian workforce was employed in agriculture which contributed 17–18% to the GDP. India ranks first in highest net cropped area followed by the United States and China.

Large quantity of food crops and vegetables are destroyed annually due to fungal pathogens. Impact of such severe economical losses is also related to global poverty. According to statistics, the world harvest figures suggest that fungal diseases cause huge yield losses in the five most important crops; wheat, rice, maize, soybean and potatoes. If such losses were diminished, those crops would have been enough to feed at least 8.5% of the seven billion populations. Moreover, in a hypothetical incidence where these five crops were affected concomitantly, around 61% of the world's population would suffer a food shortage. Therefore, proper attention to control fungal diseases in different countries, specifically in developing countries needs to be given priority.

As fungal diseases are a major threat to crop production, the application of fungicides to control fungal diseases is often considered necessary to secure the worldwide food supply. Furthermore, deliberate use of fungicides change the soil conditions and give rise to invasive fungal species. Most importantly it helps in development of fungicide resistance. The excessive use of fungicides causes health hazards to all the living creatures inhabiting both land and water, as it can enter aquatic ecosystems via drift, drainage and surface runoff from agricultural use. Despite the various risks in living systems and the environment due to excessive use of fungicides, the effects of fungicides have received far less attention. Under these circumstances, there is a need for constant search for new

environmental friendly fungicides, effective measures to prevent fungicide resistance, and more importantly novel treatment strategies by utilizing plant's own defense mechanisms through understanding plant-pathogen interactions.

Pointed gourd (*Trichosanthes dioica*) is one of the most consumed vegetable in the Asian tropical countries. It is mainly cultivated in India, Bangladesh, Pakistan, Myanmar, Nepal and Sri Lanka. In India, a total of 2,52,000 metric tons of pointed gourd was harvested from 18,000 hectares of land during 2016-2017. Different parts of the plant are used in a number of Ayurvedic preparations by the folk practitioners. Several fungal diseases have been reported to cause considerable damage to pointed gourd production in India. These include downy mildew caused by *Pseudoperonospora cubensis*, fruit rot by *Pythium aphanidermatum* and *P. cucurbitacearum*, sclerotinia stem rot by *Sclerotinia sclerotiorum*, fruit and vine rot by *Phytophthora melonis*, anthracnose by *Colletotrichum capsici* etc.

Increasing fungal attacks necessitated the use of different types of fungicides. The compulsion of alternative environment-friendly control methods are being understood by the researchers and policy makers. For this, proper understanding of the complex defense mechanisms of plants, their interaction with applied defense inducing molecules and the invading fungal pathogens are necessary in order to increase the efficiency and realize the true potential of sustainable disease control methods.

# LIST OF TABLES

- Table: 2.1.** Cloned disease resistance genes (R genes) in plants.
- Table: 3.1.** Region of collection of fruit and leaf samples of pointed gourd.
- Table: 3.2.** List of primers used for PCR amplification.
- Table: 3.3.** List of primer used for PCR amplification.
- Table: 3.4.** List of primer used for Reverse Transcriptase PCR amplification.
- Table: 4.1.** Disease incidence of pointed gourd plants in different farmer's fields of sub-Himalayan West Bengal.
- Table: 4.2.** Isolated fungal pathogens from *Trichosanthes dioica* from different locations of sub-Himalayan West Bengal.
- Table: 4.3.** Pathogenicity of six isolated fungi on whole plants of pointed gourd.
- Table: 4.4.** Pathogenicity of the 4 isolated fungi on detached leaves of pointed gourd.
- Table: 4.5.** Pathogenicity of three isolated fungi from fruit on pointed gourd fruits.
- Table: 4.6.** Growth and sporulation of *Curvularia spicifera* (KHBR) in different culture media.
- Table: 4.7.** Growth and sporulation of *Fusarium equiseti* (PG-Gua) in different culture media.
- Table: 4.8.** Growth and sporulation of *Ascochyta medicaginicola* (PGALD) in different culture media.
- Table: 4.9.** Growth and sporulation of *Periconia macrospinoso* (PGISH) in different culture media.
- Table: 4.10.** Growth and sporulation of *Fusarium oxysporum* (PG-Ph) in different culture media.

- Table: 4.11.** Growth and sporulation of *Colletotrichum orbiculare* (PG-Pha) in different culture media.
- Table: 4.12.** Growth and sporulation of *Curvularia lunata* (PG-Tau) in different culture media.
- Table: 4.13.** Growth and sporulation of *Curvularia aeria* (PG-Gar) in different culture media.
- Table: 4.14.** Growth and sporulation of *Curvularia verruciformis* (PG-Ish) in different culture media.
- Table: 4.15.** Growth and sporulation of *Alternaria alternata* (PG-WD1) in different culture media.
- Table: 4.16.** Growth and sporulation of *Alternaria destruens* (PG-WD2) in different culture media.
- Table: 4.17.** Growth and sporulation of *Alternaria tenuisima* (PG-Kra) in different culture media.
- Table: 4.18.** Growth and sporulation of *Fusarium equiseti* (PGAL) in different culture media.
- Table: 4.19.** Effect of different pH on mycelia growth of the pathogens of *Trichosanthes dioica*.
- Table: 4.20.** Effect of different incubation temperatures on mycelia growth of the pathogens of *Trichosanthes dioica*.
- Table: 4.21.** GenBank accession numbers of the pathogenic isolates with code and location of collection.
- Table: 4.22:** Disease incidence following application of abiotic inducers in pointed gourd plants against *F. equiseti*.
- Table: 4.23.** Activity of phenylalanine ammonia-lyase in pointed gourd plants pretreated with seven chemical inducers followed by *Fusarium equiseti* inoculation.

- Table: 4.24.** Peroxidase activity of Pointed gourd plants pre-treated with chemical inducers followed by challenge-inoculation with *F. equiseti*.
- Table: 4.25.** Activity of Glucanase in pointed gourd plants pretreated with seven chemical inducers followed by *Fusarium equiseti* inoculation.
- Table: 4.26.** Activity of Chitinase in pointed gourd plants pretreated with seven chemical inducers followed by *Fusarium equiseti* inoculation.
- Table: 4.27.** Activity of polyphenol oxidase in pointed gourd plants pretreated with seven chemical inducers followed by *Fusarium equiseti* inoculation.
- Table: 4.28.** Spectrometric quantification of extracted RNA from 'Treated' and 'untreated control' plants.

# LIST OF FIGURES

- Fig. 1.1:** Cultivated pointed gourd plants in farmer's field in different places of Northern West Bengal: A. Kharibari, Siliguri subdivision of Darjeeling District. B. Islampur, Uttar Dinajpur District. C. Dhupguri, Jalpaiguri district. D. Guabari, Siliguri subdivision of Darjeeling District. E. Alipurduar, Alipurduar district. and F. Tambari, Siliguri subdivision of Darjeeling District.
- Fig. 3.1:** (A) Map of West Bengal. (B) Districts of northern part of West Bengal (The present study area) where pointed gourd is grown as an important vegetable crop.
- Fig. 3.2:** Map of regions of collection of infected plant samples from different districts of North Bengal: (A) Kharibari, Siliguri subdivision of Darjeeling District. (B) Falakata, Alipurduar District. (C) Birpara, Alipurduar District. (D) Islampur, Uttar Dinajpur District. (E) Phansidewa, Siliguri subdivision of Darjeeling district and (F) Guabari, Siliguri subdivision of Darjeeling district. The red pins showing the collection spots
- Fig. 3.3:** Map of regions of collection of infected plant samples from different districts of North Bengal: (A) Phansidewa, Siliguri subdivision of Darjeeling District. (B) Kranti, Jalpaiguri District. (C) Dhupguri, Jalpaiguri District. (D) Dinhata, Coochbehar District. (E) Salkavita, Darjeeling District and (F) Islampur, Uttar Dinajpur District. The red pins showing the collection spots.
- Fig. 4.1:** Map of northern West Bengal and (1-13) regions of collection of infected plant samples from different districts of North Bengal.
- Fig. 4.2:** Naturally infected pointed gourd leaves and fruit samples: (A) Collected from Kharibari; (B) Collected from Dhupguri; (C) Collected from Ishlampur; (D) Collected from Birpara; (E) Collected from Dhupguri and (F) Collected from Phansidewa.

**Fig. 4.3:** Naturally infected pointed gourd leaves and fruit samples:(A) Collected from Ishlampur; (B) Collected from Phansidewa; (C) Collected from Salkavita; (D) Collected from Dinhata; (E) Collected from Guabari and (F) Collected from Kranti.

**Fig. 4.4:** Whole plant inoculation and disease assessment: A & B: Control plants of Swarna aloukik variety of *T. dioica*. C & D: Swarna aloukik variety plants after 6 days of C. Whole plant inoculated with fungi D. Plant after 3 days of inoculation by *Fusarium equiseti*.

**Fig. 4.5:** Detached leaf inoculation and disease assessment: A & B. Control leaves C. Leaves just after inoculation; D. Leaves after 4 days of inoculation.

**Fig. 4.6:** Fruit inoculation: Control fruits at the time of experimental setting; B. Control fruits after 3 days of experimental setting. C. Fruits just after inoculation; D. Fruits after 3 days of inoculation.

**Fig. 4.7:** Pure culture of the pathogenic isolates in PDA plates after 7days of incubation. (A) *Curvularia spicifera* (B) *Fusarium equiseti* (C) *Ascochyta medicaginicola* (D) *Periconia macrospinoso* (E) *Fusarium oxysporum* (F) *Colletotrichum orbiculare* (G) *Curvularia verruciformis* (H) *Curvularia aerea* (I) *Curvularia lunata* (J) *Alternaria alternata* (K) *Alternaria destruens* (L) *Alternaria tenuissima* (M) *Fusarium equiseti*.

**Fig. 4.8:** Spores of different fungal isolates under light microscope (40X) (A) *Curvularia spicifera* (B) *Fusarium equiseti* (C) *Ascochyta medicaginicola* (D) *Periconia macrospinoso* (E) *Fusarium oxysporum* (F) *Colletotrichum orbiculare* (G) *Curvularia verruciformis* (H) *Curvularia aerea* (I) *Curvularia lunata* (J) *Alternaria alternata* (K) *Alternaria destruens* (L) *Alternaria tenuissima* (M) *Fusarium equiseti*.

**Fig. 4.9:** A. Total DNA isolated from fungal samples on 1% agarose gel under UV-transilluminator. B-D. Amplified PCR products of three specific genes (ITS region, 28S rRNA region of large subunit and Actin gene) from fungal isolates on 1% agarose gel under

UV-transilluminator: B. Amplified with ITS1/ITS4; C. Amplified with NL1/NL4; and D. Amplified with Act1/Act2.

**Fig-4.10:** Nucleotide sequence identity matrix of *Curvularia aerea*, *Curvularia lunata*, *Curvularia spicifera* and *Curvularia verruciformis* of the present study and other *Curvularia* species following 18s rRNA sequence analysis. Identity percentages are indicated on the right side corner of the matrix.

**Fig. 4.11:** Phylogenetic tree generated by neighbour joining of different *Curvularia* species. Values at the nodes indicate percentage of bootstrap support (out of 1000 bootstrap replicates) and are indicated if greater than 50. GenBank accession numbers of the fungi have been indicated at the end of each branch.

**Fig-4.12:** Nucleotide sequence identity matrix of *Fusarium equiseti* and *Fusarium oxysporum* of the present study and other *Fusarium* species following 18s rRNA sequence analysis. Identity percentages are indicated on the right side corner of the matrix.

**Fig. 4.13:** Phylogenetic tree generated by neighbour joining of different *Fusarium* species. Values at the nodes indicate percentage of bootstrap support (out of 1000 bootstrap replicates) and are indicated if greater than 50. GenBank accession numbers of the fungi have been indicated at the end of each branch

**Fig-4.14:** Nucleotide sequence identity matrix of *Ascochyta medicaginicola* of the present study and other *Ascochyta* species following 18s rRNA sequence analysis. Identity percentages are indicated on the right side corner of the matrix.

**Fig. 4.15:** Phylogenetic tree generated by neighbour joining of different *Ascochyta* species. Values at the nodes indicate percentage of bootstrap support (out of 1000 bootstrap replicates) and are indicated if greater than 50. GenBank accession numbers of the fungi have been indicated at the end of each branch.

**Fig-4.16:** Nucleotide sequence identity matrix of *Periconia macrospinoso* of the present study and other *Periconia* species following 18s rRNA

sequence analysis. Identity percentages are indicated on the right side corner of the matrix.

**Fig. 4.17:** Phylogenetic tree generated by neighbour joining of different *Periconia* species. Values at the nodes indicate percentage of bootstrap support (out of 1000 bootstrap replicates) and are indicated if greater than 50. GenBank accession numbers of the fungi have been indicated at the end of each branch.

**Fig-4.18:** Nucleotide sequence identity matrix of *Colletotrichum orbiculare* of the present study and other *Colletotrichum* species following 18s rRNA sequence analysis. Identity percentages are indicated on the right side corner of the matrix.

**Fig. 4.19:** Phylogenetic tree generated by neighbour joining of different *Colletotrichum* species. Values at the nodes indicate percentage of bootstrap support (out of 1000 bootstrap replicates) and are indicated if greater than 50. GenBank accession numbers of the fungi have been indicated at the end of each branch.

**Fig. 4.20:** Nucleotide sequence identity matrix of *Alternaria alternata*, *Alternaria destruens*, *Alternaria tenuissima* of the present study and other *Alternaria* species following 18s rRNA sequence analysis. Identity percentages are indicated on the right side corner of the matrix.

**Fig. 4.21:** Phylogenetic tree generated by neighbour joining of different *Alternaria* species. Values at the nodes indicate percentage of bootstrap support (out of 1000 bootstrap replicates) and are indicated if greater than 50. GenBank accession numbers of the fungi have been indicated at the end of each branch.

**Fig. 4.22:** Phylogenetic relationship of present isolated PGRGA with the other RGA sequences of some plants of cucurbitaceae publish in the GenBank using the neighbor joining method. Numbers at the nodes indicate the bootstrap percentage scores out of 1000 replicates

**Fig. 4.23:** Sequence identity matrix of isolated PGRGA with other CC-NBS gene sequences of some plants of cucurbitaceae. Identity

percentage corresponding to the color matrix is indicated in the right-hand side of the figure.

**Fig. 4.24:** Sequence identity matrix of isolated PAL genes with other PAL gene sequences published in the GenBank. Identity percentage corresponding to the color matrix is indicated in the right-hand side of the figure.

**Fig. 4.25:** Phylogenetic relationship of present isolated PAL gene with the other PAL gene sequences published in the GenBank using the neighbor joining method. Numbers at the nodes indicate the bootstrap percentage scores out of 1000 replicates

**Fig. 4.26:** Sequence identity matrix of isolated Peroxidase (POD) gene with other POD gene sequences published in the GenBank. Identity percentage corresponding to the color matrix is indicated in the right-hand side of the figure.

**Fig. 4.27:** Phylogenetic relationship of present isolated Peroxidase (POD) gene with the other POD gene sequences published in the GenBank using the neighbor joining method. Numbers at the nodes indicate the bootstrap percentage scores out of 1000 replicates

**Fig. 4.28:** Sequence identity matrix of isolated Polyphenol oxidase (PPO) gene with other PPO gene sequences published in the GenBank. Identity percentage corresponding to the color matrix is indicated in the right-hand side of the figure.

**Fig. 4.29:** Phylogenetic relationship of present isolated Polyphenol oxidase (PPO) gene with the other PPO gene sequences published in the GenBank using the neighbor joining method. Numbers at the nodes indicate the bootstrap percentage scores out of 1000 replicates

**Fig. 4.30:** Sequence identity matrix of isolated Glucanase gene with other Glucanase gene sequences published in the GenBank. Identity percentage corresponding to the color matrix is indicated in the right-hand side of the figure.

# ABBREVIATIONS

<b>µg</b>	Microgram	<b>H<sub>2</sub>O<sub>2</sub></b>	Hydrogen peroxide
<b>µl</b>	Microlitre	<b>JA</b>	jasmonic acid
<b>µm</b>	Micrometre	<b>L</b>	Litre
<b>°C</b>	Degree Celcius	<b>LAR</b>	Local acquired resistance
<b>ABA</b>	abscisic acid	<b>LB</b>	Luria-Bertani
<b>AABA</b>	α-Aminobutyric acid	<b>LPS</b>	Lipopolysaccharide
<b>ACT</b>	Actin gene	<b>LRR</b>	Leucine-rich repeats
<b>avr</b>	avirulence	<b>LSU</b>	Large subunit
<b>BABA</b>	β-amino butyric acid	<b>LZ</b>	Leucine zipper
<b>BLAST</b>	Basic local alignment search tool	<b>M</b>	Mole
<b>BLASTn</b>	Nucleotide BLAST	<b>mAmp</b>	Milliampere
<b>bp</b>	Base pair	<b>MEA</b>	Malt extract agar
<b>BTH</b>	2,1,3-benzothiadiazole	<b>MEGA</b>	Molecular evolutionary genetics analysis
<b>CC</b>	Coiled-Coil	<b>mg</b>	Milligram
<b>CD</b>	Critical Difference	<b>min</b>	Minutes
<b>CDA</b>	Czapek dox agar	<b>ml</b>	Millilitre
<b>cm</b>	Centimetre	<b>mm</b>	Milimetre
<b>CTAB</b>	Cetyl trimethyl ammonium bromide	<b>mM</b>	Milimole
<b>DNA</b>	Deoxyribonucleic acid	<b>M-MuLV</b>	Moloney murine leukemia virus
<b>dNTPs</b>	Deoxyribonucleotide triphosphates	<b>MPKs</b>	mitogen-activated protein kinases
<b>EC</b>	Enzyme class	<b>mRNA</b>	Messenger RNA
<b>EDTA</b>	Ethylenediamine tetra acetic acid	<b>N</b>	Normal
<b>g</b>	Gravitational force	<b>NBS</b>	Nucleotide binding site
<b>gm</b>	Gram	<b>NCBI</b>	National Centre for Biotechnology Information
<b>GABA</b>	γ-amino butyric acid	<b>ng</b>	Nanogram
<b>h</b>	Hour	<b>nm</b>	Nanometer
<b>HR</b>	Hypersensitive response	<b>No.</b>	Number
<b>ISR</b>	Induced systemic resistance	<b>NPR1</b>	Non-expressor of pathogen-related gene 1
<b>ITS</b>	Internal transcribed spacer	<b>nt</b>	Nucleotide
<b>kb</b>	kilo bases	<b>OMA</b>	Oat meal agar
		<b>PAL</b>	Phenylalanineammonialyase

<b>PDA</b>	Potato dextrose agar	<b>SA</b>	Salicylic acid
<b>PDB</b>	Potato dextrose broth	<b>SAR</b>	Systemic acquired resistance
<b>PCR</b>	Polymerase chain reaction	<b>SDT</b>	Sequence demarcation tool
<b>PGDA</b>	Pointed gourd dextrose agar	<b>SDW</b>	Sterile distilled water
<b>PGPR</b>	Plant Growth Promoting Rhizobacteria	<b>SE</b>	Standard error
<b>PPO</b>	Polyphenol oxidase	<b>TAE</b>	Tris acetate EDTA
<b>PVP</b>	Polyvinyl pyrrolidone	<b>TIR</b>	Toll Interleukin-1 Receptor
<b>R</b>	Resistance	<b>TNL</b>	TIR-NBS-LRR
<b>RA</b>	Richards's agar	<b>TM</b>	Transmembrane region
<b>RGAs</b>	resistance gene analogs	<b>UV-VIS</b>	Ultraviolet-Visible
<b>RNA</b>	Ribonucleic acid	<b>V</b>	Volt
<b>ROS</b>	Reactive oxygen species	<b>v/v</b>	Volume by volume
<b>rpm</b>	Rotation per minute	<b>w/v</b>	Weight by volume
<b>rRNA</b>	Ribosomal RNA	<b>wt</b>	Weight
<b>RT-PCR</b>	Reverse transcription PCR	<b>YEMA</b>	Yeast extract mannitol agar

related gene product	
3.9.8. Digital analysis of Reverse Transcriptase PCR electrophoresis gel	71
3.9.9. Statistical analysis	71
<b>4. Results</b>	<b>72–169</b>
<b>4.1. Chapter-I: Fungal diseases of <i>Trichosanthes dioica</i></b>	<b>72–82</b>
4.1.1. Survey of fungal diseases in sub-Himalayan West Bengal	72
4.1.1.1. Disease symptoms of <i>T. dioica</i> of Darjeeling district	72
4.1.1.2. Disease symptoms of <i>T. dioica</i> of Jalpaiguri district	73
4.1.1.3. Disease symptoms of <i>T. dioica</i> of Alipurduar district	73
4.1.1.4. Disease symptoms of <i>T. dioica</i> of Uttar dinajpur district	74
4.1.1.5. Disease symptoms of <i>T. dioica</i> of Cooch behar district	74
<b>4.2. Chapter-II: Pathogenicity of the isolated fungi</b>	<b>83–88</b>
4.2.1. Pathogenicity of six isolated fungi on whole plants following Whole plant inoculation technique	83
4.2.2. Detached leaf inoculation	85
4.2.3. Inoculation on fruits	86
<b>4.3. Chapter III: Morphological, physiological and molecular characterization of the isolated pathogens</b>	<b>89–124</b>
4.3.1. Studies on morphology of the pathogens and their molecular characterization	89
4.3.2. Different culture conditions affecting growth and sporulation of the pathogens	94
4.3.2.1. Mycelial growth and sporulation of pathogens in different solid media	94
4.3.2.2. Effect of different pH on mycelia growth of the pathogens	101
4.3.2.3. Effect of different incubation temperatures on mycelia growth of the pathogens	102
4.3.3. Molecular Identification of the thirteen different	105

pathogenic isolates of <i>Trichosanthes dioica</i> and their phylogenetic analysis	
4.3.3.1. Molecular identification of fungal isolates	105
4.3.3.2. Phylogenetic analysis of the fungal isolates	108
4.3.3.2.1. <i>Curvularia</i> isolates	108
4.3.3.2.2. <i>Fusarium</i> isolates	109
4.3.3.2.3. <i>Ascochyta medicaginicola</i>	110
4.3.3.2.4. <i>Periconia macrospinoso</i>	110
4.3.3.2.5. <i>Colletotrichum orbiculare</i>	111
4.3.3.2.6. <i>Alternaria</i> isolates	111

**4.4. Chapter IV: Isolation and molecular characterisation of RGA and DR-genes in pointed gourd** **125–140**

4.4.1. Detection and analysis of Resistance Gene Analog (RGA)	125
4.4.2. Detection and analysis of defense related enzyme genes	128
4.5.1.1. Analysis of PAL genes isolated from pointed gourd	128
4.5.1.2. Analysis of peroxidase genes isolated from pointed gourd	128
4.5.1.3. Analysis of polyphenol oxidase (PPO) genes isolated from pointed gourd	129
4.5.1.4. Analysis of $\beta$ -1,3-glucanase genes isolated from pointed gourd	129
4.5.1.5. Analysis of chitinase genes isolated from pointed gourd	129

**4.5. Chapter V: Induction of plant defense by abiotic inducers and disease assessment** **141–162**

4.5.1. Induction of defense in pointed gourd plants by abiotic inducers and disease assessment against <i>F. equiseti</i>	141
4.5.2. Induction of defense-related enzymes in pointed gourd plant by abiotic inducers and studies on some defense related enzymes	147
4.5.2.1. Activity of phenylalanine ammonia lyase on application of abiotic inducers	147

4.5.2.2. Activity of peroxidase on application of abiotic inducers	151
4.5.2.3. Activity of $\beta$ -1,3-glucanase on application of abiotic inducers	154
4.5.2.4. Activity of chitinase on application of abiotic inducers	157
4.5.2.5. Activity of polyphenol oxidase on application of abiotic inducers	160
<b>4.6. Chapter VI: Quantification of transcripts of R and DR genes in pointed gourd plants by semi-quantitative RT-PCR</b>	<b>163–169</b>
<b>5. Discussion</b>	<b>170–186</b>
<b>6. Bibliography</b>	<b>187–237</b>
7.A. Appendix-1: Chemicals and Reagents	238–240
7.B. Appendix-2: Buffers and Solutions	241–244
7.C. Appendix-3: Growth media	245–247
7.D. Appendix-4: Sequences submitted in the GenBank	248–257
7.E. Appendix-5: List of publications	258
7.F. Appendix-6: Reprints	259