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.*

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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.

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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.

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ABBREVIATIONS

µg	Microgram
µl	Microlitre
µm	Micrometre
^{o}C	Degree Celcius
ABA	abscisic acid
AABA	α-Aminobutyric acid
ACT	Actin gene
avr	avirulence
BABA	β-amino butyric acid
BLAST	Basic local alignment search tool
BLASTn	Nucleotide BLAST
bp	Base pair
BTH	2,1,3-benzothiadiazole
CC	Coiled-Coil
CD	Critical Difference
CDA	Czapek dox agar
cm	Centimetre
CTAB	Cetyl trimethyl ammonium bromide
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleotide triphosphates
EC	Enzyme class
EDTA	Ethylenediamine tetra acetic acid
g	Gravitational force
gm	Gram
GABA	γ-amino butyric acid
h	Hour
HR	Hypersensitive response
ISR	Induced systemic resistance
ITS	Internal transcribed spacer
kb	kilo bases
H_2O_2	Hydrogen peroxide
JA	jasmonic acid
L	Litre
LAR	Local acquired resistance
LB	Luria-Bertani
LPS	Lipopolysaccharide
LRR	Leucine-rich repeats
LSU	Large subunit
LZ	Leucine zipper
M	Mole
mAmp	Milliampere
MEA	Malt extract agar
MEGA	Molecular evolutionary genetics analysis
mg	Milligram
min	Minutes
ml	Millilitre
mm	Milimetre
mM	Milimole
M-MuLV	Moloney murine leukemia virus
MPKs	mitogen-activated protein kinases
mRNA	Messenger RNA
N	Normal
NBS	Nucleotide binding site
NCBI	National Centre for Biotechnology Information
ng	Nanogram
nm	Nanometer
No.	Number
NPR1	Non-expressor of pathogen-related gene 1
nt	Nucleotide
OMA	Oat meal agar
PAL	Phenylalanine ammonia lyase

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