Antagonistic Potential of Mutants of *Trichoderma* Species

A THESIS
SUBMITTED TO THE
UTTAR BANGA KRISHI VISWAVIDYALAYA

In partial
fulfillment of the requirements for the award of the Degree of

MASTER OF SCIENCE
IN
AGRICULTURE (PLANT PATHOLOGY)

By

Tulipa De
(A-2018-046-M)

DEPARTMENT OF PLANT PATHOLOGY
FACULTY OF AGRICULTURE
UTTAR BANGA KRISHI VISWAVIDYALAYA
PUNDIBARI - 736 165, Cooch Behar, WEST BENGAL

2020

DEDICATED TO

My beloved Parents,
Brother
&
My Chairman

DEPARTMENT OF PLANT PATHOLOGY
UTTAR BANGA KRISHI VISWAVIDYALAYA
FACULTY OF AGRICULTURE
Pundibari, Cooch Behar, West Bengal – 736165, India.

Prof. (Dr.) Ayon Roy
Professor
Department of Plant Pathology

Mobile : 9434483593

Email : ayonroy.plantpathology@gmail.com / ayon@ubkv.ac.in

Date- 24-09-2020.

CERTIFICATE I

This is to certify that the work recorded in the thesis entitled, **"Antagonistic Potential of Mutants of *Trichoderma* Species"** submitted by **Miss. Tulipa De** in partial fulfillment of the requirements for the degree of M.Sc. in Agriculture (Plant Pathology) of the Uttar Banga Krishi Viswavidyalaya, Pundibari, is the faithful and bonafide research work carried out under my personal supervision and guidance. The results of the study reported in the thesis have not so far been submitted for any other degree or diploma.

The assistance and help received from various sources during the course of investigation have been duly acknowledged.

(AYON ROY)
Chairman
Advisory Committee

Place: Pundibari

Date: 24-09-2020.

DEPARTMENT OF PLANT PATHOLOGY
UTTAR BANGA KRISHI VISWAVIDYALAYA
FACULTY OF AGRICULTURE
Pundibari, Cooch Behar, West Bengal – 736165, India.

Prof. (Dr.) Ayon Roy
Professor
Department of Plant Pathology

Mobile : 9434483593

Email : ayonroy.plantpathology@gmail.com / ayon@ubkv.ac.in

Date- 22/09/2020

CERTIFICATE

Certificated that the thesis entitled "**Antagonistic Potential of Mutants of *Trichoderma* Species**" submitted by Miss. TULIPA DE bearing Registration number A-2018-046-M towards partial fulfilment of the requirement for the award of Master Degree from the Department of Plant Pathology, Faculty of Agriculture under Uttar Banga Krishi Viswavidyalaya has been checked against plagiarism through Urkund software on 22-09-2020 and the similarity index has been achieved as 1% which is below the maximum tolerable range as per stipulation of this Viswavidyalaya. The thesis of Miss. TULIPA DE may be accepted for the award of the Master Degree in M.Sc. (Agriculture) of Uttar Banga Krishi Viswavidyalaya.

Ayon Roy
(AYON ROY)
Chairman
Advisory Committee

Place: Pundibari

Date: 22-09-2020

Certificate of Originality

To

The Chairman, Advisory Committee

Department of Plant Pathology

Faculty of Agriculture

Uttar Banga Krishi Viswavidyalaya

Sub: **Undertaking regarding checking for similarity index of manuscript of submitted thesis**

Respected Sir,

I wish to submit the following:

i. Name of the student (in block letters): . TULIPA DE . . . . . .

ii. Registration number: . . . . . A-2018-046-M . . . . . .

iii. Name of Chairman of Advisory Committee: . . Prof. Ayon Roy . . .

iv. Name of the Department: . . . Plant Pathology . . .

v. Title of the Thesis: . . . Antagonistic Potential of Mutants of *Trichoderma* species . . . . . .

. . . . . .

vi. Total Pages of the thesis: . . . 50 . . . .

I do hereby certify that the above-mentioned thesis was checked against plagiarism / for similarity detection. The process and outcome are stated below:

vii. Software used: . . . URKUND . . . . . .

viii. Date: . 22.09.2020 .

ix. Similarity Index: . . . . 1% . . . . . .

The complete report is submitted for review.

Thanking you

Yours faithfully,

Signature of student: Tulipa De
22/09/2020

Name of the student (in block letters): . . . . . TULIPA DE. . . . .

Only software for checking similarity index output is certified

23/09/2020

In-Charge
CENTRAL LIBRARY
Uttar Banga Krishi Viswavidyalaya
Pundibari, Cooch Behar

(Signature of In-charge, Central Library with official seal)

Contd..../- (PTO)

Contd…from pre-page (overleaf)

The complete report of the above-mentioned thesis has been reviewed by the Advisory Committee of the student (following to be ticked as appropriate).

☐ The similarity index of the above mentioned thesis is below the accepted norms.

☐ The similarity index of the above mentioned thesis is above the accepted norms, because of the following reasons:

1 ..... NA .....

2 ..... NA .....

3 ..... NA .....

4 ..... NA .....

5 ..... NA .....

The thesis may be considered for the award of Degree (relevant software generated document attached).

Forwarded for consideration

Ayon Ray 23/09/2020

Signature of Chairman, Advisory Committee

Forwarded

23/09/2020

(Signature and Official Seal of Head of the Department)

Head
Department of Plant Pathology
Faculty of Agriculture
U.V.K.V. Pundibari, Cooch Behar

The process for submission of thesis is allowed / not allowed to commence.

(Signature and Official Seal of the Dean, Post Graduate Studies)

CERTIFICATE –II

This is to certify that the thesis entitled, **"Antagonistic Potential of Mutants of *Trichoderma* Species"** submitted by **Miss. TULIPA DE (A-2018-046-M)** to Uttar Banga Krishi Viswavidyalaya, Pundibari in partial fulfillment of the requirement of the degree in Master of Science in Agriculture (Plant Pathology) has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner. The committee recommends the thesis be accepted for the award of the degree in Master of Science in Agriculture (Plant Pathology).

Prof. B. N. Panja ..... Panja 02.10.20 .....

External Examiner

Chairman

1. **Prof. Ayon Roy**
   (Chairman, Advisory committee,
   Dept. of Plant Pathology) ..... Ayon Roy 02.10.2020

Advisory Committee Members

2. **Prof. P.M. Bhattacharya**
   (Dept. of Plant Pathology) ..... 02.10.2020.

3. **Dr. A. Debnath**
   (Department of Plant Pathology) ..... Anamika Debnath 02.10.2020

4. **Dr.Nandita Sahana**
   (Department of Biochemistry) 02/10/2020

5. **Prof. Soumen Maitra**
   (Dean for Post Graduate Studies) ..... Soumen Maitra 2.10.2020

6. **Prof. Dibyendu Mukhopadhyay**
   (Dean, Faculty of Agriculture) 02.10.2020

..... 02.10.2020

Head of the Department
(Department of Plant Pathology)

ACKNOWLEDGEMENT

*Work is worship. The almighty who lives in every soul truly manifests through work. First of all, I ascribe all glory to the gracious **"ALMIGHTY"** for his heartfelt benediction and silent blessing which always acts as my saviour in the face of all sorts of peril and paved the path of my life.*

*It is enlightened me to express my profound gratitude to my Advisor and the Chairman of my advisory committee, **Prof. Ayon Roy**, Professor, Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya for his worth mentioning dedication, talented guidance and constant support throughout the research activities and for helping me to prepare this thesis. His farsightedness, constructive criticism, dynamic visionary, correct conceptions, whole hearted efforts and motivation have deeply inspired me. He instructed me the knowhow techniques for carrying out the research and to bring out the research work in proper manner. It gave me an immense pleasure and honour to study and work under his guidance.*

*I have no words to express my heartful thanks to **Prof. Apurba Kumar Chowdhury**, Head, Department of Plant Pathology, UBKV for his valuable guidance and support during the course of investigation.*

*I am extremely grateful to **Dr. P.M. Bhattacharya**, Professor, Department of Plant Pathology and Member of my advisory committee for his valuable suggestions and general help in preparing the manuscript.*

*I feel immense pleasure to express my deep felt sense of gratitude to **Dr. Anamika Debnath**, Assistant Professor, Department of Plant Pathology and Member of my advisory committee for her generous help and guidance during investigation.*

*I am indebted to **Dr. Nandita Sahana**, Assistant Professor, Department of Plant Biochemistry and Member of my advisory committee for her constant encouragement and suggestions in preparing the thesis.*

*I am very thankful to **Dr. Hossain Ali Mondal**, Assistant Professor, Department of Genetics and Plant Breeding and I express my sincere thanks to him for constant support and close guidance and necessary experimental facilities from his well equipped laboratory.*

I am happy to express my heartiest thanks my Departmental Teachers ***Dr. Satyajit Hembram***, ***Dr. Shekhar Bandyopadhyay***, ***Dr. Surojit Khalko***, ***Mrs. Sreema Das***, *for their continuous support, endless guidance and as source of inspiration.*

I shall be failing in my duties unless I extend my heartiest thanks to ***Prof. Soumen Maitra***, *Dean, Post Graduate Studies, Faculty of Agriculture, UBKV.*

My sincere thanks go to all the ***non-teaching staff*** *of Department of Plant Pathology, UBKV for their generous help during my research work.*

I am very much grateful to all my seniors, juniors and my classmates for their encouragement and continuous support.

*I express my heartiest thanks to those who always live in my mind and soul, whose profound love will never be made up, who are closest, deepest and dearest to me, my beloved Father '****Dr. Lakshman Chandra De****', Mother '****Soma De****' and brother '****Ranit De****' who always helped me kind heartedly in my life. They are the persons whose enormous love has always been a source of inspiration in my accomplishment.*

Last but not least, I am thankful to the ***Vice Chancellor***, *Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar for providing me the merit scholarship.*

Tulipa De

Place: Pundibari

Date: 24.09.2020

(Tulipa De)

Title of the thesis	:	**Antagonistic Potential of Mutants of *Trichoderma* Species**
Name of the Student	:	Tulipa De
Admission No.	:	A-2018-046-M
Major Subject	:	Plant Pathology
Minor Subject	:	Plant Pathology
Name of the Chairman	:	Dr. Ayon Roy
Designation of the Chairman	:	Professor, Department of Plant Pathology
Degree to be awarded	:	Master of Science in Plant Pathology
Year of Award of Degree	:	2020
Total pages in Thesis	:	49 + References XXII
Name of University	:	Uttar Banga Krishi Viswavidyalaya Pundibari, Coochbehar, West Bengal-736165.

ABSTRACT

Strains UBT-18 and T21 of *Trichoderma* had been isolated from the soil of Cooch Behar. Both the strains showed different visual appearance. ITS sequencing was used for molecular characterization of native *Trichoderma* isolates using ITS1 and ITS4 primer. To identify homology between the PCR fragments and sequences in the GeneBank database, the obtained nucleotide sequences of the strains were compared with the GeneBank database using the nucleotide BLAST program and distance trees were constructed. The distance tree obtained suggest that the test isolate UBT-18 belongs to *Trichoderma harzianum* and the test isolate T21 belongs to *Trichoderma asperellum*. Different exposure duration was used when both the *Trichoderma* strains were placed under UV light and immediately transferred to BOD after treatment which were later serial diluted and plated on Petri plates containing TSM and kept on BOD for few days. Mutated colonies of *Trichoderma* were seen which was picked on PDA slants before the colonies merged with each other. The individual mutated spores from colonies were repeatedly sub-cultured in PDA slants up to 10th generation to obtain stable mutants. Some treatments died out due to segregation effect after random mutation and they were discarded from the experiment. Determination of antagonistic potential of mutated *Trichoderma* isolates as well as their respective parental strains against soil born fungal pathogens by 'Bangle method' (Singh et al., 2004) using dual culture. Among the stable mutants obtained after 10th generation, one isolate from each treatment having maximum growth rate were used against test pathogens *Fusarium oxysporum* f.sp. *lentis* and *Rhizoctonia solani*. Mutated UBT-18 (*T. harzianum*) could not give better performance due to its slow growing nature. It was able to inhibit growth over control of *Fusarium oxysporum* f.sp. *lentis* maximum up to 47.22% (T9) compared to wild one inhibiting 40.98% and of *Rhizoctonia solani* maximum up to 37.78% (T8) compared to the wild isolate inhibiting over 68.89%. T1 of strain T21 (*T. asperellum*) was most effective and exhibited higher percent inhibition of growth over control (72.78%) compared to the wild isolate (37.31%) in case of *Fusarium oxysporum* f.sp. *lentis*. In case of pathogen *Rhizoctonia solani* also, T1 showed higher percent inhibition of growth over control (70.44%) compared to the wild isolate (38.43%).

Ayon Roy.
Signature of Chairman

Advisory Committee

Tulipa De
Signature of the Student

CONTENTS

Chapter	Topic	Page
I	INTRODUCTION	1-3
II	REVIEW OF LITERATURE	4-23
III	MATERIALS AND METHODS	24-29
IV	RESULTS AND DISCUSSION	30-47
V	SUMMARY AND CONCLUSION	48-49
VI	REFERENCES	i-xxii
VII	PLAGIARISM DOCUMENT	-1-
VIII	VITA	-2-

List of Tables

Table No.	Particulars	Page No.
Table – 2.1	Potential of genus *Trichoderma* as biocontrol agent in different host pathogen interaction	5
Table – 2.2	Antibiotics isolated from *Trichoderma* spp.	11-12
Table – 2.3	Induced systemic resistance triggered by *Trichoderma* spp.	15-16
Table – 2.4	*Trichoderma* spp. against Bacterial Pathogens	19
Table – 2.5	*Trichoderma* spp. in control of weeds	19-20
Table – 2.6	Invertebrates controlled by *Trichoderma* spp.	20
Table – 2.7	*Trichoderma* sp. for bioremediation and suggested mode of growth promotion	21
Table – 2.8	List of *Trichoderma* based commercial formulation	22-23
Table – 3.1	Duration of exposure of fungal strains to UV-light	28
Table – 4.1	ITS 1 and ITS 4 sequencing of ribosomal DNA of UBT -18	30
Table – 4.2	ITS 1 and ITS 4 sequencing of ribosomal DNA of T 21	32
Table – 4.3	Mutated isolates cultured through generation	35
Table – 4.4	Antagonistic potential of *T. harzianum* (UBT-18) against soil borne plant pathogens by Dual culture method	41
Table – 4.5	Antagonistic potential of *T. asperellum* (T-21) against soil borne plant pathogens by Dual culture method	41

List of Figures

Figure No.	Particulars	Page No.
Figure 4.1	Distance tree of nucleotide sequence of UBT-18 using ITS 1 primer	31
Figure 4.2	Distance tree of nucleotide sequence of UBT-18 using ITS 4 primer	31
Figure 4.3	Distance tree of nucleotide sequence of T-21 using ITS 1 primer	32
Figure 4.4	Distance tree of nucleotide sequence of T-21 using ITS 4 primer	33

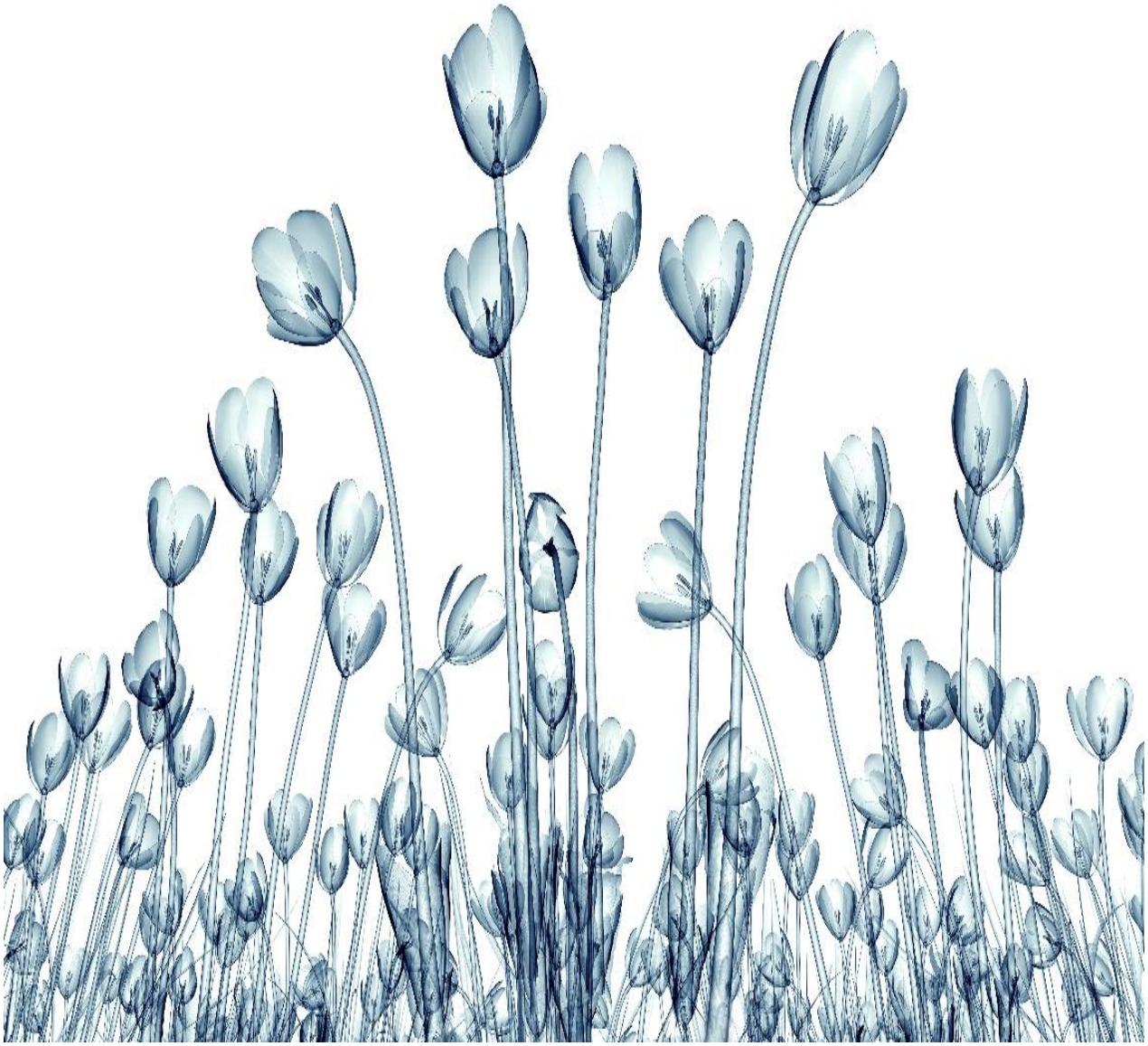
List of Plates

Table No.	Particulars	Page No.
Plate 1	*Trichoderma* and Pathogen isolates used	34
Plate 2	Generation growth of mutated UBT-18 *Trichoderma* strains	36-37
Plate 3	Generation growth of mutated T21 *Trichoderma* strains	38-39
Plate 4	Dual culture by 'Bangle method' of mutated UBT-18 *Trichoderma* strain against pathogens	42-44
Plate 5	Dual culture by 'Bangle method' of mutated T21 *Trichoderma* strain against pathogens	45-46

ABBREVIATIONS

ABC	ATP-Binding Cassette
ATP	Adenosine Tri Phosphate
BCA	Bio-Control agents
BOA	Benzoxazolinone
cAMP	Cyclic Adenosine Monophosphate
CSA	Competitive Saprophytic Ability
DDT	Dichloro Diphenyl Trichloroethane
DIBOA	2,4-dihydroxy-1,4-(2H)-benzoxazine-3-one
g	Gram
ISR	Induced Systemic Resistance
ITS	Internal Transcribed Spacer
L	Litre
LSD	Latin Square Design
MAPK	Mitogen-activated protein kinase
mg	Milligram
ml	Millilitre
nm	Nanometre
No.	Number
NTG	Nitrosoguanidine
^{o}C	Degree centigrade
PAHs	Polyaromatic hydrocarbons
psi	Pond Per Square Inch
SAR	Systemic Acquired Resistance
UV	Ultraviolet

1. INTRODUCTION



1. INTRODUCTION

Intensive agriculture generates ecological systems liable to spread epidemics in agro-ecological areas. To curb the situation, during the 19th century, over reliance on chemical input led the way to too many ill consequences like degradation of soil health, health hazards, environmental pollution, etc. which urged the need for search of alternative method of stress management. Biocontrol of plant pathogens has revealed the new vistas in the plant protection management strategies that include the use of biological control agents (BCAs) to manage existing pest populations. Among the widely used BCAs, *Trichoderma* spp. have appeared as the most potential agents for their unique biocontrol activities and for having a wide array of beneficial plant interactions (Benítez et al., 2004; Nawrocka and Malolepsz, 2013).

The worldwide emphasis on use of *Trichoderma* as biological control agent is because of its universal existence, ability of rhizosphere modification, tolerance to unfavourable weather conditions, high regeneration capacity, effective nutrient mobilization, efficient in plant growth regulations and havoc aggressiveness against phytopathogenic fungi. All these characteristics have made *Trichoderma* a unique genus distributed in any agro-ecosystem and at higher density populations. Biocontrol of plant pathogens by the use of *Trichoderma* has become an eco-friendly, effective, farmers' accepted alternative to fungicidal application for reducing the chemical extravagant in farming ecosystem (Papavizas, 1985).

Members of the fungal genus *Trichoderma* belonged to the division *Ascomycota*, order *Hypocreales* and family *Hypocreaceae* are ubiquitous in most of the ecosystem, organic rich soil system in particular and mostly encountered as plant symbionts, saprotrophs, soil inhabitants, and mycoparasites. Many species of the genus so far have been reported amidst *T. virens*, *T. viride*, *T. koningii*, *T. harzianum*, *T. asperellum*, *T. atroviride*, *T. hamatum* are few which have been studied extensively throughout the world during past few decades. As described in different findings, hyperparasitism, competitions for space, cell wall lysis are the major mechanisms involved in direct antagonism of plant pathogens. Besides, their function in producing different growth hormones, various defence related substances, pathogenesis related proteins, reclamation of heavy toxicity also help indirectly, in reducing resistance in the plants against the pathogens (Harman, 2006).

The balance between spore formation and hyphal growth is critical to the development of *Trichoderma* both in the soil and rhizosphere (Stevaert et al., 2010). In laboratory conditions, strains produce conidia and chlamydospores, and naturally some develop ascospores in perithecia. The transition from vegetative growth to conidial phase can be triggered in *Trichoderma* by the application of UV-blue light, low pH, nutrient stress, or mechanical injury to the mycelium.

Due to major impact of Trichoderma on human welfare, recent genome sequencing has targeted seven species mainly *T. atroviride*, *T. asperellum*, *T. citrinoviride*, *T. harzianum*, *T. longibrachiatum*, *T. reesei*, and *T. virens* (Grigoriev et al., 2012)

Recently, *Trichoderma* acquired tremendous importance because of its biological control ability against a number of pathogens. Pathogens that can be checked by *Trichoderma* include *Fusarium*, *Rhizoctonia*, *Pythium*, *Phytophthora*, *Sclerotinia*, and *Verticillium*. Antagonistic *Trichoderma* species can be considered as promising biocontrol agents. Expected mechanisms of antagonism acted by *Trichoderma* sp. include antibiosis, competition, mycoparasitism, hyphal interactions and enzyme secretion (Harman, 2006). Main fungal cell wall degrading enzymes secreted by *Trichoderma* are chitinase, cellulases and glucanase. These enzymes seem to play a vital role in the antagonistic properties of *Trichoderma*. The antagonistic properties of *Trichoderma* are effectively used in biological control against disease causing organisms. Antibiosis is another major component of the mechanism of biological control by *Trichoderma* where it acts synergistically to eliminate or inhibit the growth of competing microorganisms. These molecules comprise of both volatile and non-volatile metabolites with antimicrobial activities.

Mycoparasitism is another mechanism of biological control in which *Trichoderma* spp., cause a direct attack for killing the competing fungi or suppressing their growth (Li et al., 2018; Seidl et al., 2009). Tolerance to exogenous toxins, capability to perceive and attack to eliminate or inhibit the growth of other fungi, immune capacity against plant pathogens are all additional factors contributing to the commercial success of *Trichoderma* BCAs (Mukherjee et al., 2013). However, in spite of ample research on *Trichoderma* BCAs and their beneficial and diversified applications, biocontrol mechanisms are yet to be fully investigated. Improper field efficiency impairs diversified biocontrol applications and requires more research to fully know the effective

mechanisms. Above all, the antagonistic properties of BCAs can be further improved by different approaches.

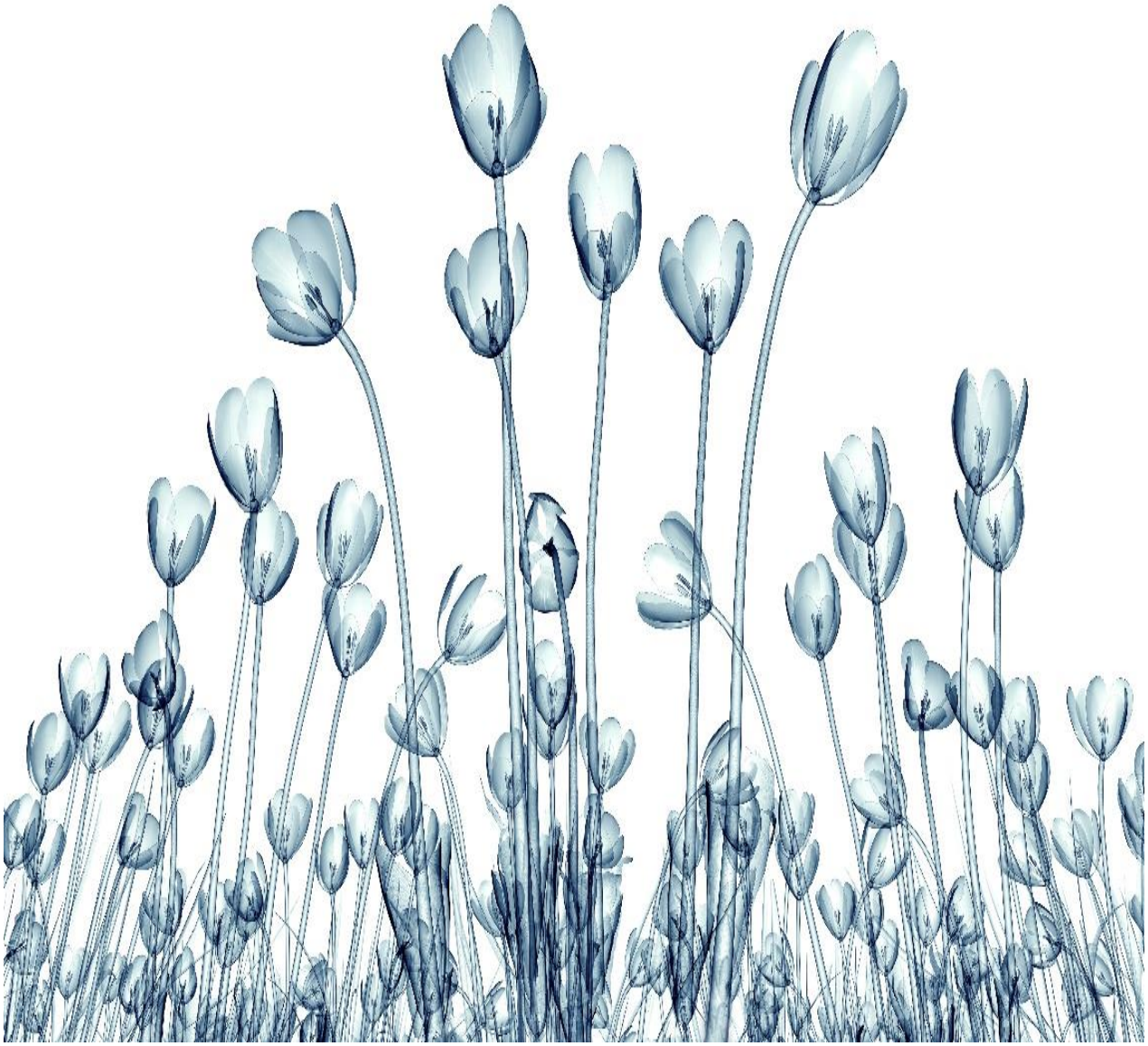
Mutagenesis is one such technique that induces diversification of genotypes of targeted organisms. Mutagen induced changes could either be random or targeted based upon the suitable mutagenesis technique. Biocontrol-associated characters of mutants obtained by successful screening and analysis can be significantly improved.

During the past few decades, genetic improvement of *Trichoderma* spp. by induced mutation using physical and chemical mutagens have been attempted successfully to ameliorate the efficacy of native strains (Mukherjee and Mukhopadhyay, 1993, Hazra, 2003, Walnuj and Jhon, 2013). Such mutation brings about changes in various morphological features and different physical and biochemical attributes as well as potential increase in antifungal metabolites of the selected mutants as compared to their wild parents has also been observed. Certain mutants of *Trichoderma* spp. have been to have better rhizosphere competence compared to their parent strains. Selection of such beneficial mutants may be a better avenue for the management of plant pathogens

In the present investigation, an attempt has been made to generate UV induced mutants of two native isolates of *Trichoderma* species whose antagonistic potentials have already been evaluated. The objectives laid down for the present study include

a. Molecular characterization through ITS sequencing of the native isolates of *Trichoderma* species.
b. Exposure of the isolates to different doses of UV radiation to develop mutants and also to study the stability of the mutants in subsequent generations.
c. Evaluation of *in-vitro* antagonistic property of the stable mutants against the soil-borne fungal pathogens *Fusarium oxysporum* f.sp. *lentis* and *Rhizoctonia solani* by dual culture method.

2. REVIEW OF LITERATURE



2. REVIEW OF LITERATURE

The pertinent writing obtainable on the current work, "Antagonistic potential of mutants of *Trichoderma* species" is evaluated hereafter with more prominence on neoteric evolution in *Trichoderma* research in a more feasible way.

2.1 *Trichoderma* as Biological Control agent

Trichoderma species belonging to the Class Ascomycetes are omnipresent soil colonizing fungi (Domsch et al., 1980; Christensen, 1981) which represents around one third of all fungal species used for biological control of plant pathogens (Chernin and Chet, 2002). The teleomorph of the genus is *Hypocrea* (Samuels, 1996) with order Hypocreales and family Hypocreaceae. In 1932, the importance of the genus *Trichoderma* as biological control agent was established by Weindling (Pandya et al., 2011). The genus was first suggested by Persoon in 1974 and the first field success of biocontrol using *Trichoderma* against *Sclerotium rolfsii* was seen in the 1970s. The fungi appear as green spores with sweet aroma due to the presence of volatile compound 6-pentyl-a-pyrone. Some important species of the genus includes *T. harzianum, T. viride, T. koningii, T. hamatum, T. aureofaciens, T. asperellum, T. atroviride,* etc.

Trichoderma plays an immense role in ecosystem health through production of elicitors and induction of plant resistance through root colonization. Root symbiosis instigates Induced Systemic Resistance (ISR) and to some extent Systemic Acquired Resistance (SAR). The agents colonize and grow as endophytes (between of living cells), and emerge in plant litter, soil organic matter (saprophytes) (Harman et al., 2004; Samuels., 2006) and in mammalian tissues as human pathogens. *Trichoderma* species shows antagonistic activity towards a number of soil pathogens (Wright, 1956). The potential for biocontrol of plant pathogenic fungi (Papavizas, 1985) was detected when *Trichoderma* spp. showed antagonism to *Fusarium solani* and *Fusarium oxysporum* in field and pot tests (Kudryavtseva, 1980; Sychev and Shaposhnik, 1982). They degrade xenobiotic pesticides (Harman, 2006) reducing harmful effects on the environment and are used for suppressing plant diseases or increasing industrial enzyme production (Benitez et al., 2004; Mukherjee et al., 2013).

2.2 Plant Pathogen Control by *Trichoderma* spp.

Repeated research on *Trichoderma* bio-control potential exploitation revealed its effectiveness in controlling various plant diseases through its antagonistic activity on

pathogens. There are also reports of multiple actions (Saha and Pan, 1996; Bell et al., 1982; Sarmah and Mukhopadhyay, 1999). *Trichoderma* spp. elevates yield of vegetable crops besides minimizing disease incidence (Tchameni et al., 2011).

Table 2.1. Potential of genus *Trichoderma* as biocontrol agent in different host pathogen interaction

Crop	Pathogen/ Disease	Trichoderma spp.	References
Soybean	*Rhizoctonia solani*	*T. harzianum*	Singh et al., 1973
Sesame	*Curvularia lunata*	*T. viride*	Tamimi and Hadvan, 1985
Black gram	*Macrophomina phaseolina*	*T. viride*	Raghuchander et al., 1997
Groundnut	*Thievaliopsis basicola*	*T. harzianum*	Biswas and Sen, 2000
Wheat	*Rhizoctonia* sp.	*T. atroviridae*	Innocenti et al., 2003
Cabbage	*R. solani*	*T. viride*	Sharma et al., 2003
Bean	*Sclerotinia sclerotiorum*	*T. koningii*	El- Fiky et al., 2006
Tomato	*F. oxysporum*	*T. viride*	Singh, 2007
Capsicum	*Alternaria alternata*	*T. virde*	Kapoor, 2008
Cumin	*F. oxysporum f spcumini*	*T. viride*	Chawla and Gangopadhyay, 2009
Potato	*R. solani*	*T. harzianum*	Basu, 2009
Rice	*R. solani*	*T. harianum*	Naeimi et al., 2010
Ginger	*Pythium aphanidermatum*	*T. harzianum*	Gupta et al., 2010
Spinach	*Pythium sp.*	*T. virens*	Siameto et al., 2010
Brinjal	*F. solani*	*T. viride*	Balaji and Ahir, 2011
Pigeon pea	*Fusarium udum*	*T. viride*	Hukma and Pandey, 2011
Cowpea	*R. solani*	*T. harzianum*	Pan andDas, 2011
Garden sorrel	*Alternaria tenuissima*	*T. viride*	Ambuse et al., 2012
Cauliflower	*R. solani*	*T. viride*	Rehman et al., 2012
Mushroom	*Rhizopus stolonifer*	*T. viride*	Rawal et al., 2013
Tobacco	*R. solani*	*T. viride*	Sumana et al., 2013
Fenugreek	*Alternaria brassicae*	*T. viride*	Trivedi et al., 2013
Safflower	*Fusarium moniliforme*	*T. viride*	Jat and Agalve, 2013
Chili	*Colletotrichum capsici*	*T. virens*	Rahman et al., 2013
Onion	*Colletotrichum circinans*	*T. harzianum*	Abo-Elyousr et al., 2014
Taro	*Phytophthora colocasiae*	*T. asperellum*	Nath et al., 2013
Chickpea	*Sclerotium rolfsii*	*T. harzianum*	Saxena et al., 2014
Lettuce	*R. solani*	*Trichoderma spp.*	Pinto et al., 2014
Garlic	*Sclerotium cepivorum*	*T. harzianum*	Dilbo et al., 2015
Cucumber	*S. rolfsii*	*T. aureoviride*	Kotasthane et al., 2015
Pea	*Erysiphe pisi*	*Trichoderma spp.*	Patel et al., 2016

2.3 Growth and Sporulation of *Trichoderma*

2.3.1 Growth

Potential of *Trichoderma* is governed by the existence of higher levels of plant roots on which they colonize promptly. The species does not thrive at temperature above $28^{O}C$. Carbon and energy requirement of *Trichoderma* is fulfilled by monosaccharides, disaccharides, complex polysaccharides, pyrimidine, purine, aldehydes, amino acids, organic acid, long chain fatty acid, methanol and formate. Ammonium serves as a source of nitrogen in buffer media and various other nitrogen sources like urea, amino acids, nitrite and nitrate aid in vegetative growth. Sodium chloride decreases growth of *Trichoderma*, whereas; inorganic salts (salts of magnesium) increases growth of *T. viride*. Effects of CO_2 on growth depend on pH of the media and concentration of CO_2, where rapid growth of *Trichoderma* is observed at higher CO_2 concentration and in alkaline medium. HCO_3^- has a greater effect on *Trichoderma* growth than CO_2 does.

2.3.2 Sporulation

Trichoderma spp. are photosensitive and they sporulate on artificial and natural substrates. In response to alteration of light and darkness, they emerge as a concentric pattern of alternating rings. Sporulation increases when agar plates are subjected to light for 20-30 sec of 85-90 lux intensity. Maximum photoinduction occurs at 380 and 440 nm. Photoinduced sporulation is inhibited by Azaguazine, Actinomycin D, Ebr, Phenyl alcohol. Formation of chlamydospores by *T. harzianum*, *T. viride*, *T.hamatum* and *Gliocladium virens* eventuates in solid and liquid fermentation media.

2.4 Environmental Factors Affecting Action of *Trichoderma*

Successful biological control of plant pathogens can be achieved when an organism persist, grows and carries out its metabolic activity in an introduced environmental condition. Fundamental knowledge of ecological relationship of the divergent population of a species is required for effective control of plant pathogens.

2.4.1 Effect of Temperature

A wide domain of temperature relation is revealed by various *Trichoderma* isolates. Sixteen *Trichoderma* isolates showed growth at optimum temperature from 22 to $34^{O}C$ and

along with a continuity of temperature maxima with a range from 28 to 42°C, using poly-temp thermostat (Danielson and Davey, 1973a). A large no. of isolates shows growth inhibition at 35°C, and maxima above 38°C was observed in a small number of populations. Isolates of *T. saturnisporum*, *T. pseudokoningii* and *T. harzianum* had higher temperature maxima. Maximum temperature between 30 and 31°C is observed in white *Trichoderma* strains (*Pachybasium*) (Gutter, 1957). *T. viride* and *T. polysporum* thrive in low temperature as low as 2^{O}C, whereas growth stops below 5°C in case of *T. longibrachiatum* and *T. harzianum*.

Volatile and non-volatile antibiotic metabolites are produced at 10°C and 5°C by *Trichoderma* isolates along with interaction of hyphae and mycelium of test fungi (Tronsmo and Dennis, 1978). Kohl and Scholsser (1989) noticed more than 94% average germination at 24°C after 24 h in 24 isolates of *Trichoderma* kept at different temperatures of 5°, 10° and 24°C. Only the cold tolerant isolates showed growth and germination after 4 days at 5°C. Thus, biological control activity of *Trichoderma*sp is effective in both high and lower temperature conditions.

2.4.2 Effect of Light

Gressel and Hartmann (1968) noticed the photosensitive nature of *Trichoderma* which sporulates on various substrates with specific patterns due to diurnal change in light and darkness during the growth period. Sporulation stops below 254nm or above 1,100 nm with maximum activity at 380nm and 440nm. Exposure to daylight for 3 min or to near UV radiation (366nm) for duration of10-30 sec is best of phialoconidiogenesis photoinduction (Betina and Spisiakova, 1976).

2.4.3 Effect of pH Concentration

pH of the soil determines the fungal propagule's density. pH range from 3.7 to 4.7 is optimum for *Trichoderma* growth (Danielson and Davey, 1973). Extreme acidity and neutrality is detrimental on the growth of *T. saturnisporum* and *T. pseudokoningii*. Less than 10% growth in media of pH 6.2 was observed with an exception for *T. hamatum* and *T. harzianum*. pH 6 of clay loam soil along with 10 *Trichoderma* propagules/ g soil is advantageous for disease development. Columbia soil with high organic content holds 10^{8} propagules/ g soil (Chet and Baker, 1981). Martin et al. (1985) suggested the high level of antagonist propagule in acidic soil.

2.4.4 Effect of Carbon dioxide and Bicarbonate

In 1962**,** Waid observed the inhibition of *Trichoderma* by 3-5% CO_2 whereas; Macauley and Giffin (1969) found relatively no effect by 10 or 20% CO_2 levels. Danielson and Davey (1973b) reported the proper growth of *Trichoderma* in high level of CO_2 in basic medium than on an acidic medium. They also saw the inhibition of *Trichoderma* with addition of HCO_3^- to an alkaline medium. Extremely high pH values showed positive or negative effect on growth (Macauley and Griffin, 1969b).

2.4.5 Effect of biocides/fungicides

Instead of ineffective single control strategy (Martin et al., 1985), IPM practices can extend the duration and effectiveness of control. There are reports of *Trichoderma* dominance in soil due to use of biocides like methyl bromide (Elad et al., 1982), metham sodium (Henis and Papvizas, 1983), etc. Captan, chloroneb, vinclozolin, chlorothalonil, iprodione, pyrocymidone have no inhibitory action on *Trichoderma* (Abd-El Moity et al., 1982; Ordentlich and Chet, 1989). Treatment with Thiram and Metalaxyl shows positive compatibility with *Trichoderma* spp. (Davet and Papavizas, 1981).

Disease control of radish damping off was obtained by use of a combination of *T. harzianum* and PCNB, which reduced the inoculum potential of *R.solani* (Henis et al. 1978). *T. harzianum* alone or in combination with Vitavax-200 fungicide was reported to control chickpea wilt complexes (Kaur and Mukhopadhyay, 1992).

2.5 Strain improvement in *Trichoderma*

Biocontrol agent including *Trichoderma* by own nature is not as efficient as chemical fungicides but can provide sustainable control of pathogens of interest. This advantage encouraged the microbiologists and plant pathologists to explore different biotechnological approaches to enhance their better utilization (Monte, 2001). The antagonistic potential of different hyperparasitic fungi can be enhanced by different methods like mutation, protoplast fusion and genetic transformation (Fahmi et al., 2012). Strain improvement of wild type *Trichoderma* by mutation is a successful method to enhance the antagonistic activity, rhizosphere competence, antibiotic production, production of cell wall degrading enzymes like chitinases, glucanases, cellulases in addition to growth promotion and induction of resistance in plants against various stresses (Hazra, 2003, Roy, 2003, Villena and Guttierezz-Correa, 2006). Further the strategy can also be employed to develop the strain more tolerant

to the fungicides that are widely used in plant protection (Shi and Feng, 2006). Mutation as a natural phenomena leads to genetic variability which needs proper attention. Advances in molecular technology have paved the way to create improved biocontrol agents through physical and chemical mutagenesis. Induction of random mutation by physical mutagens like UV, X and gamma radiation, and chemical mutagens such as ethyl methane sulfonate and N-methyl-N-nitro-N-nitrosoguanidine have been used as useful tools to manipulate antagonists genetically (Cattlet et al., 2003).

2.5.1. UV Ray as Mutagen

The non-ionizing ultraviolet rays having wavelength between 1000 to 4000 Å causes excitation and photochemical reaction which results in increased chemical reactivity inside the tissue on exposure. These rays are produced in mercury vapour lamps and the desired plants are exposed to tubes designed for UV rays treatment.

Trichoderma spp. mutated utilizing UV light, produces two new antifungal compounds along with antibiotic produce by the parental strain (Graeme-Cook et al., 1991). Mutated forms of *T. harzianum* are more rhizosphere competent with exorbitant cellulase activity than non-competent ones (Baker, 1988) and mutants with velvet protein (Vel 1) deleted, generate more chlamydospores under nutrient stress condition in contrast with the wild type (Mukherjee et al., 2013). In certain case the increased antibiotic levels slow down the colonisation of pathogens like *Fusarium oxysporum* and *Rhizoctonia solani* showing great variability depending on the kind of the *Trichoderma* isolate and on the test fungus (Graeme-Cook et al., 1991).

Mutation is employed in *Trichoderma* to improve various properties like survival ability, fungicide tolerance, antagonistic potentiality, genetic variability, colonizing ability and competitive saprophytic ability (CSA) (Pecchia and Anne, 1989; Mukherjee and Mukhopadhyaqy, 1993; Migheli et al., 1998, Mukherjee et al., 1999; Haggag et al., 2010) and strain improvement. *Trichoderma* species (*T. ressei* QM9414) treated with UV radiation and sodium nitrate (0.5 mg/ml) for duration of 45 min shows hyper-production of cellulase (1.5-1.75 fold) (Gadgil et al., 1995). Four mutant strains produced by UV irradiation of conidia of *T. viride* revealed morphological differences with the parent strain and with 2 to 3 times higher level of beta- glucosidase secretion (Farkas et al., 1981). 3.3 times extracellular protein and 3 times endoglucanase secretion were reported from the mutant strains of *T. pseudokoningii* (Zaldivar et al., 1987). Greater tolerance to Carbendazim than wild parental

strain was found in four stable mutants of *T. viride* and *T. lignorum* obtained through UV radiation (Dutta and Chatterjee, 2004). Selvakumar et al. (2000) used UV light and ethyl-methane sulfonate to develop carboxin tolerant mutant of *T. viride*. Superiority over parental strains in antagonistic activity against root rot and damping off pathogens was observed in three mutants of *T. koningii* and four of *T. ressei* when treated with a combination of UV light and nitrate (Mohamed and Haggag, 2010). Mutagenic treatment with UV light, diethyl sulphate, CO and nitrosoguanidine (NTG) resulted in high cellulase yielding mutants in case of *T. pseudokoningii*.

2.6 Mechanism of *Trichoderma* Action

Various modes of action of *Trichoderma* like antibiosis, mycoparasitism, competition, induced resistance, tolerance to biotic and abiotic stress, etc may operate independently or together to suppress plant pathogens.

2.6.1 Antibiosis

It is the phenomenon where secretion of certain chemicals by one microorganism affects the growth and reproduction of another one. It involves direct lysis of pathogen. Weindling in 1934 first reported this mechanism of *Trichoderma* against *R. solani* which he later named as 'gliotoxin'. Antibiotics produced by *Trichoderma* are very specific in their reaction and inhibit growth of microorganisms (Benitez et al., 2004). The availability of N and C sources of media determines the amount of volatile antibiotics i.e. pyran, acetaldehyde and ethanol etc. The chemical in secondary metabolites produced by *Trichoderma* helps it to compete with other micro and macro organisms, metal transport, symbiosis, differentiation, *etc*. (Demain and Fang, 2000).

Toxic metabolites produced by *Trichoderma* strain like viridin, gliovirin, massoilactone, harzianic acid, tricholin, glisoprenin, heptelidic acid hinder colonization of other microorganisms (Vey et al., 2001; Reino et al., 2008). *Trichoderma* produces three types of secondary metabolites: (a) volatile antibiotic of isocyanide derivative like 6-pentyl-a-pyrone, (b) water soluble compounds like koningic acid or heptelidic acid, and (c) peptaibols which are 15-22 amino acids long linear oligopeptides (Le Doan e al., 1986; Rebuffat et al., 1989).

Secondary metabolism is process that is linked to reproduction in fungi. Structural classification of secondary metabolites produced by *Trichoderma* was done by Luckner

(1990) and Turner and Aldridge (1983). Peptaibols in tobacco plants provides defence against Tobacco mosaic virus (Wiest et al., 2002). Howell in 2003 reviewed antibiosis and secondary metabolites production by *Trichoderma*. Polyketides forms the largest fungal secondary metabolites group. They help in competition for nutrients by reducing the competitor capability and establishing chemical communication with other surrounding organisms (Khosla, 2009; Mukherjee et al., 2012). The most abundant and widely distributed metabolites of secondary origin is terpenes (Claydon et al., 1987). Daguerre et al.(2014) summarized and illustrated various pathways which produce secondary metabolites like pyrone biosynthesis pathway, peptaibol biosynthesis pathway, polyketide biosynthesis pathway, flocculosin, gliotoxin biosynthesis pathway, terpenoid and gliovirin biosynthesis pathways.

Hydrophobic peptides trichaziannines are produced by UV treatment of *T. harzianum* which induce membrane permeability. Mutants which produce toxic chemical in small amount have less antagonistic potentiality (Wilhite et al., 1994). Certain mutants remain infective even in lack of mycoparasitism and toxin producing capacities (Howell, 2002; Howell et al., 2000).

Table 2.2 Antibiotics isolated from *Trichoderma* spp.

Antibiotic	**Type**	***Trichoderma* spp.**	**Target Organism**	**References**
6-n-pentenyl-2 H pyran-2 one	Volatile	*T. harzianum*	*Pythium, Macrophomina, Sclerotium, Rhizoctonia*	Claydon et al. (1987)
Trichodermin	Non- volatile	*T. ressei, T. virde*	*Rhizoctoniasolani, Fusariumoxysporum, Botrytis cinerea*	Haggag and Mohamed (2002)
Dermadin	Non- volatile	*T. harzianum, T. koningii*	Fungi, gram + ve& gram – ve bacteria	Pyke and Dietz (1996)
Trichoviridin	Non-volatile	*T. viride*	*Rhizoctonia, Fusarium, Slerotium*	Yamano et al. (1970)
Heptalic acid	Non-volatile	*T. hamatum, T. virens*	*Fusarium, Rhizoctonia*	Itoh et al. (1980)
Trichorzianines	Non-volatile	*T. harzianum*	*Rhizoctonia, Sclerotium*	Baldwin et al. (1981)

Antibiotic	Type	*Trichoderma* spp.	Target Organism	References
Isonitrin A, B	Non-volatile	*T. viride, T. harzianum*	*Macrophoina, Sclerotinia, Sclerotium*	Baldwin et al. (1991)
Gliovirin	Non-volatile	*T. virens*	*Pythiumultimum*	Howell and Stipanovic et al. (1983)
Gliotoxin	Non-volatile	*T. virens*	*Rhizoctoniasolani, Macrophominaphaseolia*	Pahl et al. (1996); Tanaka et al. (1998)
Viridin	Non-volatile	*T. virens, T. viride*	*Botrytis allii, Colletotrichumlini, Fusariumcaeruleum*	Dodge et al. (1995); Brian and Mc Gowan (1945)
Viridiol	Non-volatile	*T. virens, T. hamatum*	*Macrophomina, Rhizoctonia, Sclerotium*	Sakuno et al (2000)
Cerinolactone	-	*T. cerinum*	*Pythiumultimum, Rhizoctoniasolani, Botrytis cinerea*	Vinale et al. (2006)

2.6.2 Mycoparasitism

The mechanism of mycoparasitism involves the existence of one fungus in intimate association with another from which it derives all or a small amount of its nutrient while providing no return benefit. This mechanism is one of the main forms involved in antagonism of *Trichoderma*. Druzhinina et al. (2011) defined the concept as the ability of *Trichoderma* to feed on dead fungi. The whole process involves chemotropic growth of *Trichoderma*, host recognition, extracellular enzymes secretion, hyphal penetrations and host lysis (Zeilinger et al., 1999). Different interactive sessions takes place in this mechanism like accumulation of parasite organelle in the parasitizing cells, coiling of hyphae, deposition of extracellular fibrillar, production of sheath matrix and haustoria formation (Tu, 1980; Chu and Wu, 1981; Tu and Vartaaja, 1981; Elad et al., 1983; D' Ercole et al., 1981; Wu et al., 1986; Kumar et al., 1998; Gupta et al., 1999; Anitha and Murugesan, 2001). The host destruction and flow of nutrients in mycoparasite is due to the production of extracellular enzymes chitinase, beta-1,3 glucanase, cellulase, lipase and protease (Sharma et al., 2012). Growth of *Trichoderma* towards hosts by recognizing them is due to the production of these pathogenesis related proteins (Harman et al., 2004). All these enzymes attack the tips of the hyphae of pathogen and result in the cell wall thinning, turgor pressure imbalance and finally the protrusion and bursting of hyphal tips. Gajera et al. (2013) observed the fungal pathogen growth inhibition

due to secretion of exo-chitinases resulting in fungal cell wall degradation at lower level of enzyme secretion. Pathogenesis related peptides from appressorial content released into the pathogen help in hyphal entry and cell wall digestion of *Trichoderma* (Howell, 2003). Mycoparasitism and nutrient uptake are done by the ATP- binding cassette (ABC) transporter protein of *Trichoderma* (Ciliento et al., 2006).

The mycoparasitic relationships towards hosts are mainly of 2 types: one where the interaction is biotrophic (feeding on fungal pathogens without causing its death) and the second one is necrotrophic (aggressive reaction towards prey). Due to broad antagonistic capacities, *Trichoderma* genera are considered to exert necrotrophic mycoparasitism for their effectiveness as biological control agents. High concentration of glycerol (osmotic solutes) is found in appressorium like structures (McIntyre et al., 2004). *Trichoderma* hyphal entry in lumen of parasitized fungus is due to appressoria formation and CWDEs production along with peptaibols (Howell, 2003). McIntyre et al. (2004) investigated the signal transduction pathways of *T. atroviride* which led to the isolation of various components like cAMP (cyclic adenosine monophosphate) and MAPK (mitogen- activated protein kinase). All these components play their role in enzyme secretion, antibiotic production and hyphal coiling. *T. harzianum* secretes pachybasis and emodin which can parasitize *R. solani* through cAMP accumulation in *Trichoderma* (Lin et al., 2012). Cell wall degrading hydrolytic enzymes are important components for chitin degradation of fungi for nutritional purposes (Gruber and Seidl-Seiboth, 2012). In different *Trichoderma* spp. proteases were found for cell wall degradation of fungi (Geremia et al., 1993; Seidl et al., 2009; Suarez et al., 2007). Szekeres et al. (2004) isolated protease overproducing *Trichoderma* strains (*T. harzianum*) playing their role in mycoparasitism.

Mutants of *Trichoderma* are unable to form coils around host hyphae due to the effect in gene (tga3) which resulted in production of low cAMP intracellular levels (Zeilinger et al., 2005). Certain mutants irradiated by UV radiation were effective against *Fusarium culmorum* and *R. solani*. Daguerre et al. (2014) studied the mycoparasitism process in *Trichoderma* sp. using gene-for-gene experiments and which needs further studies. Certain mutants lack capacity in their antagonistic potentiality due to low toxin production (Wilhite et al., 1994).

2.6.3 Competition

Competition is the injurious effect of one organism on another due to utilization of resources of the environment (Clark, 1968). *Trichoderma* sp. protects crops from pathogens

with its ability to compete with later for food and space (Alexannder, 1982; Cook and Baker, 1983). *Trichoderma* competes for carbon which is essential for viability (Sarroco et al., 2009; Alabouvette et al., 2009). Population level of 1×10^6 units/g of soil is required for antagonist to control rhizome rot disease. This was seen while in a field experiment was conducted using *T. viride* and *T. harzianum*. Soil properties like temperature, pH, moisture content and available iron content affect the competitive ability vis-a-vis biocontrol potentiality of *Trichoderma*. On the other hand, *Trichoderma* can survive in a competitive environment by producing structures like conidia and chlamydospores.

In low iron content of soil, aerobic and facultative anaerobic micro-organisms produces low molecular weight, small sized ferric-iron chelators called siderophores. Siderophores produced by *Trichoderma* are able to restrict the growth of harmful fungi at plant roots (Chet and Inbar, 1994). Germination of fungal spores of pathogen is inhibited due to iron starvation in the rhizosphere and rhizoplane.

Botrytis cinerea is effectively controlled due to competition from *Trichoderma* (Latorre et al., 2001). Proteins plays an important role in *Trichoderma* by root colonization activity and helping in competing with various root colonizers (Saloheimo et al., 2002; Viterbo et al., 2004; Brotman et al., 2008) and some establish symbiotic relation with host plant (Samolski et al., 2012).

Different rhizosphere competent strains of *Trichoderma* are obtained by mutagenic agent treatment. They show correlation between competitive saprophytic ability (CSA) and rhizosphere competence and ability to utilize cellulose associated with root. *Trichoderma* utilizes ATP from different sources of environment like glucan, cellulose, chitin and others and turns them to glucose (Chet et al., 1997). In Europe, carbon disulfide is used for control of *Armillaria mellea* as the sublethal dose of the chemical gives. *Trichoderma* provides a competitive advantage as antagonist to the *A. mellea*. Antagonist mainly encircles the infection court during competition with pathogens for space (Papavizas, 1985; Barbosa et al., 2001).

2.6.4 Endophytism

Endophytes are indulged in stimulating plant growth by reducing drought stress and blocking pathogens (Piotrowski and Volmer, 2006). In this case hyphae grow between cortical cells without causing harm to the host. Endophytic activity of *Trichoderma* isolates

against woody plants is noticed recently (Gazis and Chaverri, 2010; Chaverri and Gazis, 2011). Alteration of pattern of gene expression in shoot is seen due to such association which may improve traits in plants like abiotic/biotic stress resistance, photosynthetic efficiency and higher yields (Chaverri and Samuels, 2013; Harman et al., 2012). All endophytic species are classified by phylogenetic analysis. Certain *Trichoderma* sp like *T. koningiopsis, T. stromaticum, T. stilbohypoxyli* showing exception within their group at terminal position explains the recent evolvement of endophytism trait in *Trichoderma* sp (Chaverri et al., 2011; Samuels et al., 2006; Samuels and Ismiel, 2009; Druzhinina et al., 2011).

2.6.5 Induced Resistance

Plants (mono and dicotyledonous) show high degree of resistance when pre-treated with *Trichoderma* (Harman et al., 2004). *T. harzianum* strain T-39 which is a active ingredient in Trichodex controls diseases caused by *Botrytis cinerea* and *Colletotrichum lindemuthianum* on root application (Biigirimana et al., 1997). De Mayer et al. (1998) saw similar activity from *B. cinerea* to other dicots. Ten different dicots and monocots were resistant to various fungi (*R. solani, Alternaria spp., B. cinerea, Magnaporthe grisea*, etc), bacteria (*Pseudomonas syringae, Xanthomonas* spp., etc) and viruses like CMV.

In model plants, only the strain T-22 of *T. harzianum* induces systemic resistance (Contreras-Cornejo et al., 2011; Salas-Marina et al., 2011; Yoshioka et al., 2012). Harman (2006) believed that the induced systemic resistance was one of the most important mechanisms in *Trichoderma*. *Trichoderma* spp. in treated roots penetrate into the roots and are restricted to epidermis and cortex and thus strengthen them. Bacterial spot reduction to the extent of 69.32% was observed in tomato plants (cv. Sida cultivar) after 14 days post inoculation with isolate of *T. harzianum* (T9) (Saksirirat et al., 2009). Many similar examples can be seen like reduction in 19.23% spots (grey leaf spot: *Stemphylium solani*) after 10 days post inoculation with *T. asperellum* (T18). Various terpenoid phytoalexin synthase protects cotton against the pathogen *R. solani* through artificial inoculation. Oxidative stress brings about the production of various enzymes and Pathogenesis Related Proteins (PRs) for resistance development in plants (Gajera et al., 2013).

Table 2.3 Induced systemic resistance triggered by *Trichoderma* spp.

***Trichoderma* species and strain**	**Crop**	**Pathogens**	**References**
*T. harzianum*T-39	Bean	*B. cinerea, Colletotrichum lindemuthianum*	Bigirimana et al., 1997

***Trichoderma* species and strain**	**Crop**	**Pathogens**	**References**
T. harzianum T-39	Tobacco, tomato, bean	*B. cinerea*	De Meyer, 1998
T. virens T3; *T. harzianum*T-1 & T22	Cucumber	*Green-mottle, mosaic virus*	Lo et al., 2000
Trichoderma GT3-2	Cucumber	*P. syringae pv. lachrymans*	Koike et al., 2001
T. harzianum T-22	Tomato	*A. solani*	Seaman, 2003
T. harzianum T-22; *T. virens* T3	Bean	*B. cinerea, X.campestris pv. phaseoli*	Harman et al., 2004
T. asperellum (T203)	Cucumber	*P. syringae pv. lachrymans*	Shoresh et al., 2005
T. harzianum	Pepper	*Phytophthora capsici*	Ahmed et al., 2009
T. virens G-6, G-6-5 and G-11	Cotton	*R. solani*	Howell et al., 2009
T. harzianum T-39	Grapes	*Plasmopara viticola*	Perazzoli et al., 2012
T. Asperellum SKT-1	*Arabidopsis thaliana*	*P. syringae pv. tomato* DC3000	Yoshioka et al., 2012
T. harzianum Tr6, and *Pseudomonas* sp. Ps14	Cucumber and *A. thliana*	In cucumber- *F. oxysporum f.sp. radicis-cucumerinum* and in *A. thaliana* against *B. cinerea*	Alizadeha et al., 2013
T. asperellum SKT-1	*A. thaliana*	*Cucumber mosaic virus*	Elsharkawy et al., 2013
T. harzianum T-39	Tomato	*B. cinerea*	Meller et al., 2013
T. harzianum T-39	Bean, tomato, cucumber	*B. cinerea* and *Podospaera xanthi*	Levy et al., 2015
T. atroviride and *T. virens*	Tomato	*A. solani, B. cinerea* and *P. syringae pv. tomato (*Pst DC3000)	Salas-Marina et al., 2015

2.7 Importance of *Trichoderma* in Agriculture

2.7.1 In Food and Textiles Industries

Trichoderma species are used for commercial production of cellulases and other enzymes due to their capability to efficiently produce extracellular enzymes (Kredics et al., 2005). These enzymes are used in the food and textile industries due to their ability to degrade complex polysaccharides. They are used for brewing process (beta-glucanases), livestock feed additives (xylanases), in fruit juice production as macerating enzymes (pectinases, hemicellulases, cellulases). Cellulases play their role in malting, baking and grain alcohol production. For food preservation *T. harzianum* is used due to their antifungal effect. The first product with characteristic coconut-like aroma (6-pentyl-alpha-pyrone) having antibiotic properties was isolated from *T. viride*, the improved concentration of which now is obtained from *T. harzianum* (Oda et al., 2009). Biostoning of denim fabrics is done using

cellulases to make soft, white and stone-washed denim. For textile enzymes production *T .ressei* is said to be the most important species (Puranen et al., 2014). In poultry farming also these enzymes are used as feed in order to increase the digestibility of hemicelluloses from various crops.

2.7.2 Wood Preservation

Trichoderma spp. is used to protect wood by controlling brown rot fungi, *Antrodia carbonia* and *Neolentinus lepideus* (Highley and Ricard, 1988) and dry rot fungus, *S. lacrymans* (Score and Palfreyman, 1994). For successful field application of biocontrol agent, *in-vitro* application techniques play a major role. Various nutrient compositions in wood may affect the interactions of antagonists (Score and Palfreyman, 1994). Rate of hyphal growth of *Trichoderma* spp. may be affected by such nutrients and studies on components like tannin and lignin should be taken into view.

For over a period of 11 months, *T. viride* was able to control the decay of wood by *Gloeophyllum* sp. and *G. sepiarium* in field conditions in tropical environments (Ejechi, 1997). When compared with *Penicillium*, *Trichoderma* spp. gives more efficient protection against wood decay fungi (Bruce and Highley, 1991; Highley and Ricard, 1988). Against certain basidiomycetes, *Trichoderma* spp. are efficient in wood protection studied using soil burial test systems (Tucker et al., 1997) and modified versions of American (1997) and European standard (1980).

2.7.3 As Source of Transgenes

Genes cloned from *Trichoderma* spp. produces plant disease resistant crops. These transgenic strains have recombinant nucleic acid molecule which in turn encodes a bioactive molecule to control plant diseases. They are applied to plant or plant seed under conditions (European Patent Office, 2005).

2.7.4 Fruits and Vegetables

Investigation of Mortuza and Ilag (1999) using 10 *Trichoderma* isolates to control fruit rot of banana (*Lasiodiplodia theobromae*) confirmed the association of mycoparasitism with antibiosis and competition for substrate. *T. harzianum* and *T. viride* were present in such 10 isolates list. On working with *T. viride* for controlling mango stem-end rot, Moreno and Paningbatan (1995) reported the mycoparasitism and antagonism as major biocontrol activities. To control post-harvest decay of apple, Batta (2004) examined the action of *T.*

harzianum formulation against blue mold infection in which invert emulsion application mode turned out to be best for fungi like *Trichoderma*. Greenhouse strawberries could be protected from post-harvest rotting using *Trichoderma* spp. application (Hjeljord et al., 2000). Potential antagonism of *Trichoderma* in controlling stem disease of potato was studied by Brewer and Larkin (2005). A study showed the 300-700% increased metabolite production in *Trichoderma* sp. due to the presence of pathogens (Cooney and Lauren, 1998).

2.7.5 Plant Growth Promotion

Trichoderma has ability to promote plant growth (Whipps, 2001; Punja and Utkhede, 2003; Benhamou et al., 1999; Harman and Bjorjmann, 1998). *Trichoderma* spp. produce growth hormones (Harman et al., 2004; Anderson et al., 1993) and help in mineral transfer to rhizosphere (Harman et al., 2004). Due to growth promoting activity of *T. viride* in rhizosphere, the growth promotion of gnotobiotic tomato takes place (Lindsey and Baker, 1967). Due to ectomycorrhizal association of *Trichoderma* spp., they procure substrates like glucosamine, protein- tannin from soil (Heraux et al., 2005; Bae and Knudsen, 2005; Vargas-Garcia et al., 2005). Application of *Trichoderma* spp. in composting wastes in agricultural field influence yield of plants (Heraux et al., 2005; Hutchinson, 1999; Vargas-Garcia et al., 2005).

Resistance induction in plants against pathogens by *Trichoderma* fungi is an indirect growth promotion factor (Yedidia et al., 2000; Benhamou et al., 1999; Zeilinger et al., 1999; Shoresh et al., 2005). Associaion of *Trichoderma* with roots and lytic enzymes was suggested by the authors, which was induced by a plant defense mechanism to demolish pathogen cell wall.

2.7.6 Crops/Seeds

Trichoderma spp. act as fungal antagonists of crops/seeds pathogens. Pre-treatment of cowpea seeds in *T. viride* spore suspension controls brown blotch disease (*Colletotrichum truncatum*) (Bankole and Adebanjo, 1996). Studies showed that fungal diseases of wheat (Perello et al., 2003) and sunflower (Escande, 2002) were controlled by mycoparasitic and antibiosis activities of *Trichoderma* spp. Pre-treated cotton seedlings with *T. virens* restrain pathogen germination (Howell and Puckhaber, 2005).

2.7.7 Anti-Bacterial Potential

There is only meagre application in pathogenic bacterial control using *Trichoderma* spp. The actions are mainly based on the antibiosis activity of *Trichoderma* but no physical interactions with pathogens (Hanson and Howell, 2002; Utkhede and Koch, 2004; Itoh et al., 1980).

Table 2.4. *Trichoderma* spp. against Bacterial Pathogens

Trichoderma sp.	Bacterial Pathogens	Responsible factors	References
T. viride	*Bacteroides fragilis*	Heptelidic acid	Stipanovic and Howell, 1983
T. hamatum	*Escherichia coli, Streptococcus bovis, Ruminococcus albus, Megasphaerae lsdenii* (Rumen bacteria)	Isocyanide metabolite-3 propionic acid	Liss et al., 1985
T. viride	*Serratia* sp. (Post harvest yam rot)	Non volatile and volatile antibiotics	Okigbo and Oikediugwu, 2000
T. harzianum	*Clavibacter michiganensis* (Bacterial canker)	Unknown	Utkhede and Koch, 2004
T. virens, T. koningii	*Bacillus subtilis*	Gliotoxin, viridiol, viridin, dimethyl gliotoxin	Hanson and Howell, 2002

2.7.8 Herbicidal Action

Trichoderma spp. even shows herbicidal activity (Heraux et al., 2005; Hutchinson, 1999; Evans, 1998). *T. virens* is known to control grass weeds either when used independently or in combination with compost manure and rye cover crop (Heraux et al., 2005). Herbicidal activity is also shown by *T. virens* composted with rye cover crop and chicken manure (Heraux et al., 2005; Hutchinson, 1999). Various compounds like viridiol, (3H)- benzoxazolinone (BOA) and 2,4-dihydroxy-1,4-(2H)-benzoxazine-3-one (DIBOA) were found to be release by *T. virens* for its activity (Heraux et al., 2005). More examinations are required to make the products economically feasible.

Table 2.5. *Trichoderma* spp. in control of weeds

Trichoderma spp.	Weeds	Responsible factors	References
T. virens	*Setaria viridis* (broadleaf), *Amaranthus retroflexus* (grass weeds)	Allelochemicals, phytotoxin, viridian, viridiol, gliovirin	Heraux et al., 2005; Hutchinson, 1999
T. viride	Invasive weed (*Lantana*	Lytic enzymes and	Evans, 1998

Trichoderma spp.	Weeds	Responsible factors	References
	camara, Mikania micrantha)	phytotoxins	
T. harzianum	*Phytophthora capsici* (*Capsicum annuum* rot)	Antibiosis; enzymes	Khan et al., 2004 Etebarian et al., 2000
T. virens	*Verticillium dahliae* (Wilt of cotton)	Terpenoids induction	Hanson, 2000
T. koningii	*Gaeumannomyces graminis* var. *tritic i*(Take all of wheat)	Anti-fungal compounds	Simon, 1989; Duffy et al., 1997

2.7.9 Control of Invertebrates (Insects and Nematodes)

Trichoderma spp. have the ability to produce chitin degrading enzymes (exochitinases and endochitinases) to destroy the chitin structure in invertebrates (Cruz and Llobell, 1999; Hoell et al., 2005; Felse and Panda, 1999). *Trichoderma* occupies the rhizosphere zone of soil similar to the ones occupied by nematodes (Kerry, 1981; Windham et al., 1989; Saifullah and Thomas, 1996; Spiegel and Chet, 1998; Sharon et al., 2001; Eapen et al., 2005). *Trichoderma* spp. attack the chitin rich cellular structure of nematodes. Due to increased permeability of nematode eggshell, staining of eggs becomes easy for microscopy (Eapen et al., 2005).

T. harzianum shows insecticidal activity on aphids (Ganassi et al., 2001). It produces toxins inside the cuticle of insects.

Table 2.6. Invertebrates controlled by *Trichoderma* spp.

Trichoderma spp.	Invertebrates	Responsible factors	References
Trichoderma spp.	*Caenorhabditis elegans*; *Meloidogyne incognita* (root knot nematodes)	Antibiosis; enzymes	Eapen et al., 2005
T. viride, T. koningii, T. hamatum, T. longibrachiatum	*Atta cephalotes* (leaf cutting, fungus growing ant)	Proteases, chitinases, lipases, 1,3-beta-Glucanases	Ortiz and Orduz, 2001; Lopez and Orduz, 2003
T. harzianum ITEM 908 and ITEM 910	*Schizahis graminum* (Aphid)	Proteases, lipases, polysaccharide lyases	-

2.7.10 Bioremediation

Trichoderma spp. are used for bioremediation of soil contaminated with pollutants by degrading polycyclic aromatic hydrocarbons (PAHs) (Azcbn-Aguilar and Barea, 1997). *Trichoderma* spp. removes 75% pyrene (4-ring PAH) at 5 mg/l which serves as a carbon

source (Saraswathy and Hallberg, 2002). This genus also degrades synthetic dyes, endosulfan, pentachlorophenol and DDT (Katayama and Matsumara, 1991). This procedure can improve soil and plant health and also increase crop yield.

Table 2.7.*Trichoderma* sp. for bioremediation and suggested mode of growth promotion

***Trichoderma* sp.**	**Target compounds/ plant name**	**Target compound concentration/ Growth promoters**	**Bioremediation/ suggested plant growth mechanism**	**References**
T. pseudokoningii strain (O10), *T. harzianum* (O90, O77, O80 and O82)	Cyanide	Cyanide to ammonia	Co-metabolism of cyanide in the presence of glucose	Ezzi and Lynch, 2005
T. virens, T. viride, T. harzianum	Chickpea	Nitrogen and phosphorus	Nutrient supplementation	Rudresh et al., 2005
T. hamatum, T. koningii, T. harzianum	Fluorene	100µg/g soil	Co-metabolism in the presence of glucose	Garon et al., 2004
T. koningii, T. viride, T. harzianum, T. longibrachiatum	Pine	NH4+-N and NO3—N	Competitive inhibition	Celar, 2003
Trichoderma sp.	Polyaromatic hydrocarbons (PAHs)	800-1100mg PAHs/kg soil	Co-metabolism in the presence of glycerol	Potin et al., 2004
Trichoderma sp.	P. resinosa Ait (red pine)	NH4+-N; organic N (amino acid)	Saprotrophic fungus, *Trichoderma* sp. mobilized N from organic compounds via ectomycorrhizal fungi incapable of directly uptaking organic N from soil	Koide et al., 1998; Wu et al., 2003
T. harzianum DB 11	Strawberry, Fragariaananassa	Rhizosphere enhancers-N and P	Mycorrhizal fixation of phosphorus and nitrogen	Vestberg et al., 2004

2.7.11 Commercial Formulations of *Trichoderma* spp.

Commercial products of *Trichoderma* spp. enhance soil and boosts plant growth by having spores as active ingredients (Whipps and Lumsden, 2001; Punja and Utkhede, 2003; Batta, 2004). Mycelia, conidia and chlamydospores have characteristics of stability, production and biocontrol activity. So the best selection of *Trichoderma* spp. propagules are required (Amsellem et al., 1999; Lewis and Papavizas, 1983; Pelczar Jr. et al., 1993). Conidia production of *Trichoderma* sp. is preferred since chlamydospores cannot survive in drying processes (Lewis and Papavizas, 1983), whereas, conidia are less susceptible to various environmental conditions and produced in faster rate and in large amounts (Verma et al., 2005).

Table 2.8. List of *Trichoderma* based commercial formulation

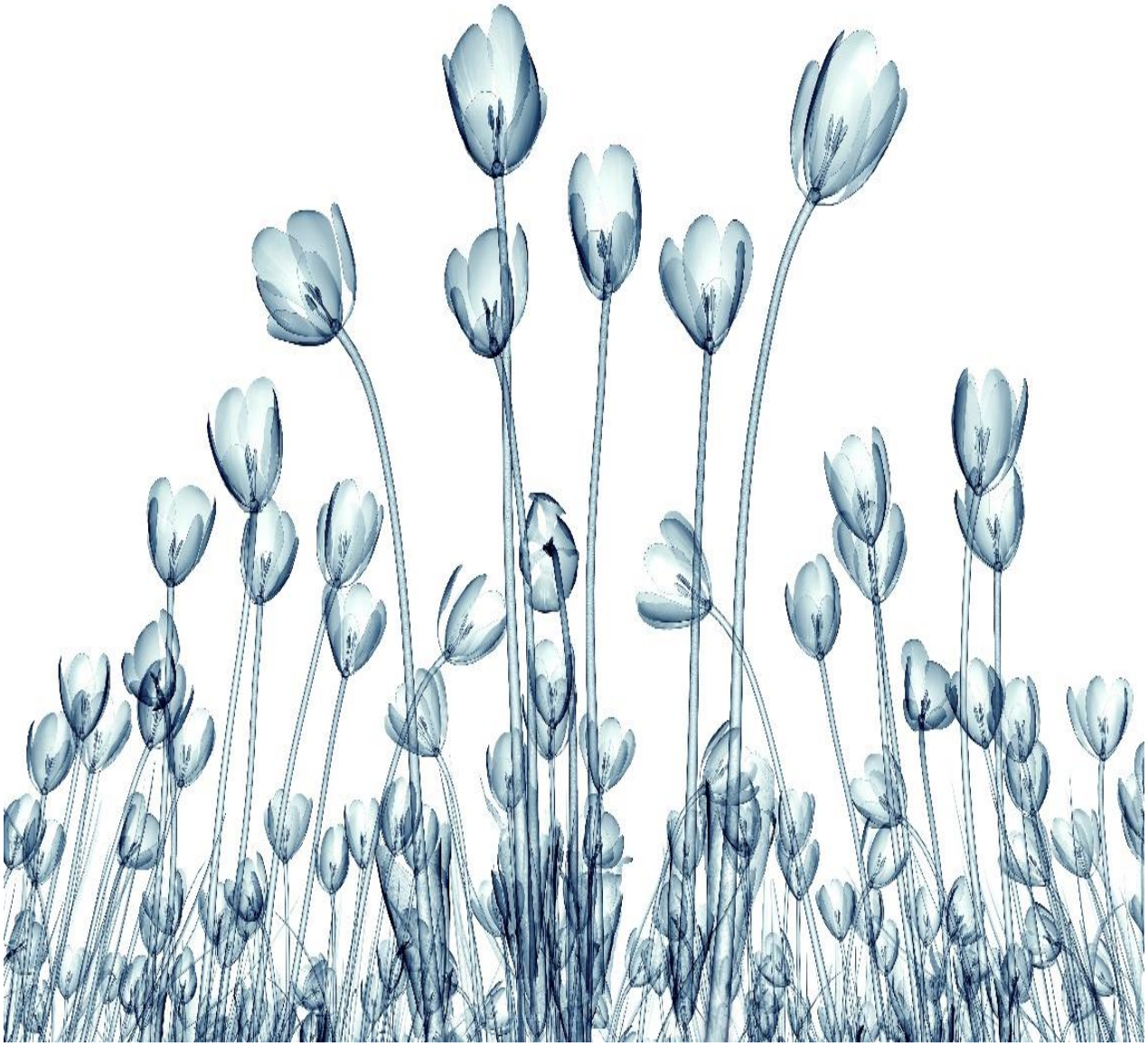
Product Name	Species / Strains	Manufacturer
Antagon T.V.	*T. viride*	Green Tech Agro Product, Coimbatore
BINAB-T	*Trichoderma* spp.	Bio-innovation AB, Sweden
Bioderma	*T. viride*	Biotech International Ltd.
Biocure	*T. viride*	T. Stances & Company Ltd.
BiosparkTrichoderma	*T. pseudokoningii, T. parceramosum*	Biospark Corporation, Philippines
Bip T	*T. viride*	Poland
Biobus 1.00WP	*T. viride*	Nam Bac, Vietnam
Biocon	*T. viride*	Tocklai Experimental Station Tea Research Association, India
Bio-HumaxinSenVàng 6SC	*Trichoderma* spp.	An Hung Tuong, Vietnam
Binap-T&W	*T. harzianum* + *T. polysporum*	Bio Innovation AB, Toreboda, Sweden
Defence	*T. viride*	Wockhardt Life Science Ltd.
Ecoderma	*T. viride*	P.J. Margo Pvt. Ltd.
Ecovit	*T. viride*	Bayer Industries Ltd.
Ecofit	*Trichoderma* spp.	Agro Evo, Mumbai, India
ECO T/T22	*T. harzianum*	PHP Ltd., South-Africa
F Stop	*T. harzianum*	Eastman Kodak Co., USA
Fulhumaxin 5.15SC	*Trichoderma* spp.	An Hung Tuong, Vietnam
Funginil	*T. viride*	Crop Health Bioproduct Research Centre, India
Guard	*T. viride*	Amit Biotech Ltd.
Glioguard	*T. virens*	W.R. Grace & Co., USA
Harzian 10, Harzian 20	*T. harzianum*	Natural Plant Protection , Noguerres, France
Monitor	*Trichoderma* spp.	Agricultural & Biotech Pvt. Ltd., Gujarat
NLU-Tri	*T. virens*	Ho Chi Minh University of Agriculture and Forestry, Vietnam
Plant Shield	*T. harzianum*	Bioworks, Geneva, USA
Plant biocontrol agent-	*T. harzianum*	University of Agriculture &

Product Name	Species / Strains	Manufacturer
1		Technology, Panatnagar, Uttarakhand
Plant helper	*T. atroviride*	Ampac, California
PromotPlusWP, PromotPlusDD	*T. koningii, T. harzianum*	Tan Quy
Root Pro	*T. harzianum*	EfalAgri, Israel
Soldier	*T. viride*	Agronovo Manufacturing Research & Development Pvt. Ltd.
Sanjeevani	*T. viride*	Pannacea India Ltd.
Soil Guard 12 G	*T. virens*	Thermo Trilogy Corp. USA
Superesivit	*Trichoderma* spp.	Bioplant, Denmark
Trichodermin	*Trichoderma* spp.	Bulgaria and Soviet Governments
Trichoderma	*Trichoderma* spp.	Innovative Pest Control Laboratory, Bangalore
Trichiguard	*T. viride*	Anu Product Ltd.
Trichoderma-6	*T. viride*	Rajcho Pesticide & Chemicals
TrishuBioguard	*T. viride*	KrishiRasayan Export Pvt. Ltd.
T-22B, T-22G	*T. harzianum* T-22	TGT Inc. New York, USA
T-35	*T. harzianum*	Makhteshim-Agan Chemical Israel
Tricho-X	*T. viride*	Excel Industries Ltd., India
Trichodex	*T. harzianum*	MakhteshimChemicak Works, Isreal
Trichopel	*Trichoderma* spp.	Agrimm Technologies, New Zealand
Trieco	*T. viride*	Ecosense Laboratories, India
TRICO-DHCT	*Trichoderma* spp.	Can Tho University, Vietnam
Trichotech	*Trichoderma* spp.	Finlay International Kenya Ltd. Dudutech, Laboratory
Vi-DK	*Trichoderma* spp.	Pesticide Corp.
WRC-GV	*T. virens*	-

2.7.12 Integrated Pest Management with *Trichoderma* spp.

Trichoderma based biocontrol agents are compared with chemical fungicides in terms of efficiency (Brewer and Larkin, 2005; Jensen et al., 2002; Khan et al., 2004; Dubey et al., 2007; Lewis and Papavizas, 1991). According to Fisher's LSD test (P=0.05) comparison of *T. virens* with fungicide azoxystrobin disclosed that *Trichoderma* causesd as low as 33.3% disease infestation with severity of 0.3% (Brewer and Larkin, 2005). Many researches convey the greater biocontrol activity of *Trichoderma* sp. than that of chemical fungicides. The reports were much better when *Trichoderma* based biocontrol agents and chemical fungicides were combined (integrated control) than either of the two alone. Elevated seed germination (12.0-14.0%) and grain yield (42.6-72.9%) with lessened disease of wilt (44.1-60.3%) were noticed when *T. harzianum* (10^5 spores/ml/g seed) and carboxin (2g/kg seed) were used in union for seed treatment (Dubey et al., 2007). This approach is more sustainable and has economic boon (Jensen et al., 2002; Dubey et al., 2007; Lewis and Papavizas, 1991).

3. MATERIALS AND METHODS



3. MATERIALS & METHODS

The details of experimental procedures adopted, materials used and techniques followed during the course of present investigation are described in this chapter.

3.1 Media used

3.1.1 Potato Dextrose Agar (PDA, Riker and Riker, 1936)

It is a semi synthetic growth media for routine culture of the test biocontrol agent *Trichoderma* sp.

Compositions	**g/L**
Potatoes, infusion from	200.0
Dextrose anhydrous	20.0
Agar agar	20.0
Distilled water	1L

Final pH (at 25°C) 5.6±0.2

3.1.2 Potato dextrose broth

When agar is omitted in the composition of PDA then we get broth of potato. It is helpful for sporulation of test pathogen and in *Trichoderma* mass multiplication.

3.1.3 *Trichoderma* specific medium (TSM, Elad et al., 1981; modified by Saha and Pan, 1997)

Due to low glucose level of this medium, rapid growth and sporulation of fungus takes place. The medium was used for selective isolation of *Trichoderma* from soil. In present investigation the medium was also used for isolation of mutated *Trichoderma* isolates after exposure to different doses of UV radiation. The viability of mutated isolates was also examined on this medium and then studied through generation study.

Components	**g/L**
Glucose	3.0
KCl	0.15
$MgSO_4$	0.25
NH_4NO_3	1.0

K_2HPO_4	0.9
Agar Agar	15
Distilled water	1L
Rose Bengal	0.033
Captan	0.1
Methyl Orange	0.3
Chloramphenicol	0.25
pH	5.0-5.5

3.2 Sterilization of glass wares and medium

Using chromic acid and detergent, the cleaning of glasswares like flasks, Petri plates, pipette, beaker, microscopic slides was routinely done to prevent any type of contamination. Dry heat treatment for sterilization of glassware was done using a hot air oven. Due to this method any pathogen growth is inhibited due to proteins denaturation and oxidation of biomolecules. All the glasswares were wrapped in paper before being sterilized at hot air oven at 160° C for 1 hr. Distilled water, cotton plugs, semi solid and liquid medium, micropipette, glass bangles were sterilized using moist heat of autoclave at 121°C and 15 psi for 15-20 min. Forcep tip, tips of tubes and flasks, inoculation needles were sterilized in flame after dipping in ethyl alcohol. For the purpose of hand sterilization, 70% ethyl alcohol was used and was even effective for surface sterilization of the working inoculation chamber.

3.3 Native *Trichoderma* strains used

Two best strains of *Trichoderma* i.e. UBT-18 and T21 were used. These strains were isolated from soil of Cooch Behar (vegetable plots, tea plantation).

3.3.1 Pure culture of *Trichoderma*

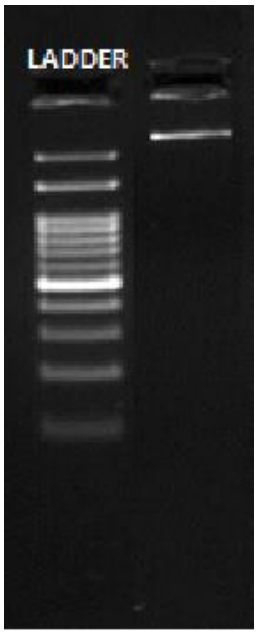
Single spore of *Trichoderma* strains is taken to get pure culture from the previous available culture. The spore is placed on fresh PDA medium on sterile Petri plate. The Petri plates are properly wrapped and incubated in BOD at 28±1° C for 5-7 days. The process can be repeated after 15-20 days to maintain the culture for future use.

3.3.2 Transfer on test tubes

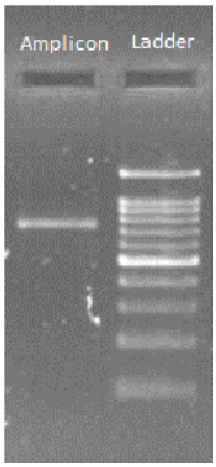
From pure culture Petriplates, *Trichoderma* was transferred on PDA slants that were then kept in BOD at 28±1° C for 5-7 days and later maintained at 4° C as pure culture.

3.4 Molecular characterization of native *Trichoderma* isolates by ITS sequencing

a. Genomic DNA was isolated from the culture using the method described by Raeder and Broda (1985). Its quality was evaluated on 1.0% Agarose Gel, a single band of high-molecular weight DNA has been observed.



b. Fragment of ITS region was amplified by PCR. A single discrete PCR amplicon band of ~700 bp was observed when resolved on agarose.



c. The PCR amplicon was purified to remove contaminants.

d. Forward and reverse reactions of PCR amplification of target DNA segment were carried out with ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 primers (5′-TCC TCC

GCT TAT TGA TAT GC-3′) described by White et al. (1990) using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer.

e. The ribosomal DNA (rDNA) sequencing data were compared with the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/), using the nucleotide BLAST program to identify homology between the PCR fragments and sequences in the GenBank database. Hundred sequences were selected and distance trees for ITS 1 and ITS 4 sequences for both the strains were constructed with a maximum sequence difference of 75% using BLAST pairwise alignment and Neighbour joining method between a query (yellow marked) and database searched.

3.5. Fungal pathogens used

Two major soil borne pathogens viz., *F. oxysporum* f.sp. *lentis* and *R. solani* were isolated from diseased plants of lentil and rice, respectively on PDA for studying the antagonistic effect of mutated *Trichoderma* strains against them.

3.4.1 Isolation of *F. oxysporum* f.sp. *lentis*

Infected tissues of lentil plant were taken and surface sterilized with 0.1% mercuric chloride to remove any other unwanted bacterial contamination in the culture. The tissue was placed on a sterile Petri plate containing PDA medium and then incubated at 28±1 ^{o} C till the growth of mycelia was observed. The presence of micro and macro conidia observed under microscope confirmed the presence of *F. oxysporum* f.sp. *lentis* in the culture. After every 3 months sub culturing was done to avoid the loss of culture due to certain contamination and kept at 4° C for its use in the study.

3.4.2 Isolation of *R. solani*

The pathogen was isolated from infected rice plant. Modified Ko and Hara's medium was used for the purpose of isolation and then transferred on sterile Petri plates having PDA. They were maintained on PDA slants in test tubes at 4° C for future use. Sub culturing in every 3 months is done to avoid contaminations. Under microscope hyphal growth of *Rhizoctonia solani* is seen and mycelium branches at right angle to each other.

3.5 UV Mutagenesis

The mutagenesis of both the *Trichoderma* strains was done in UV2 sterilizing PCR Workstation Cabinet having 2 UV sources of 254 nm shortwave UV tubes. Timer with 10-15

min was used first to decontaminate the chamber before use. The exterior dimension of the model (W×D×H) is 737 × 610 × 729 mm and interior dimensions (W×D) of 500 × 544 mm and the distance between the Petri plates to the UV light source was 700mm.

Petri plates containing *Trichoderma* strains were placed under UV light with different duration of exposures i.e. for strain UBT-18 starting from 15 min to 2 hr 30 min at 15 min interval forming 10 different treatments. For strain T21, 5 different treatments starting from duration of 1 hr 30 min to 2 hr 30 min keeping 15 min interval between each (Table 3.1). The treated Petri plates were immediately transferred in BOD at 28±1 ^{o} C for 18-19 hr approximately.

Table 3.1 Duration of exposure of fungal strains to UV-light

Fungal strain/mutant UBT-18 Treatment	Duration	Fungal strain/mutant T21 Treatment	Duration
T1	15 min	T1	1hr 30 min
T2	30 min	T2	1 hr 45 min
T3	45 min	T3	2hr
T4	1hr	T4	2hr 15 min
T5	1hr 15 min	T5	2hr 30 min
T6	1 hr 30 min		
T7	1hr 45 min		
T8	2hr		
T9	2hr 15 min		
T10	2hr 30min		

3.5.1 Serial dilution of UV treated *Trichoderma* strains

After UV treatment and BOD placement, the cultures from UV treated plates were serial diluted and plated on Petri plates containing TSM. Conical flask of 50 ml was taken and filled with 45 ml distilled water and sterilized. In a single flask a single treatment of the *Trichoderma* strain was used. From treated *Trichoderma* plates a portion of culture using cork borer is taken and dipped into the conical flask and shaken properly. Aliquot of 5 ml from the conical flask is taken using pipette and transferred into another conical flask containing only 45 ml distilled sterilized water. From the second conical flask 1 ml of the solution is taken using micropipette and placed on a sterilized Petri plate containing TSM.

Sufficient replication was maintained for a single treatment of a *Trichoderma* strain and for other treatments also the same method was carried out. The plates were then kept in BOD at 28±1° C until single colony of viable *Trichoderma* spores grew into small mutated colony which subsequently was picked before merging with one another based on difference in colony appearance of native strains and transferred to test tube containing PDA slants and incubated in BOD for growth.

3.6 Generation growth of mutated *Trichoderma*

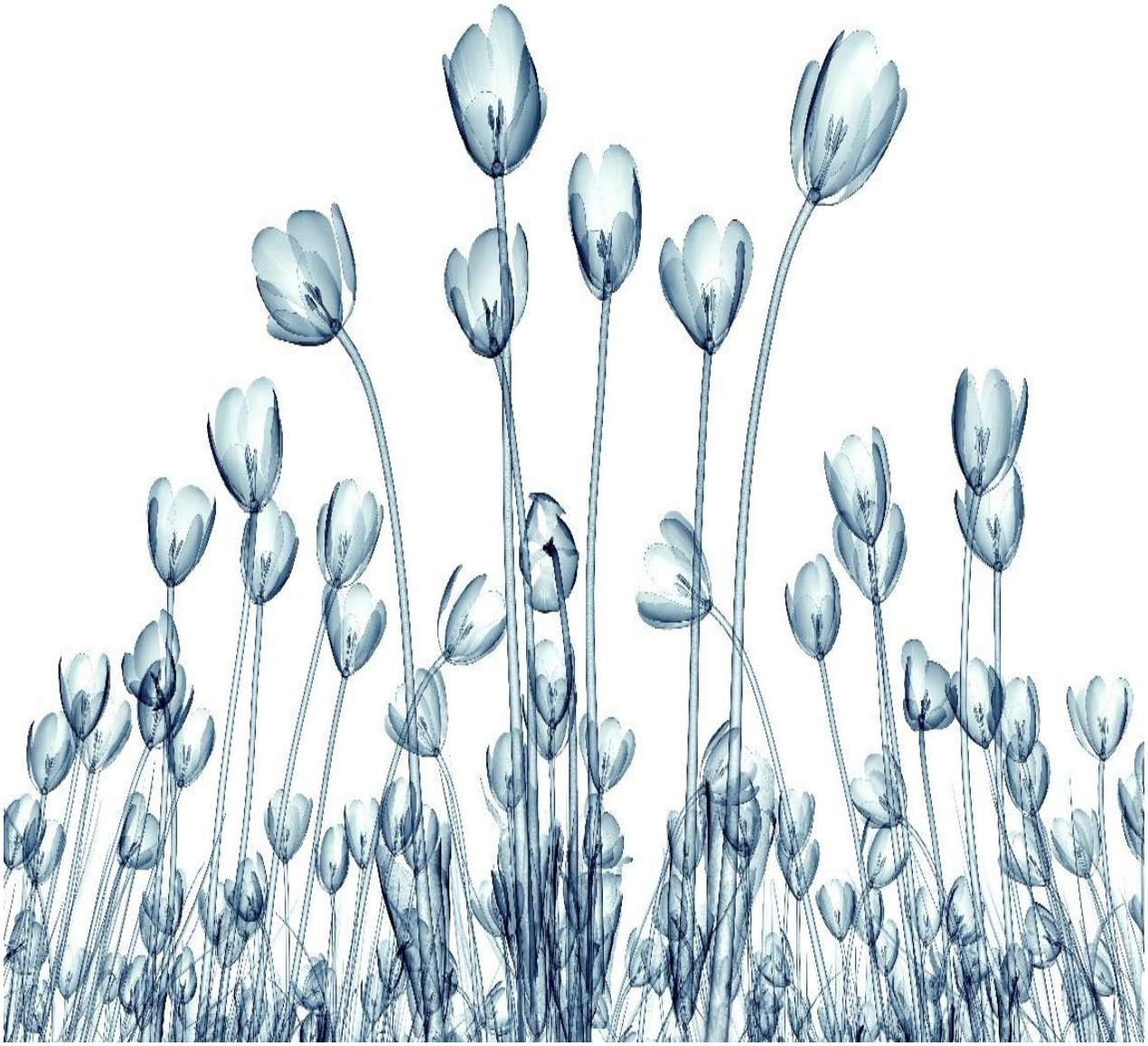
The colonies from individual mutated spores of *Trichoderma* strains were repeatedly sub-cultured in test tubes up to 10th generation. For this purpose, spores from the previous test tubes are taken and inoculated onto fresh PDA slant tubes for growth. Some *Trichoderma* colonies under some treatments even died out before reaching last generation growth due to segregation effect. During each generation of treatments, the growth pattern and visual colour changes if any was observed carefully.

3.7. Antagonistic potential determination of *Trichoderma* using 'Bangle method'

The 'Bangle method' (Singh et al., 2004) was used for determining antagonistic potential of mutated *Trichoderma* isolates as well as their respective parental strains against the test pathogens. In this method a pathogen disc (8-9 mm diameter) having mycelia was cut by using cork borer from the periphery of the colony and inoculated centrally in a Petri plate containing 20 ml sterilized PDA. Around this pathogen disc, a sterilized bangle dipped in conidial suspension of *Trichoderma* was placed. Requisite replications (3-4) of each treatment of *Trichoderma* strains were maintained. In control plates, only free growth of the pathogens was allowed or sterilized bangles were placed without *Trichoderma* suspensions. Both control plates and antagonist inoculated Petri plates were incubated in BOD at 28±1° C. Inhibition by *Trichoderma* on growth of pathogen with respect to control was calculated to get the inhibition percentage using the formula given below.

% of inhibition = {(Growth of pathogen in control plate – growth of pathogen in *Trichoderma* treated plate)/ growth of pathogen in control plate} ×100

4. RESULTS AND DISCUSSION



4. RESULTS AND DISCUSSION

4.1. Molecular characterization of native *Trichoderma* isolates by ITS sequencing

Molecular characterization of native *Trichoderma* isolates by using ITS1and ITS 4 primers were performed following the method described in section 3.4. The results obtained have been discussed below.

4.1.1. Characterization of UBT-18

The results of nucleotide sequences of UBT 18 obtained from PCR amplicon by using ITS 1 and ITS 4 primers have been presented in Table 4.1.

Table 4.1. ITS 1 and ITS 4 sequencing of ribosomal DNA of UBT 18

ITS 1 sequencing GGCGTAAGGTCTCGAAAGGTGAACCAGCGGAAGGATCATTACCGAGTTTACAACTCCCA AACCCAATGTGAACGTTACCAAACTGTTGCCTCGGCGGGATCTCTGCCCCGGGTGCGTC GCAGCCCCGGACCAAGGCGCCCGCCGGAGGACCAACCAAAACTCTTATTGTATACCCCC TCGCGGGTTTTTTTATAATCTGAGCCTTCTCGGCGCCTCTCGTAGGCGTTTCGAAAATGA ATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTG CGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCT CCGGGGGGTCGGCGTTGGGGATCGGCCCTGCCTTGGCGGTGGCCGTCTCCGAAATACAG TGGCGGTCTCGCCGCAGCCTCTCCTGCGCAGTAGTTTGCACACTCGCATCGGGAGCGCG GCGCGTCCACAGCCGTTAAACACCCAACTTCTGAAATGTTGACCTCGGATCAGGTAGGA ATACCCGCTGAACTTAAGCATATCAATAAGGCGGAGGAAAG
ITS 4 sequencing ACCCCTGGTTCGTACTGATCCGAGGTCACATTTCAGAAGTTGGGTGTTTAACGGCTGTGG ACGCGCCGCGCTCCCGATGCGAGTGTGCAAACTACTGCGCAGGAGAGGCTGCGGCGAG ACCGCCACTGTATTTCGGAGACGGCCACCGCCAAGGCAGGGCCGATCCCCAACGCCGAC CCCCCGGAGGGGTTCGAGGGTTGAAATGACGCTCGGACAGGCATGCCCGCCAGAATACT GGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTAC TTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGT TTTGATTCATTTTCGAAACGCCTACGAGAGGCGCCGAGAAGGCTCAGATTATAAAAAAA CCCGCGAGGGGGTATACAATAAGAGTTTTGGTTGGTCCTCCGGCGGGCGCCTTGGTCCG GGGCTGCGACGCACCCGGGGCAGAGATCCCGCCGAGGCAACAGTTTGGTAACGTTCACA TTGGGTTTGGGAGTTGTAAACTCGGTAATGATCCCTCCGCTGGTTCACCAACGGAGACCT TGTTACGACTTTTACTTCCTCTAATTGACCACAGACTT

The above sequences were compared with the GenBank database using the nucleotide BLAST program to identify homology between the PCR fragments and sequences in the GenBank database and the distance tree were constructed which have been presented below. (Fig 4.1 and Fig. 4.2)

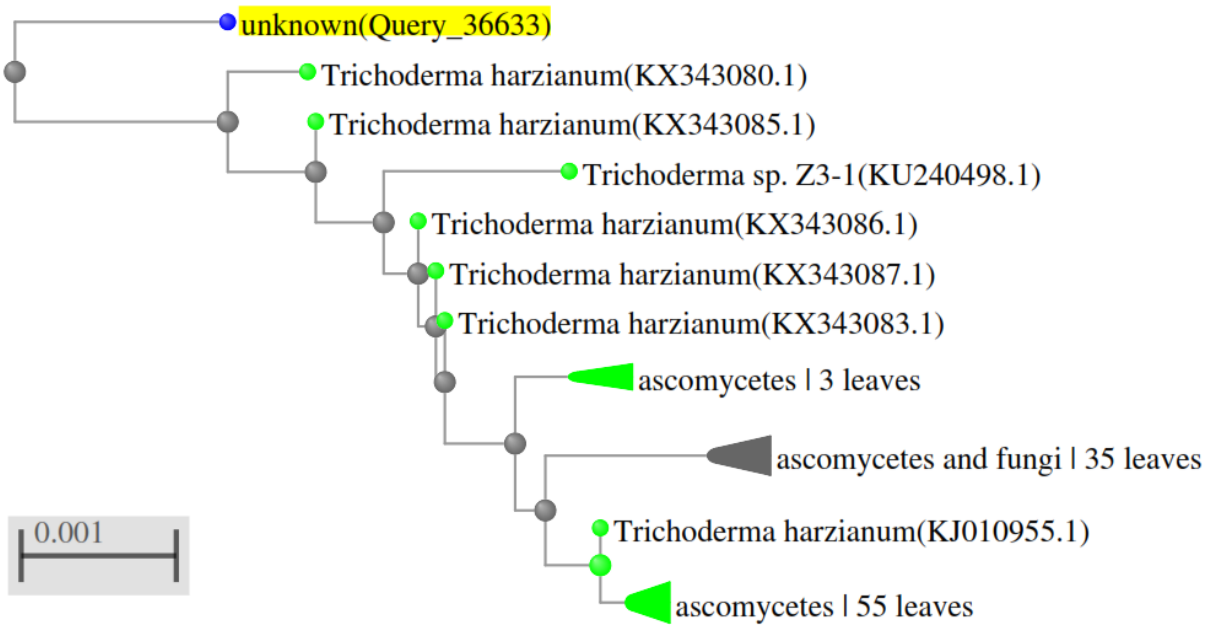


Fig. 4.1. Distance tree of nucleotide sequence of UBT 18 using ITS 1 primer

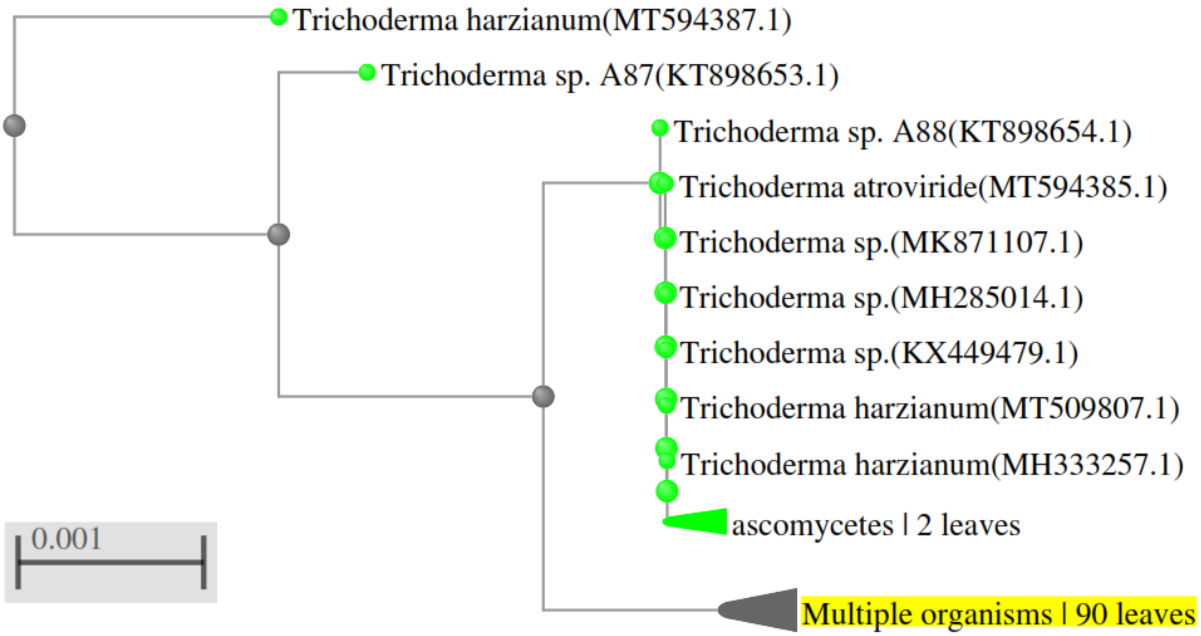


Fig. 4.2. Distance tree of nucleotide sequence of UBT 18 using ITS 4 primer

The above figures (4.1. and 4.2) suggest that the test isolate belongs to *Trichoderma harzianum*. The ITS1 sequence data was submitted to NCBI Gene Bank and the accession no. MT876632 has been obtained.

4.1.2. Characterization of T-21

The results of nucleotide sequences of T 21 obtained from PCR amplicon by using ITS 1 and ITS 4 primers have been presented in Table 4.2.

Table 4.2. ITS 1 and ITS 4 sequencing of ribosomal DNA of T 21

ITS 1 sequencing GGGTCGGTCTCGTTGGTGAACCAGCGGAAGGATCATTACCGAGTTTACAACTCCCAAAC CCAATGTGAACGTTACCAAACTGTTGCCTCGGCGGGGTCACGCCCCGGGTGCGTCGCAG CCCCGGAACCAGGCGCCCGCCGGAGGAACCAACCAAACTCTTTCTGTAGTCCCCTCGCG GACGTATTTCTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTTTCAACAACGG ATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTG CAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCG GGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCCGGGGGATCGGCGTTGGG GATCGGGACCCCTCACACGGGTGCCGGCCCCGAAATACAGTGGCGGTCTCGCCGCAGCC TCTCCTGCGCAGTAGTTTGCACAACTCGCACCGGGAGCGCGGCGCGTCCACGTCCGTAA AACACCCAACTTTCTGAAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAA GCATATCAATAGGCCGGAGGAAAA
ITS 4 sequencing AGGAGCGGGTTCCTACCTGATCCGAGGTCACATTTCAGAAAGTTGGGTGTTTTACGGAC GTGGACGCGCCGCGCTCCCGGTGCGAGTTGTGCAAACTACTGCGCAGGAGAGGCTGCGG CGAGACCGCCACTGTATTTCGGGGCCGGCACCCGTGTGAGGGGTCCCGATCCCCAACGC CGATCCCCCGGAGGGGTTCGAGGGTTGAAATGACGCTCGGACAGGCATGCCCGCCAGA ATACTGGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCAC ATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTG AAAGTTTTGATTCATTTTGAATTTTTGCTCAGAGCTGTAAGAAATACGTCCGCGAGGGGA CTACAGAAAGAGTTTGGTTGGTTCCTCCGGCGGGCGCCTGGTTCCGGGGCTGCGACGCA CCCGGGGCGTGACCCCGCCGAGGCAACAGTTTGGTAACGTTCACATTGGGTTTGGGAGT TGTAAACTCGGTAATGATCCCTCCGCTGGTTCACCAACGGAGACCTTGTTACGACTTTTA CTTCCTCTAATTGGACCAAGAAG

The above sequences were compared with the GenBank database using the nucleotide BLAST program to identify homology between the PCR fragments and sequences in the GenBank database and the distance tree were constructed which have been presented below. (Fig 4.3 and Fig. 4.4)

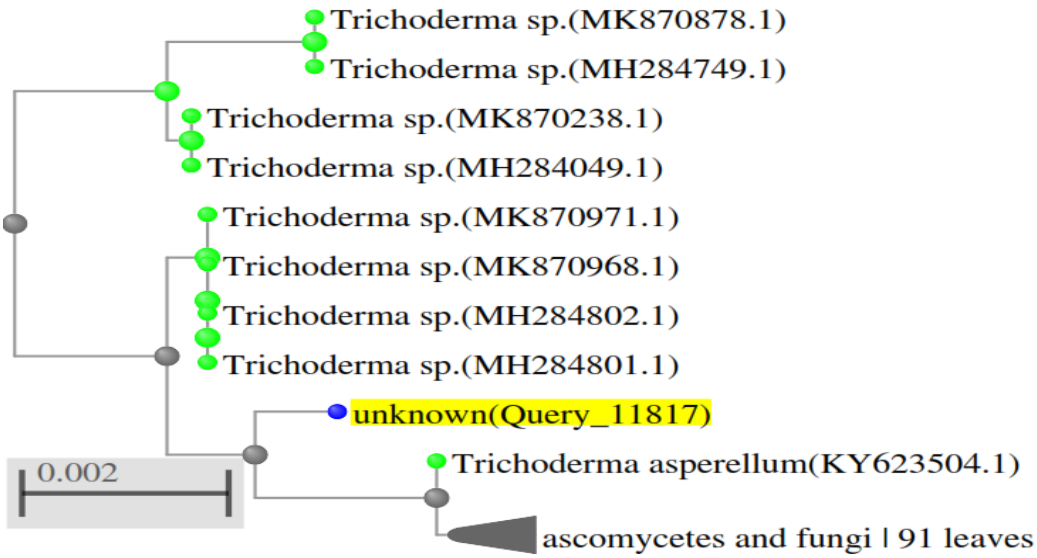


Fig. 4.3. Distance tree of nucleotide sequence of T 21 using ITS 1 primer

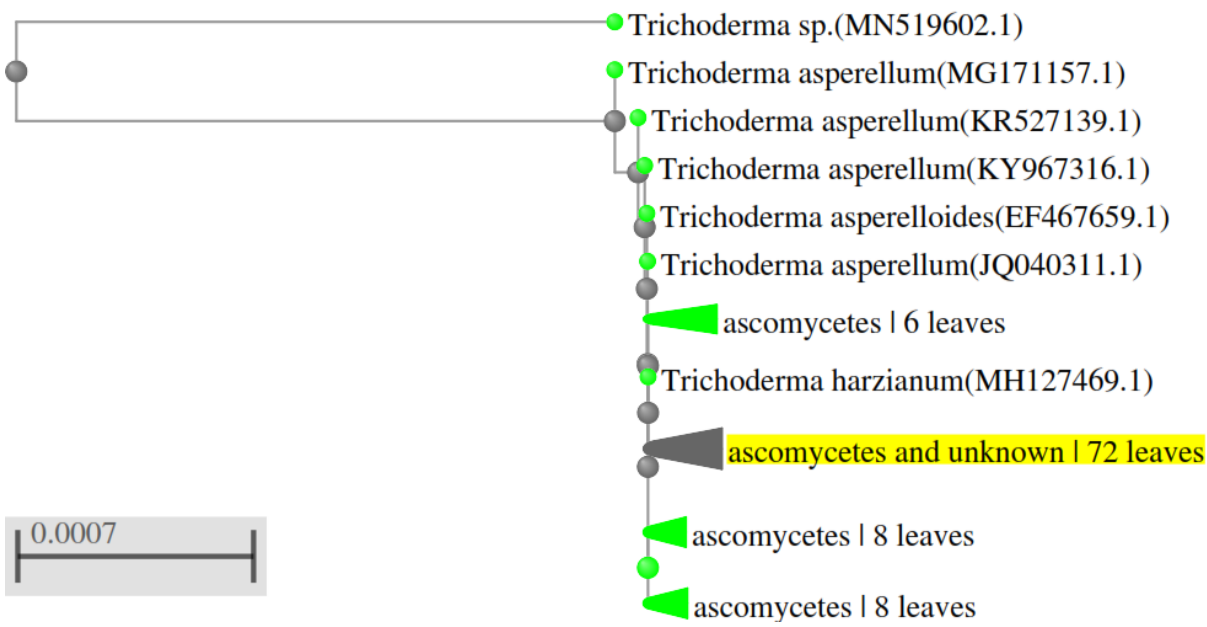
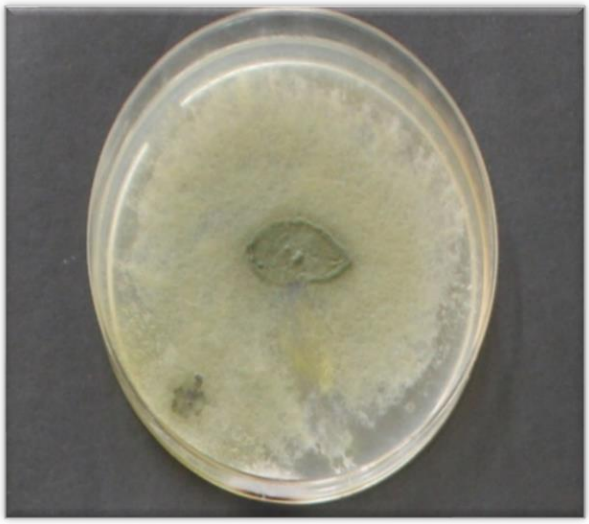


Fig. 4.4. Distance tree of nucleotide sequence of T 21 using ITS 4 primer

The above figures (4.3. and 4.4) suggest that the test isolate belongs to *Trichoderma asperellum*. The ITS1 sequence data was submitted to NCBI Gene Bank and the accession no. MT951635 has been obtained.

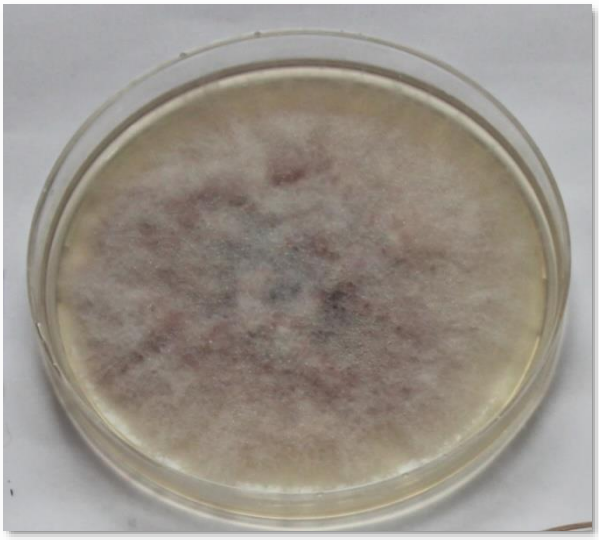
Species of the *Trichoderma* genus are all-pervasive in nature and normally found as soil-borne or wood-decaying fungi. Some species are economically significant because of having capability to produce industrial enzymes (cellulase and hemicellulase) and antibiotics or act as biocontrol agents (Hassan 2014 ; Fahmi et al. 2016). For that reason, accurate findings of *Trichoderma* at the species level is necessary. Morphological identification of species has proven unreliable (Sharma et al. 2009 and Shahid et al. 2013) because of their high degrees of similarity. By the reason of sensitivities to environmental components, recognition depending on host inclination and morphological distinction is not dependable. Hence, molecular methods have been introduced recently for accurate identification. Molecular patterns in genomic DNA are hypothetically not affected by these variations and can be useful for species identification (Gajera and Vakharia 2010 and Błaszczyk et al. 2011). The ribosomal RNA genes (rDNA) possess characteristics that are appropriate for the identification of fungal isolates at the species level. These rDNAs are highly stable and exhibit a mosaic of conserved and diverse regions within the genome (Hibett 1992). They also occur in multiple copies with up to 200 copies per haploid genome arranged in tandem repeats with each repeat consisting of the 18S small subunit, the 5.8S, and the 28S large subunit genes.

Plate 1: *Trichoderma* and Pathogen isolates used



Trichoderma – UBT-18 (*T. harzianum*)

Trichoderma – T21 (*T. asperellum*)



Pathogen – *Fusarium oxysporum* f.sp. *lentis*

Pathogen – *Rhizoctonia solani*

Many other researchers have used ITS sequences to identify *Trichoderma* spp. (Rifai 1969 and Fahmi et al. 2016). Kuhls et al. (1997) used sequence analysis to differentiate between *T. reesei* and *T. longibrachiatum*. The present findings are also in line of observation of several workers who observed the amplified rDNA fragment of approximately 500 to600 bp by ITS-PCR in *Trichoderma* (Lieckfiledt et al 1999, Harmosa et al. 2000). Chakraborty et al. (2010) also reported that the ITS regions of ribosomal genes for the construction of primers can be used to identify *Trichoderma* spp. ITS region of rDNA was amplified using genus specific ITS-1 and ITS-4 primers.

4.2 Mutagenic Treatment

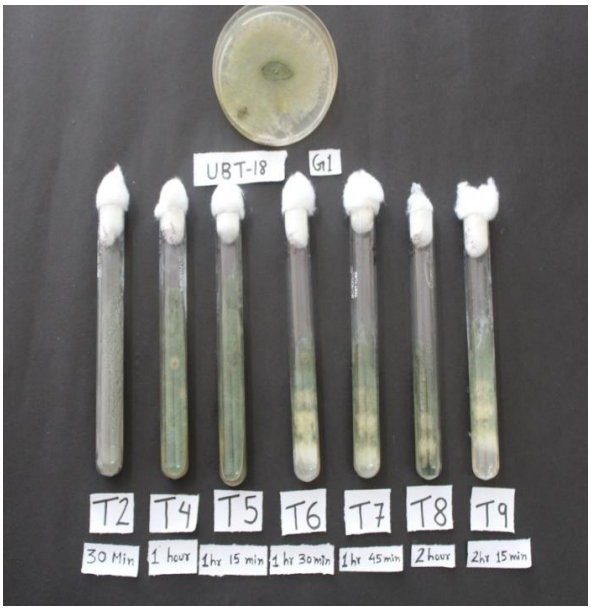
The above two *Trichoderma* spp. were taken which showed different visual appearances. *T. harzianum* (UBT-18) was light yellowish green in colour with low sporulation rate. On the other hand, *T. asperellum* (T 21) exhibited dark green spores, high growth rate, granular and with marginal mycelia growth. Both were exposed to UV rays following the method described in section 3.5. After incubation of 18-19hrs upon exposure the mutated conidia were plated for isolation. Based on difference in visual appearance of single colony of various exposures, following numbers of mutated isolates were transferred to PDA slants for generation studies.

Table 4.3. Mutated isolates cultured through generation

***T. harzianum* (UBT-18)**		***T. asperellum* (T 21)**	
Treatment	**No. of single mutated colony isolated**	**Treatment**	**No. of single mutated colony isolated**
T1	No colonies isolated due to absence of variation in colony appearance from native culture	T1	3
T2	3	T2	4
T3	Same as T1	T3	2
T4	4	T4	3
T5	5	T5	4
T6	5		
T7	4		
T8	3		
T9	3		
T10	No colony appeared		

The mutated isolates were repeatedly sub-cultured up to 10th generation to observe the effect of segregation after random mutation. The purpose was also to get the stable mutants. During the study few mutants lost their viability and were excluded from the experiment. The

Plate 2: Generation growth of mutated UBT-18 *Trichoderma* strains



UBT-18 (*T. harzianum*) - 1st Generation

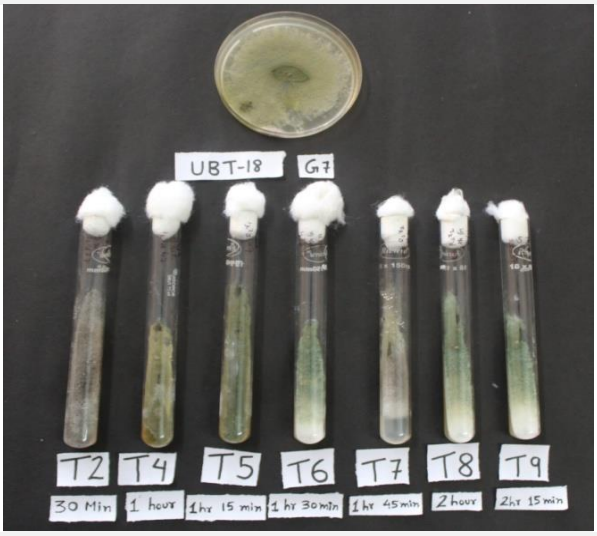
UBT-18 (*T. harzianum*) - 2nd Generation

UBT-18 (*T. harzianum*) – 3rd Generation

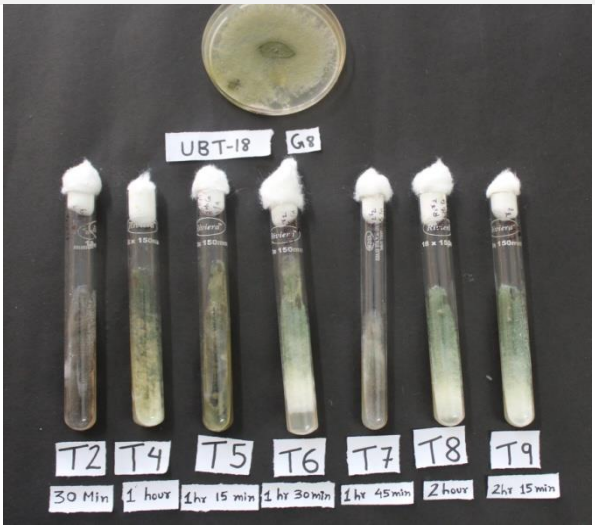
UBT-18 (*T. harzianum*) – 4th Generation

UBT-18 (*T. harzianum*) – 5th Generation

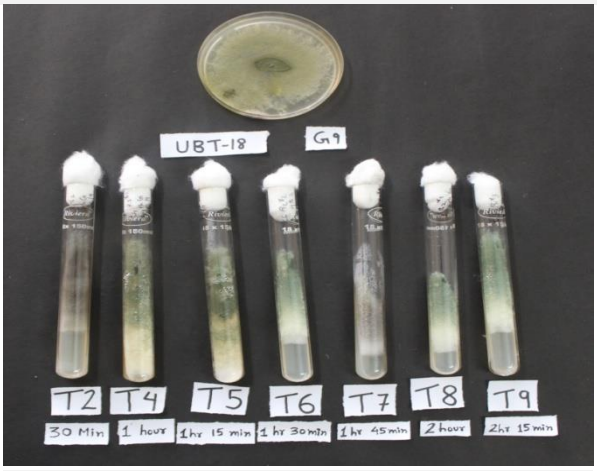
UBT-18 (*T. harzianum*) – 6th Generation



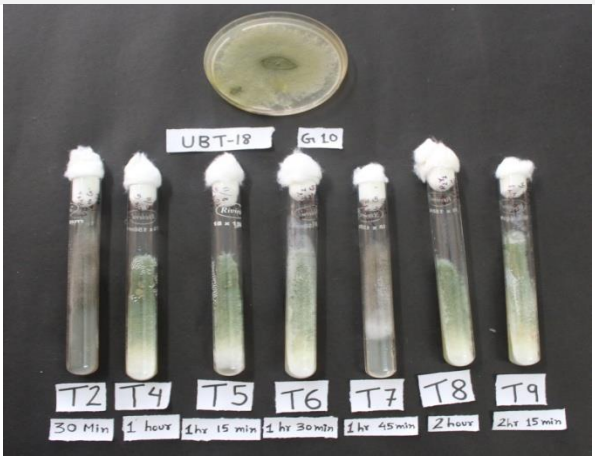
UBT-18 (*T. harzianum*) – 7th Generation



UBT-18 (*T. harzianum*) – 8th Generation

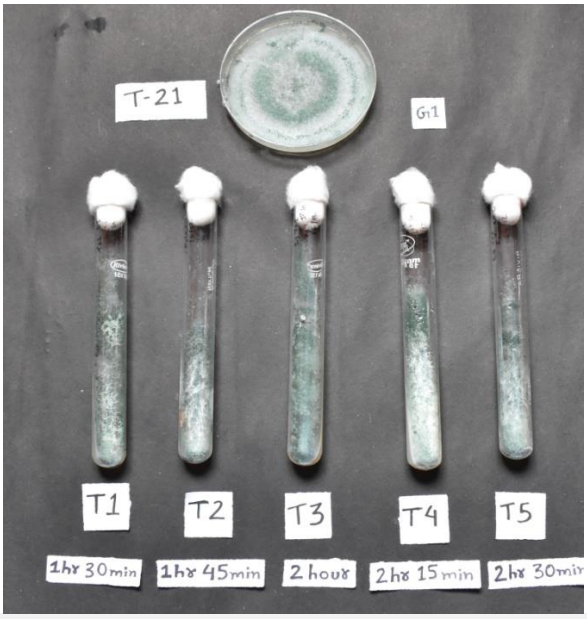


UBT-18 (*T. harzianum*) – 9th Generation

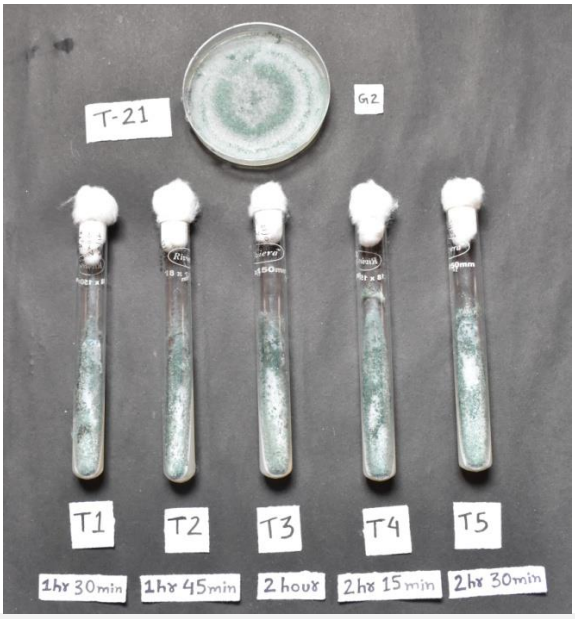


UBT-18 (*T. harzianum*) – 10th Generation

Plate 3: Generation Growth of mutated T21 *Trichoderma* strains



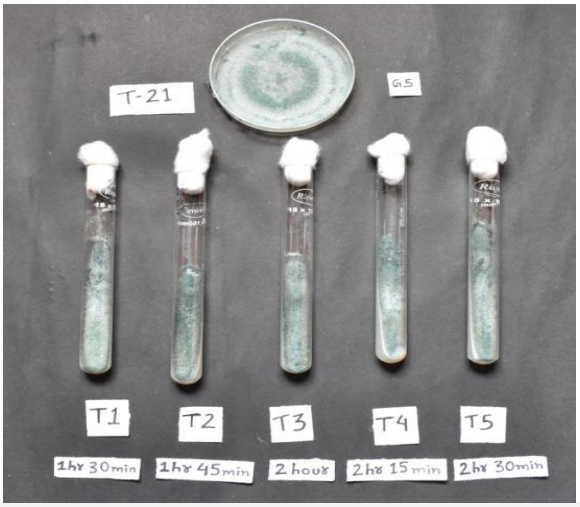
T21 (*T. asperellum*) – 1st Generation



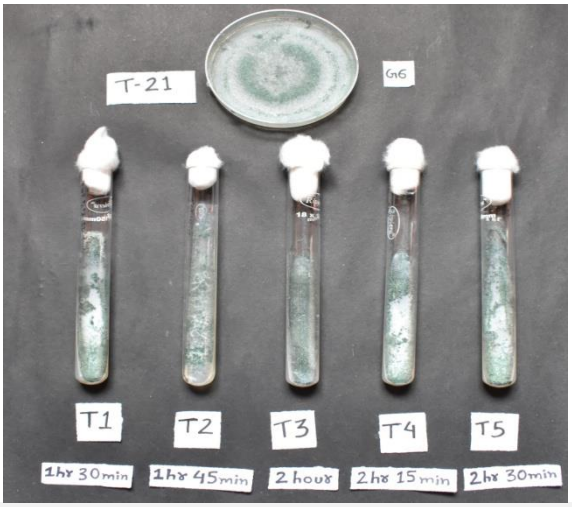
T21 (*T. asperellum*) – 2nd Generation

T21 (*T. asperellum*) – 3rd Generation

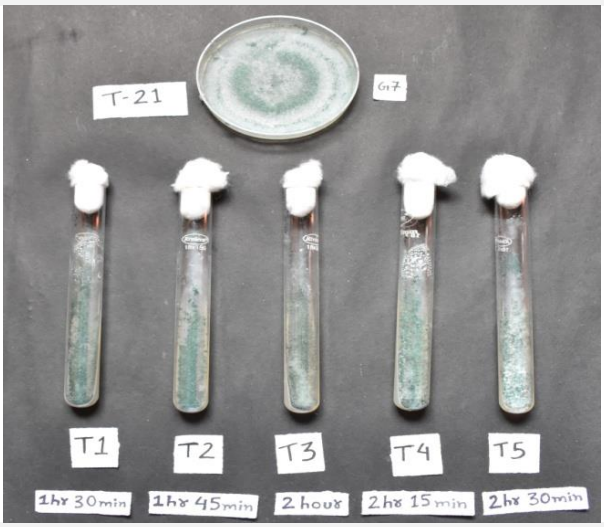
T21 – (*T. asperellum*) 4th Generation



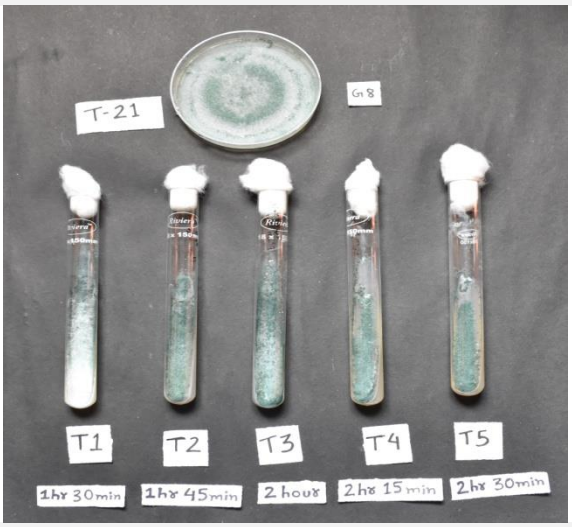
T21 (*T. asperellum*) – 5th Generation



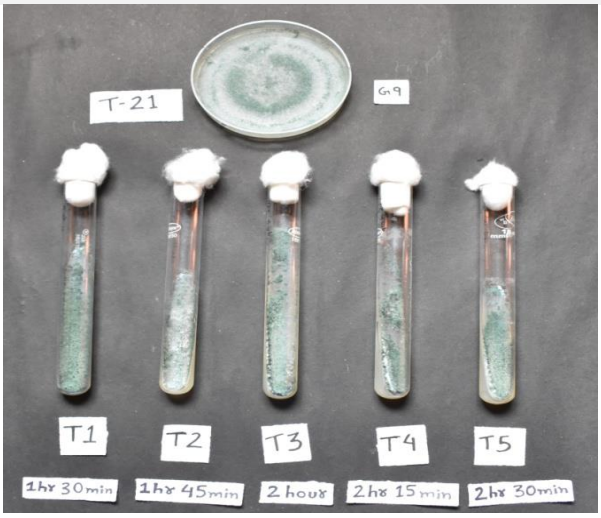
T21 (*T. asperellum*) – 6th Generation



T21 (*T. asperellum*) – 7th Generation



T21 (*T. asperellum*) – 8th Generation



T21(*T. asperellum*) - 9th Generation

T21 (*T. asperellum*) – 10th Generation

various generations from 1st to 10th showed visual differences in the pigmentation of the spores which have been presented in **plate (2 and 3)**. Among the stable mutated isolates obtained after 10th generation, one isolate from each treatment having maximum growth rate was selected to study *in-vitro* antagonistic potential against fungal pathogens.

The mutagenic effect of UV light on DNA structure results in variations in cultures of *Trichoderma* spp. Nodvig et al. (2015) gave the mutagenesis techniques for filamentous fungi. Certain morphological changes observed due to mutagenic consequences include the change in colony appearance, colony colour, pigmentation and sporulation rate (Ikehata and Ono, 2011; Pfeifer et al., 2005). The mutagenic consequence seems to change with change in UV treatments. The UV-surviving mutants can induce stress due to photoreactivation, post-replication repair, excision repair and cellular repair system (Ganesan, 1974; Seeberg et al., 1995; Yasui et al., 2003). But cell death can occur due to excessive damage in the form of numerous DNA cross-linkages, multiple breaks in strand and cyclobutane pyrimidine dimers (Lin and Wang, 2001).

4.3 Antagonistic Potential of the stable mutants

Dual culture done using the Bangle method is described in 3.7 portion and results are presented in Table 4.4 and 4.5. The biocontrol efficacy was tested against two test pathogens. It is evident from table 4.4 that the treatment T9 of UBT-18 (*T. harzianum*) exhibits higher percentage inhibition of growth over control (47.22%) compared to wild UBT-18 (40.98%) in case of *Fusarium oxysporum* f.sp. *lentis*. The lowest percent inhibition of growth over control is shown by T2 (41.67). Therefore, treatment T9 exhibits 6.24% increase of inhibition over wild isolate. On the other hand, UBT-18 does not show better inhibition against *Rhizoctonia solani* where the wild UBT-18 shows 68.89% inhibition of growth over control but the maximum inhibition percentage was seen in case of T8 reaching only 37.78%. T5 shows the lowest percent inhibition of growth over control (20.11%). In this case, treatment T8 shows 31.11% decrease of inhibition over wild isolate. From the Table 4.5 it has been clearly visible that treatment T1 of T-21 (*T. asperellum*) exhibits higher percent inhibition of growth of *Fusarium oxysporum* f.sp. *lentis* over control (72.78%) compared to the wild isolate showing only 37.31% inhibition. T1 shows 35.47% increase of inhibition over wild isolate. The lowest inhibition percentage of growth over control was shown by treatment T4 (62.11%) but still it has 24.80% increase of inhibition over wild type. This explains that all mutants of T-21 were more efficient in controlling *Fusarium oxysporum* f.sp. *lentis*. In case of the pathogen

Rhizoctonia solani also, treatment T1 shows higher percent inhibition of growth over control (70.44%) compared to the wild isolate (38.43%). Here, T1 has 32.01% increase of inhibition over wild isolate. But T5 shows only 33.56% inhibition of growth over control exhibiting 4.87% decrease of inhibition over wild isolate.

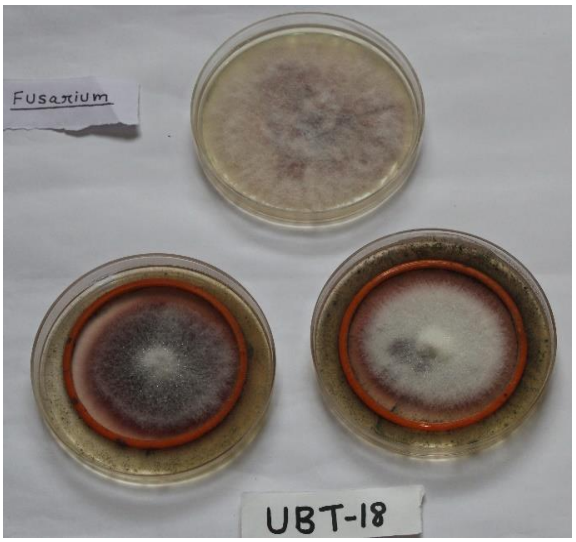
Table 4.4: Antagonistic potential of *T. harzianum* (UBT-18) against soil borne plant pathogens by Dual culture method

Fungal strain/mutant *T. harzianum* (UBT-18)	***Fusarium oxysporum* f.sp. *lentis***		***Rhizoctonia solani***	
	% inhibition of growth over control	*% increase or decrease of inhibition over wild isolate*	*% inhibition of growth over control*	*% increase or decrease of inhibition over wild isolate*
Wild UBT 18	40.98		68.89	
T2	41.67	0.69	22.89	(-) 46.00
T4	46.00	5.02	23.78	(-) 45.11
T5	44.44	3.46	20.11	(-) 48.78
T6	42.22	3.24	22.44	(-) 46.45
T7	44.44	3.46	22.67	(-) 46.22
T8	45.33	4.35	37.78	(-) 31.11
T9	47.22	6.24	32.22	(-) 36.67
SEm±	1.14		3.59	
CD (P=0.05)	NS		10.54	

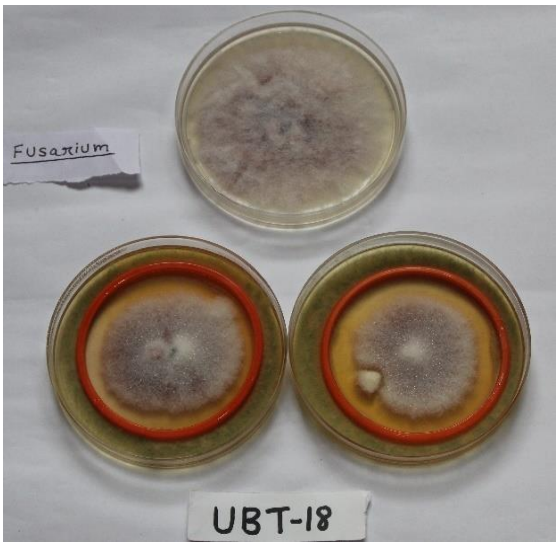
Table 4.5: Antagonistic potential of *T. asperellum* (T-21) against soil borne plant pathogens by Dual culture method

Fungal strain/mutant *T. asperellum* (T-21)	***Fusarium oxysporum* f.sp. *lentis***		***Rhizoctonia solani***	
	% inhibition of growth over control	*% increase or decrease of inhibition over wild isolate*	*% inhibition of growth over control*	*% increase or decrease of inhibition over wild isolate*
Wild T-21	37.31		38.43	
T1	72.78	35.47	70.44	32.01
T2	68.33	31.02	43.56	5.13
T3	70.56	33.25	45.78	7.35
T4	62.11	24.80	52.22	13.79
T5	66.67	29.36	33.56	(-) 4.87
SEm±	0.66		2.65	
CD (P=0.05)	2.00		8.02	

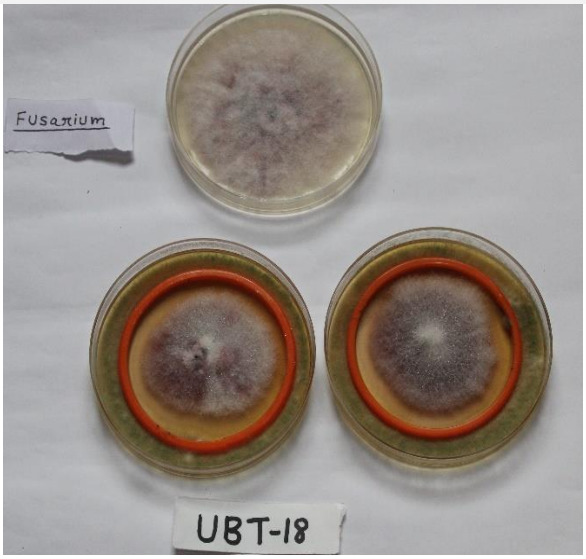
Plate 4: Dual culture by 'Bangle method' of mutated UBT-18 *Trichoderma* strain against pathogens



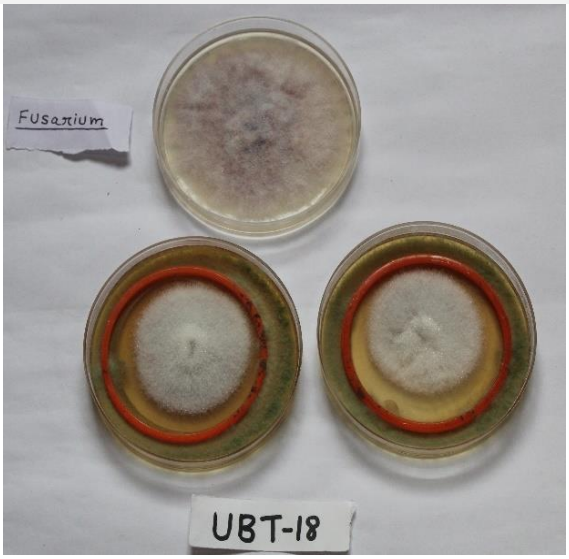
Fusarium – UBT-18 (*T. harzianum*) (T2)



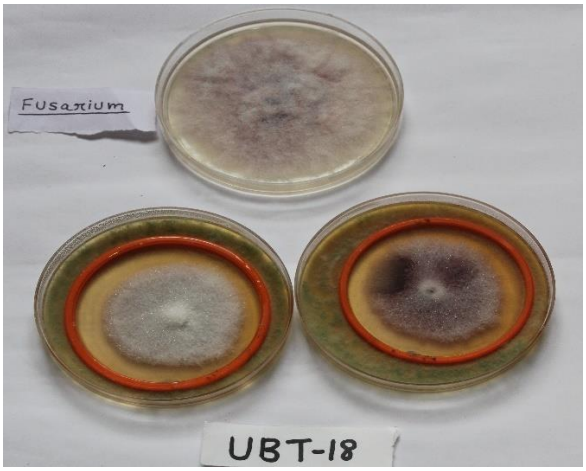
Fusarium – UBT-18 (*T. harzianum*) (T4)



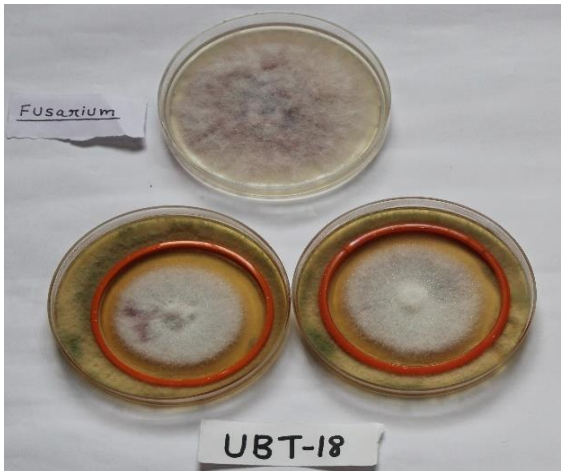
Fusarium – UBT-18 (*T. harzianum*) (T5)



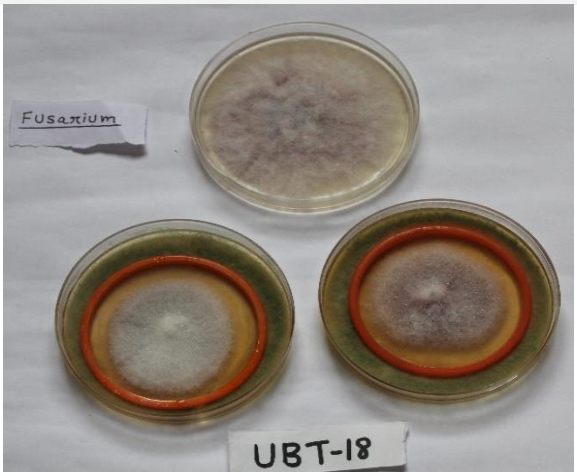
Fusarium – UBT-18 (*T. harzianum*) (T6)



Fusarium – UBT-18 (*T. harzianum*) (T7)



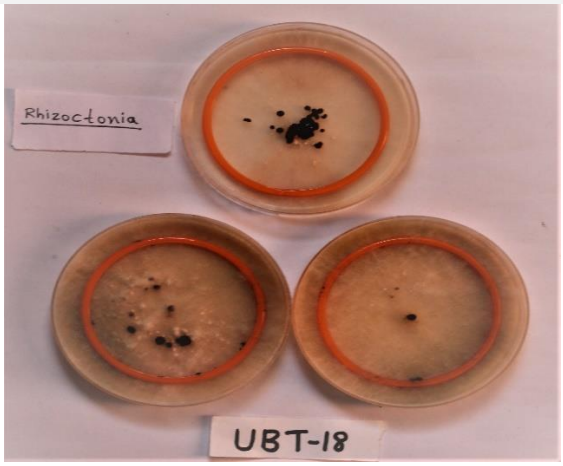
Fusarium – UBT-18 (*T. harzianum*) (T8)



Fusarium – UBT-18 (*T. harzianum*) (T9)

Rhizoctonia – UBT-18 (*T. harzianum*) (T2)

Rhizoctonia – UBT-18 (*T. harzianum*) (T4)



Rhizoctonia – UBT-18 (*T. harzianum*) (T5)

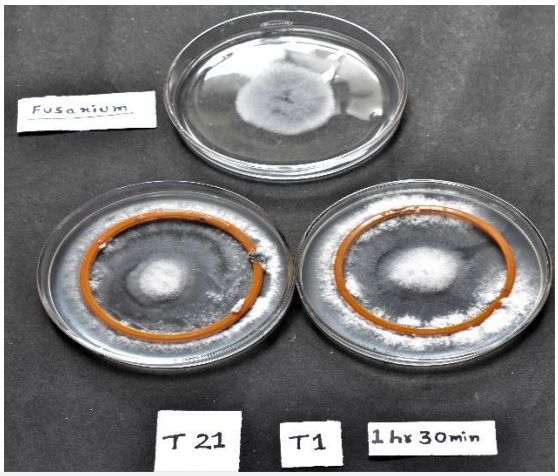
Rhizoctonia – UBT-18 (*T. harzianum*) (T6)

Rhizoctonia – UBT-18 (*T. harzianum*) (T7)

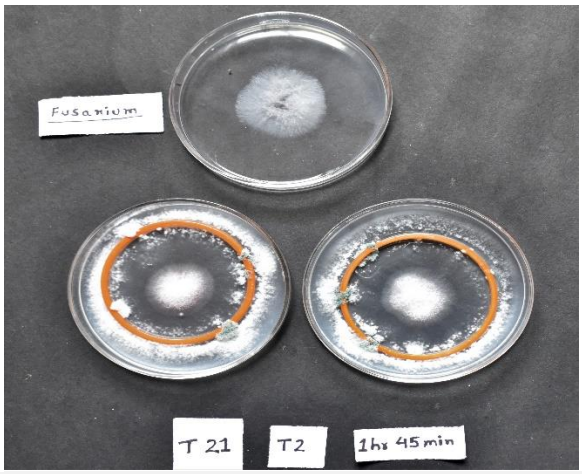
Rhizoctonia – UBT-18 (*T. harzianum*) (T8)

Rhizoctonia – UBT-18 (*T. harzianum*) (T9)

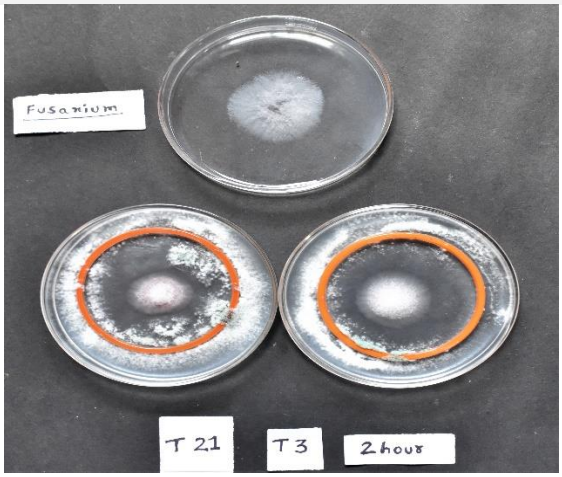
Plate 5: Dual culture by 'Bangle method' of mutated T21 *Trichoderma* strain against pathogens



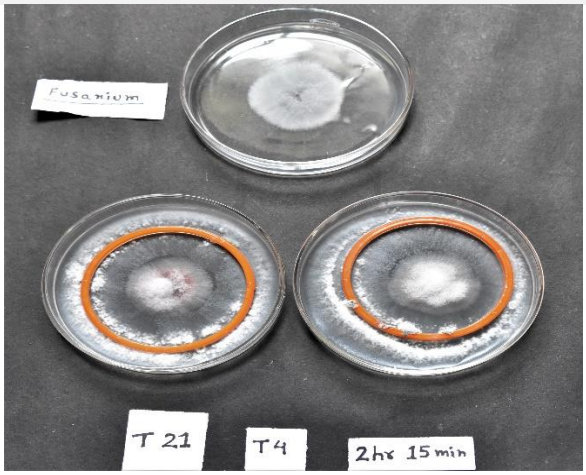
Fusarium – T21(*T. asperellum*) (T1)



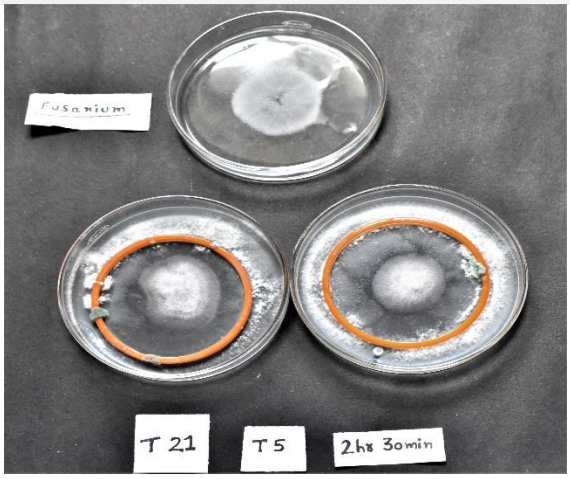
Fusarium – T21(*T. asperellum*) (T2)



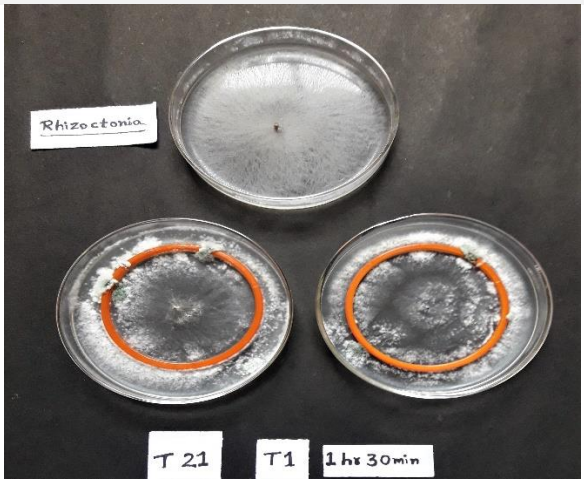
Fusarium – T21 (*T. asperellum*) (T3)



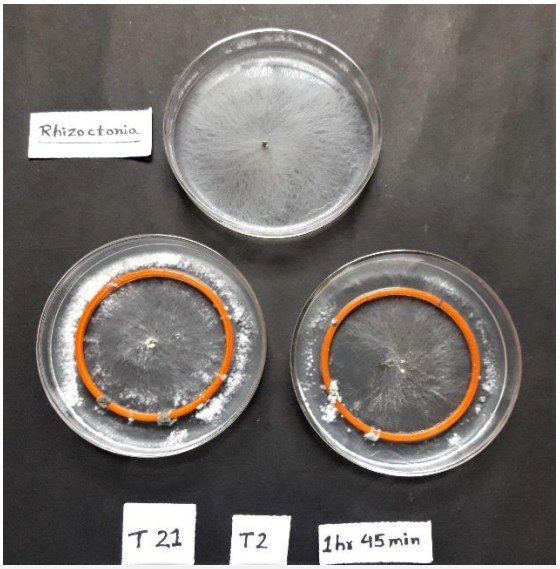
Fusarium – T21(*T. asperellum*) (T4)



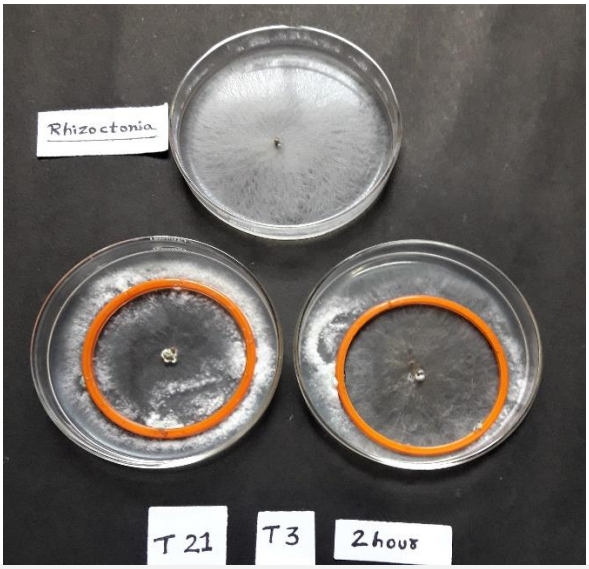
Fusarium – T21(*T. asperellum*) (T5)



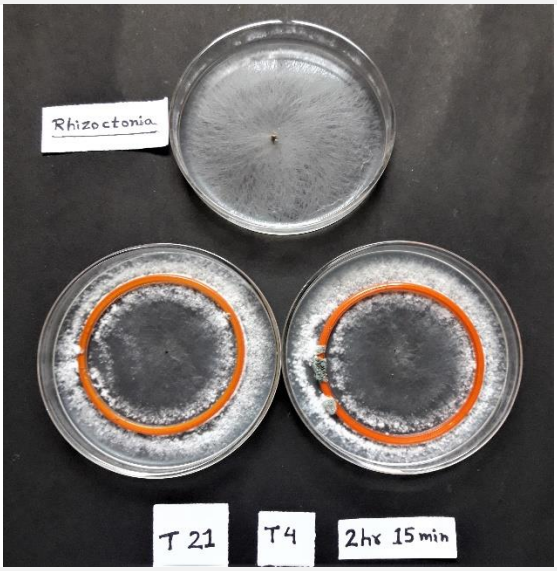
Rhizoctonia – T21(*T. asperellum*) (T1)



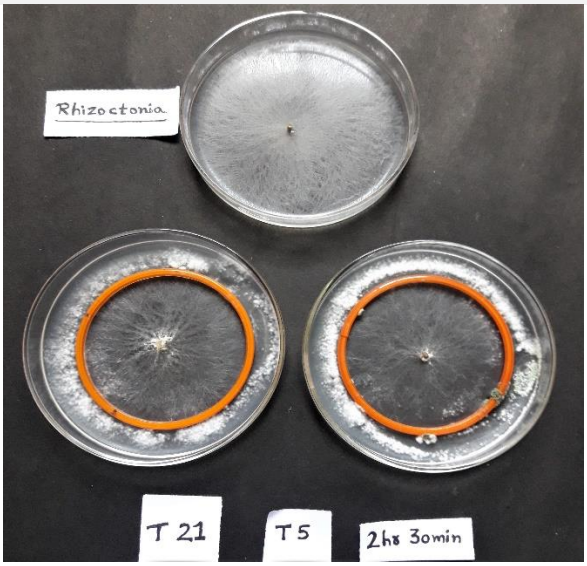
Rhizoctonia – T21(*T. asperellum*) (T2)



Rhizoctonia – T21(*T. asperellum*) (T3)



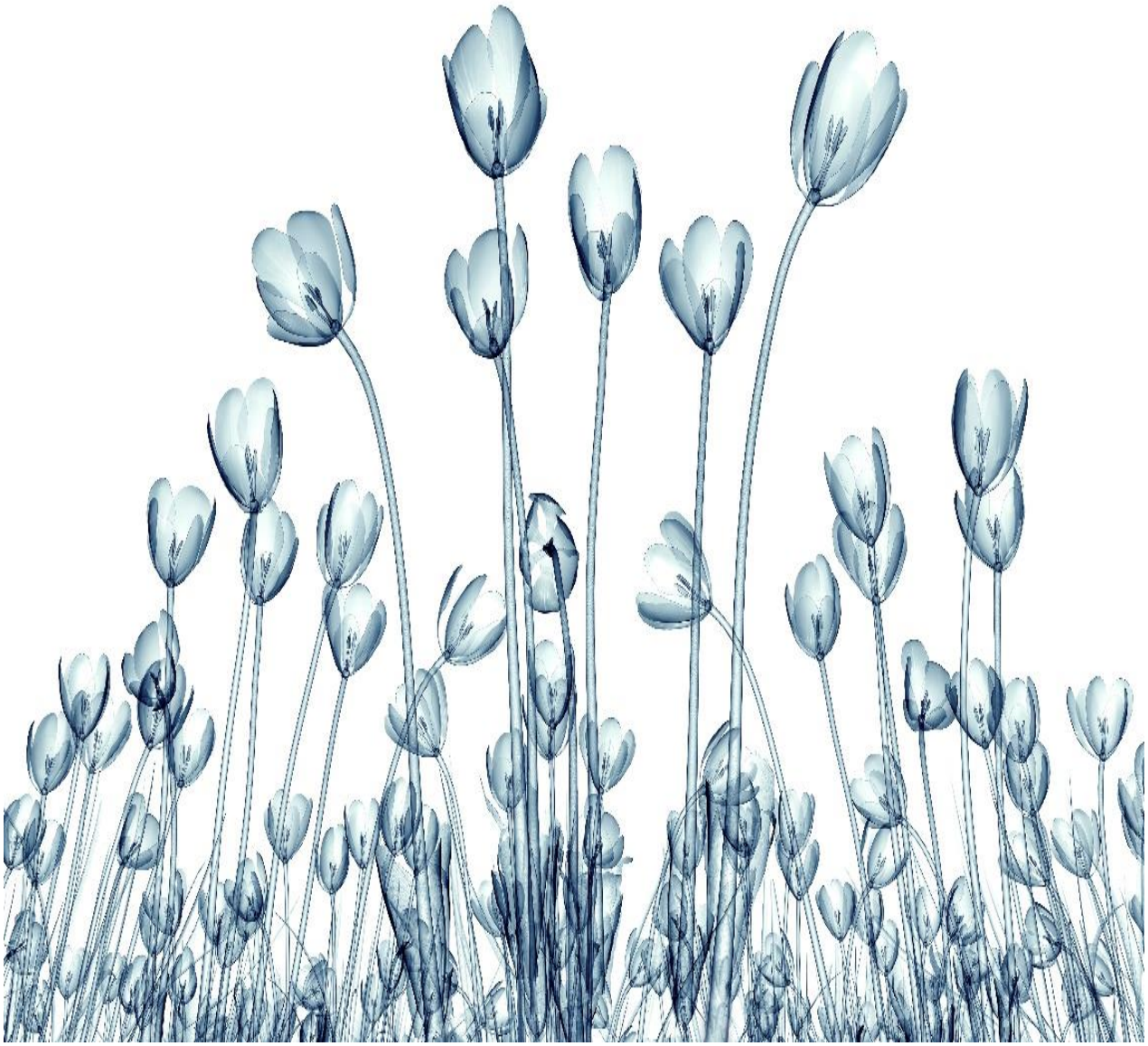
Rhizoctonia – T21(*T. asperellum*) (T4)



Rhizoctonia – T21(*T. asperellum*) (T5)

In summary, T1 strain of T21 shows highest inhibition ability against both the test pathogen. UBT-18 could not give better performance due to its slow growth. Sufficient competitive ability is not present in bio control agents to replace a pathogen which is already established. (Campbell, 1988). Therefore, co-inoculation of pathogens was done at the same time with the antagonist to get better efficacies. Against different pathogens, *Trichoderma* isolates show different antagonistic activities (Dennis and Webster, 1971; Papavizas and Lumsden, 1980), and even different strains of the same pathogen species (Bell et al., 1982). Mutagenesis is an approach that induces the genetic variations of targeted organisms. Induced changes could either be random or targeted depending on the selected mutagenesis technique. Polyketide synthase is a multidomain enzyme that is responsible for the production of the secondary metabolite polyketides. Hertweck (2009) showed a five-fold increase of polyketide synthase in the mutant strain compared to the parental strain. The investigation of Zhang et al. (2013) relayed that the mutant *T. harzianum* T-E5 manifested 30.2% exalted indole acetic acid yield compared to its wild kind. This rise enhanced plant-root colonization and plant biomass. It was reported that a UV-induced single point mutation localized to a regulatory domain in a UV-derived T. reesei mutant, had significantly increased xylanase expression and protein secretion. The observations of Besoain (2007), Wagh (2015), Alfiky (2019) stated the possible mechanisms of T1 isolate of T21 against *Fusarium oxysporum* f. sp. *lentis.*

5. SUMMARY AND CONCLUSION



5. SUMMARY AND CONCLUSION

The current study on "Antagonistic potential of mutants of *Trichoderma* species" was accomplished with the aim for (a) Molecular characterization through ITS sequencing of the native isolates of *Trichoderma* species (b) Exposure of the isolates to different doses of UV radiation to develop mutants and also to study the stability of the mutants in subsequent generations (c) Evaluation of *in-vitro* antagonistic property of the stable mutants against the soil-borne fungal pathogens *Fusarium oxysporum* f.sp. *lentis* and *Rhizoctonia solani* by dual culture method. The results acquired have been summed up herein.

(i) Two best strains of *Trichoderma* (UBT-18 and T21) isolated from soil of Cooch Behar were used. Both strains showed different visual appearance i.e. UBT-18 was light yellowish green in colour with low sporulation rate and on the other hand T21 exhibited dark green spores, high growth rate and marginal mycelial growth.

(ii) Molecular characterization of native *Trichoderma* isolates was done by ITS sequencing using ITS1 and ITS4 primers. The obtained nucleotide sequences of the strains were compared with the GeneBank database using the nucleotide BLAST program to identify homology between the PCR fragments and sequences in the GeneBank database and the distance tree was constructed. The distance tree obtained suggest that the test isolate UBT-18 belongs to *Trichoderma harzianum* and the test isolate T21 belongs to *Trichoderma asperellum*.

(iii) Both the *Trichoderma* spp. were exposed to UV light for different durations, immediately transferred to BOD after treatment and were later serial diluted and plated on Petri plates containing TSM. After few days of growth on BOD, mutated colonies of *Trichoderma* spores were picked on PDA slants before the colonies merged with each other.

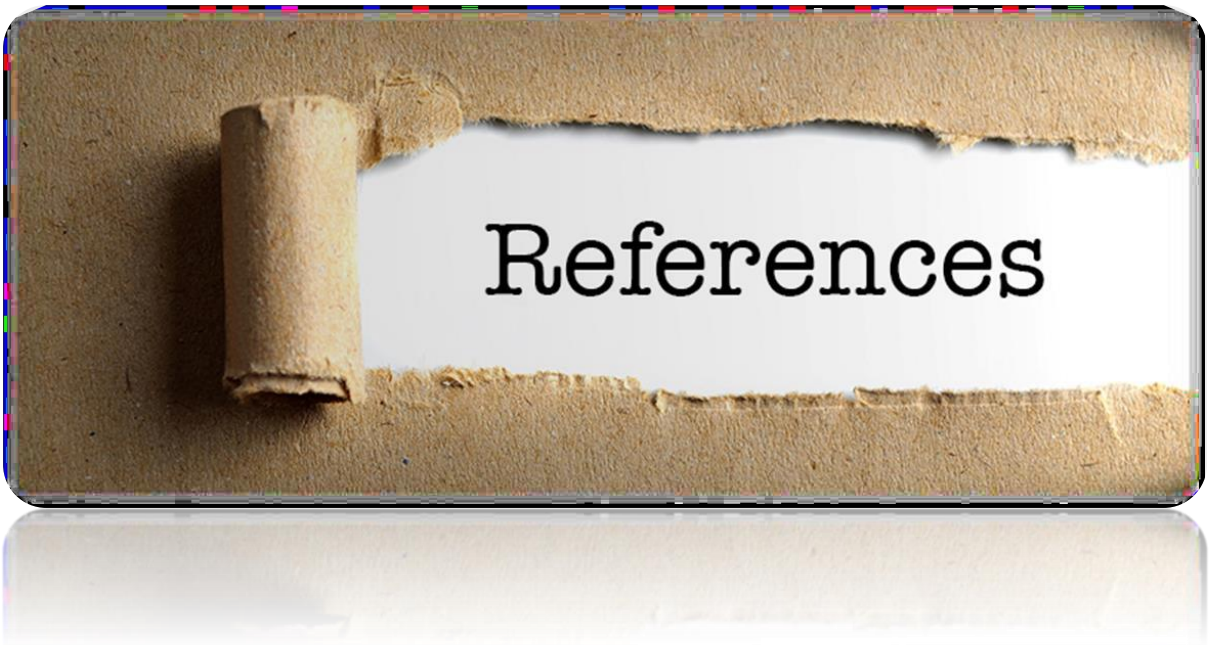
(iv) The colonies from individual mutated spores of *Trichoderma* strains were repeatedly sub-cultured in PDA slants up to 10th generation to obtain stable mutants. Some *Trichoderma* strains died out before reaching last generation growth due to segregation effect after random mutation. Such viability losing mutants were excluded from the experiment. During each generation of treatments, the differences in growth pattern and visual colour if any were observed carefully.

(v) The 'Bangle method' was used for determining antagonistic potential of mutated *Trichoderma* isolates as well their respective parental strains against the soil borne fungal pathogens *Fusarium oxysporum* f.sp. *lentis* and *Rhizoctonia solani* using dual culture. Among

the stable mutated isolated obtained after 10th generation, one isolate from each treatment having maximum growth rate was used.

(vi) Treatment T9 of UBT-18 (*T. harzianum*) exhibited higher percentage inhibition of growth over control (47.22%) compared to the wild UBT-18 (40.98%) thus showing 6.24 % increase of inhibition over wild isolate in case of pathogen *Fusarium oxysporum* f.sp. *lentis*. On the other hand, treatment T8 of UBT-18 was able to inhibit growth over control by 37.78% which was less compared to the wild one inhibiting over 68.89% in case of *Rhizoctonia solani*. Thus, T8 has shown 31.11% decrease of inhibition over wild isolate.

(vii) In case of *Fusarium oxysporum* f.sp. *lentis*, treatment T1 of T21 (*T. asperellum*) exhibit higher percent inhibition of growth over control (72.78%) compared to the wild isolate showing only 37.31% inhibition. Thus, T1 has 35.47% increase of inhibition over wild isolate. All the mutants of T21 were more efficient in controlling *Fusarium oxysporum* f.sp. *lentis* compared to the wild one. In case of pathogen *Rhizoctonia solani* also, treatment T1 showed higher percentage of inhibition of growth over control (70.44%) compared to the wild isolate (38.43%) thus exhibiting 32.01% increase of inhibition over wild isolate. But T5 have shown only 33.56% inhibition of growth over control exhibiting 4.87% decrease of inhibition over wild isolate. In summary, T1 strain of T21 was most effective in its inhibition ability against both the pathogens but UBT-18 could not give better performance due to its slow growth.


References

REFERENCES

Abd-EL M T H, Papavizas G C and Shatla M N (1982) Introduction of new isolates of *Trichoderma harzianum* tolerant to fungicides and their experimental use for control of white rot of onion. *Phytopathology* **72:** 369-400.

Abo-Elyousr K A M, Abdel Hafez S I and Abdel Rahim I (2014) Isolation of *Trichoderma* and evalation of their antagonistic potential against *Alternaria porri. J. Phytopathology* **162:** 567-574.

Ahmed, Sid A, Ezziyyani M and Sanchez C P (2003) Effect of chitin on biological control activity of *Bacillus* spp. and *Trichoderma harzianum* against root rot disease in pepper chilli (*Capsicum annum*) plants. *European Journal of Plant Pathology* **109(6):** 633-637.

Alabouvette C, Olivain C, Migheli Q, Steinberg C (2009) Microbiological control of soil-born phytopathogenic fungi with special emphasis on wilt-inducing *Fusarium oxysporum*. *New Phytol*. **184:** 529-544.

Alexander M (1982) *Introduction to Soil Microbiology*. pp 467. Wiley, New York.

Alfiky A (2019) Effects of ultraviolet irradiation on the in vitro antagonistic potential of *Trichoderma* spp. against soil-borne fungal pathogens. *Heliyon* **5(7)**, e02111.

Alizadeh H, Behboudi K, Ahmadzadeh M, Javan-Nikkhah M, Zamioudis C, Pieterse C M and Bakker P A (2013) Induced systemic resistance in cucumber and *Arabidopsis thaliana* by the combination f *Trichoderma harzianum* Tr6 and *Pseudomonas* sp. Ps 14. *Boil. Cont* **65(1):** 14-23.

Ambuse M G, Chatage V S and Bhale U N (2012) Influence of *Trichoderma* spp. against *Alternaria tenuissima* inciting leaf spot of *Rumex acetosa* L. *Bioscience Discovery* **3(2):** 259-262.

Amsellem Z, Zidack N K and Quimby P C Jr. (1999) Long-term dry preservation of viable mycelia of two mycoherbicidal oganisms.*Crop Protection* **18:** 643-649.

Anderson J D, Bailey B A, Taylor R, Sharon A, Avni A, Mattoo A K and Fuchs Y (eds) (1993) *Cellular and molecular aspects of the plant hormone ethylene*. pp 197-204. Kluwer, Dordrecht, The Netherlands.

Anitha R and Murugesan K (2001) Mechanism of action of *Gliocladium virens* on *Alternaria helianthi. Indian Phytopathology* **54:** 449-452.

Bae Y S and Knudsen G R (2005) Soil microbial biomass on growth and biocontrol efficacy of *Trichoderma harzianum. Biological Control* **32:** 236-242.

Baker R and Ahmad J S (1988) Implications of rhizosphere competence of *Trichoderma harzianum*. *Canadian Journal of Microbiology* **34(3):** 229-234.

Balaji L P and Ahir R R (2011) Evaluation of plant extracts and biocontrol agents against leaf spot disease of brinjal. *Indian Phytopath* **64(4):** 378-380.

Baldwin J E, O'Neil I A and Russell A T (1991) Isonitrin a: revision of the structure and total synthesis in racemic form. *Synlett* **8:** 551-552.

Bankole S A and Adebanjo A (1996) Biocontrol of brown blotch of cowpea caused by *Colletotrichum trunncatum* with *Trichoderma viride. Crop Protection* **15:** 633-636.

Barbosa M A G, Rehm K G, Menzes M and Mariano R L R (2001) Antagonism of *Trichoderma* species on *Cladosporium herbarium* and their enzymatic characterization. *Brazilian Journal of Microbiology* **32(2):** 98-104.

Basu A (2009) Employing eco-friendly potato disease management allows organic tropical Indian production systems to prosper. *As. J. Food Ag-Ind.* **Special Iss:** S80-S87.

Batta Y A (2004) Postharvest biological control of apple gray mold by *Trichoderma harzianum* Rifai formulated in invert emulsion. *Crop Protection* **23:** 19-26.

Bell D K, Wells H D and Markham C R (1982) In vitro antagonism of *Trichoderma* species against six fungal plant pathgens. *Phytopathology* **72:** 379-382.

Benitez T, Rincon A M, Limon M C, Codon A C (2004) Biocontrol Mechanisms of *Trichoderma* strains. *International Microbiology* **7:** 249-260.

Besoain X, Perez L M, Araya A, Lefever L, Sanguinetti M and Montealegre J (2007) New strains obtained after UV treatment and protoplast fusion of native *Trichoderma harzianum*: their biocontrol effect on *Pyrenochaete lycopersici. Electronic Journal of Biotechnology* **10(4):** 604-617.

Betina V and Spisiakova J (1976) Suppression of photo-induced sporulation in *Trichoderma viride* by inhibitors. *Folia Microbio.* **21:** 362-370.

Bigirimana J, De Meyer G, Poppe J, Elad Y, Hofte M (1997) Induction of systemic resistance on bean (*Phaseolus vulgaris*) by *Trichoderma harzianum. Med. Fac. Landbouww Univ. Gent.* **62:** 1001-1007.

Biswas K K and Sen C (2000) Management of stem rot of groundnut caused by *Sclerotium rolfsii* through *Trichoderma harzianum. Indian Phytopath* **53(3):** 290-295.

Blaszczyk L, Popiel D, Chelkowski J, Koczyk G, Samuels G J, Sobieralski K and Siwulski (2011) Species diversity of *Trichoderma* in Poland. *J. Appl Genetics* **52:** 233-243.

Brewer M T and Larkin R P (2005) Efficacy of several potential biocontrol organisms against *Rhizoctonia solani* on potato. *Crop Prot* **24:** 939-950.

Brian P W and McGowan J C (1945) Viridin. A highly fungistatic substance produced by *Trichoderma viride. Nature* **156:** 144-145.

Brotman Y, Briff E, Viterbo A, Chet I (2008) Role of swollenin, an expansin-like protein from *Trichoderma*, in plant root colonization. *Plant Physiol.* **147:** 779-789.

Bruce A and Highley T (1991) Control of growth of wood decay basidiomycetes by Trichoderma spp. and other potentially antagonistic fungi. *Forest Products Journal* **41(2):** 63-67.

Campbell R, Wilson M and Crawford E K (1988) Biological control by *Trichoderma harzianum* of damping-off of lettuce caused by *Rhizoctonia solani*. *EPPO Bulletin* **18(1):** 83-89.

Catlett N, Lee B N, Yoder O C and Turgeon B G (2003) Split marker recombination for efficient targeted detection of fungal genes. *Fungal Genetics Newsletter* **50:** 9-11.

Celar F A (2003) Competition for ammonium and nitrate forms of nitrogen between some phytopathogenic and antagonistic soil fungi. *Biological Control* **28(1):** 19-24.

Chakraborty B N, Chakraborty U, Dey P L, Saha A and Sunar K (2010) Molecular characterization of *Trichoderma viride* and *Trichoderma harzianum* isolated from soils of North Bengal based on rDNA markers and analysis of their PCR-RAPD profiles. *Global J. Biochem* **5(1):** 55-61.

Chaverri P, Gazis R, Samuels G J (2011) *Trichoderma amazonicum*, a new endophytic species on *Hevea brasiliensis* and *Hevea guianensis* from the Amazon basin. *Mycologia* **103:** 139-151.

Chaverri P and Samuels G J (2013) Evolution of habitat preference and nutrition mode in a cosmopolitan fungal genus with evidence of inter kingdom host jumps and major shifts in ecology. *Evolution* **67:** 2823-2837.

Chawla N and Gangopadhyay S (2009) Integration of organic amendments and bioagents in suppressing cumin wilt caused by *Fusarium oxysporum* f. sp. *cumini. Indian Phytopath* **62(2):** 209-216.

Chernin L and Chet I (2002) (eds.) *Enzymes in the Environment: Activity, Ecology and Applications*. pp 171-225. Marcel Dekker, Inc., New York.

Chet I, Harman G E and Baker R (1981) *Trichoderma hamatum:* Its hyphal interactions with *Rhizoctonia solanii* and *Pythium* spp. *Microb. Ecol.* **7:** 29-38.

Chet I and Inbar J (1994) Biological control of fungal pathogens. *Applied Biochemistry and Biotechnology* **48:** 37-43.

Chet I, Inbar J, Hardar I (1997) *The Mycota IV: Environmental and microbial relationships.* pp 165-184. Springer-Verlag, Berlin.

Christensen M (1981) (eds.) *The Fungal Community: its Organization and Role in the Ecosystem.* pp 201-232. Marcel Dekker, Inc. New York.

Chu F F and Wu W S (1981) Antagonistic action of *Trichoderma* spp. and *Penicillium* spp. on *Rhizoctonia solani*. *Memoirs of the College of Agriculture* **21:** 4-18.

Ciliento R, Lanzuise S, Gigante S, Rocco M, Anacleria M, Woo S L, Marra R, Vinale F, Ferraioli S, Ambrosino P, Soriente I, Turra D, Scala F and Lorito M (2006) The tabc2 abc transporter gene is involved in Trichoderma atroviride mycoparasitic and nutritional processes. *Proceedings*. Ninth International Workshop on Trichoderma and Gliocladium Vienna, Austria.

Clark F E (1965) (eds.) *Ecology of Soil-Borne Pathogens*. pp 339. University of California Press, Berkeley.

Claydon N, Allan M, Hanson J R and Avent A G (1987) Antifungal alkyl pyrones of *Trichoderma harzianum. Transactions of the British Mycological Society* **88(4):** 503-513.

Contreras-Cornejo H A, Macias-Rodriguez L, Beltran-Pena E, Herrera-Estrella A, Lopez-Bucio J (2011) *Trichoderma*-induced plant immunity likely involves both hormonal and camalexin dependent mechanisms in *Arabidopsis thaliana* and confers resistance against necrotrophic fungi *Botrytis cinerea*. *Plant Signal. Behav.* **6:** 1554–63.

Cook R and Baker K (1983) The nature and practice of biological control of plant pathogens. *Am. Phytopathol. Soc.* St Paul, MN.

Cooney J M and Lauren D R (1998) *Trichoderma* / pathogen interactions: Measurement of antagonistic chemicals produced at the antagonist / pathogen interface using a tubular bioassay. *Letters in Applied Microbiology* **27(5):** 283-286.

Cruz J and Llobell (1999) Purification and properties of a basic endo-β-1,6-glucanase (BGN16.1) from the antagonistic fungus *Trichoderma harzianum*. *European Journal of Biochemistry* **265:** 145-151.

Daguerre Y, Siegel K, Edel-Hermann V and Steinberg C (2014) Fungal proteins and genes associated with biocontrol mechanisms of soil-borne pathogens: a review. *Fungal Biology Reviews* **28:** 97-125.

Danielson R M and Davey C B (1973a) The abundance of *Trichoderma* propagules and the distribution of species in forest soil. *Soil Biol Biochem* **5:** 485-494.

Danielson R M and Davey C B (1973b) Nonnutritional factors affecting the growth of *Trichoderma* in culture. *Soil Biol. Biochem.* **5:** 495-504.

Davet P (1981) Effect, de quelques pesticides sun la colonisation d'un substract par le *Trichoderma harzianum* Rifai en presence des autres champignons du sol. *Soil Bio. Biochem.* **13:** 513-517.

D' Ercole N, Sportelli M and Nipoti P (1983) In vitro trials of antagonism between fungi of plant pathological interest. *Informatore Fitopathologico* **33:** 55-58.

Demain A L and Fang A (2000) The natural functions of secondary metabolites. *Adv. Bio. Engi. and Biotech***. 69:** 1-39.

De Meyer G, Bigirimana J, Elad Y, Hofte M (1998) Induced systemic resistance in *Trichoderma harzianum* T39 biocontrol of *Botrytis cinerea*. *Eur. J. Plant Pathol*. **104:** 279-286.

Dennis C and Webster J (1971) Antagonistic potential of species group of *Trichoderma*. *Trans. Br. Mycol. Soc* 57 41-IN4.

Dilbo C, Alemu M, Lencho A and Hunduma T (2015) Integrated management of garic white rot (*Sclerotium cepivorum* Berk) using some fungicides and antifungal *Trichoderma* species. *Journal of Plant Pathology & Microbiology* **6(1):** 1.

Dodge J A, Sato M and Vlahos C J (1995) Inhibition of phosphatidylinositol 3- kinase with viridin ananalogs thereof. *Eur. Patent Appl*. 648492.

Domsch K H, Gams W and Anderson T H (1980) *Compendium of Soil fungi*. pp 794-809. Academic Press, London.

Druzhinina I S, Seidl-Seiboth V, Herrera-Estrella A, Horwitz B A, Kenerley C M, et al. (2011) *Trichoderma*: the genomics of opportunistic success. *Nat. Rev. Microbiol.* **9:** 749–59.

Dubey SC, Suresh M, Singh B (2007) Evalaution of *Trichoderma* species agasint *Fusarium oxysporum* f.sp. *ciceris* for integrated management of chickpea wilt. *Biol. Control* **40:**118-127.

Duffy B K, Ownley B H and Weller D M (1997) Soil chemical and physical properties associated with suppression of take-all of wheat by *Trichoderma koningii. Phytopathology* **87:** 1118-1124.

Dutta S and Chatterjee N C (2004) Raising of carbendazim-tolerant mutants of *Trichoderma* and variations in their hydrolytic enzyme activity in relation to mycoparasitic action against *Rhizopus stolonifer*. *Journal of Plant Diseases and Protection* **111(6):** 557-565.

Eapen S, Beena B and Ramana K V (2005) Tropical soil microflora of spice-based ropping systems as potential antagonists of root-knot nematodes. *Journal of Invertebrate Pathology* **88(3):** 218-5.

Ejechi B O (1997) Biological control of wood decay in an open tropical environment with *Penicillium* spp. and *Trichoderma viride. Int. Biodeterior. Biodegrad*. **39:** 295-299.

Elad Y, Chet I, Boyle P, Henis Y (1983) Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii* scanning electron microscopy and fluorescence microscopy. *Phytopathology* **73:** 85–88.

Elad Y, Chet I and Heni Y (1981) A selective medium for improving quantitative isolation of *Trichoderma* spp. From soil. *Phytoparasitica* **9:** 59-67.

Elad Y, Chet I and Henis Y (1982) Degradation of plant pathogenic fungal *Trichoderma harzianum. Canad. J. Microbiol.* **28:** 719-725

Elad Y, Harder Y, Chet I and Henis Y (1982). Prevention with *Trichoderma harzianum* Rifai aggr., of reinfection by *Sclerotium rolfsii* sacc and *Rhizoctonia solanii* kuhn of soil fumigated with methyl bromide, and improvement of disease control in tomatoes and peanuts. *Crop Prot.* **1:** 199-211.

El-Fiky Z A, Shalaby O Y and Ahmed N F (2006) Characterization of some *Trichoderma* isolates antagonistic to *Rhizoctonia solani* the causal of bean root rot. Proc 2nd *Farm Integrated Pest Management 16-18 Jan* pp. 154-171.

Elsharkawy M M, Shimizu M, Hideki T, Kouichi O and Mitsuro H (2013) Induction of systemic resistance against cucumber mosaic virus in *Arabidopsis thaliana* by *Trichoderma asperellum* SKT-1. *Plant Pathol. J.* **29:** 193-200.

Escande A, Laich F and Pedraza M V (2002) Field testing of honeybee-dispersed *Trichoderma* spp. to manage sunflower head rot (*Sclerotinia sclerotium*). *Plant Pathology* **51(3):** 346-351.

Etebarian H, Scott E and Wicks T (2000) *Trichoderma harzianum* T39 and *Trichoderma virens* DAR 74290 as potential biological control agents for *Phytophthora erythroseptica*. *Eur. J. Plant Pathol*. **106:** 329-337.

Evans H C (2000) Evaluting plant pathogens for biological control of weeds: an alternative view of pest risk assessment. *Australasian Plant Pathology* **29:** 1-14.

Ezzi M and Lynch J (2005) Biodegradation of cyanide by *Trichoderma* spp. and *Fusarium* spp. *Enzyme and Microbial Technology* **36(7):** 849-854.

Fahmi A I, Al-Talhi, A D and Hassan, M M (2012) Protoplast fusion enhances antagonistic activity in *Trichoderma* spp. *Nature and Science* **10(5):** 100-106.

Fahmi A I, Eissa R A, El-Halfawi K A, Hamza H A and Helwa M S (2016) Identification of *Trichoderma* spp. by DNA barcode and screening for cellulolytic activity. *J Microb Biochem Technol* **8(3):** 202-209.

Farkas V, Labudova I and Ferenczy L (1981) Preparation of mutants of *Trichoderma viride* with increases production of cellulase. *Folia Microbiologica* **26(2):** 129-132.

Felse P A and Panda T (1999) Self-directing optimization of parameters for extracellular chitinase production by *Trichoderma harzianum* in batch mode. *Process Biochemistry* **34(6-7):** 563-566.

Gadgil N, Daginawala H F, Chokrabarti T and Khanna P (1995) Enhanced cellulase production by a mutant of *Trichoderma reesei*. *Enzyme Microbial Technol*. **17:** 942-946.

Gajera H, Domadiya R, Patel S, Kapopara M, Golakiya B (2013) Molecular mechanism of *Trichoderma* as bio-control agents against phytopathogen system – a review. *Curr. Res. Microbiol. Biotechnol*. **1:** 133-142.

Gajera H P and Vakharia D N (2010) Molecular and biochemical characterization of *Trichoderma* isolates inhibiting a phytopathogenic fungi *Aspergillus niger* Van Tieghem. *Physiological and Molecular Plant Pathology* **74(3-4):** 274-282.

Ganassi as, Moretti A, Stornelli C and Sabatini M A (2001) Effect of *Fusarium paecilomyces* and *Trichoderma* formulations against aphid *Schizaphis graminum. Mycopathologia* **151(3):** 131-138.

Ganesan A K (1974) Persistence of pyrimidine dimers during post-replication repair in ultraviolet light-irradiated *Escherichia coli* K12. *J. Mol. Biol*. **87:** 103-119.

Garon D and Sage L (2004) Effects of fungal bioaugmentation and cyclodextrin amendment on fluorene degradation in soil slurry. *Biodegradation* **15(1):** 1-8.

Gazis R and Chaverri P (2010) Diversity of fungal endophytes in leaves and stems of rubber trees (*Hevea brasiliensis*) in Tambopata, Peru. *Fungal Ecol.* **4:** 94-102.

Geremia R A, Goldman G H, Jacobs D, Ardiles W, Vila S B, Van Montagu M and Hrrera-Estrella A (1993) Molecular characterization of the proteinase-encoding gene, *prb*1, related to mycoparasitism by *Trichoderma harzianum. Mol. Microbiol.* **8:** 603-613.

Graeme-Cook K A and Faull J L (1991) Effect of ultraviolet induced mutants of *Trichoderma harzianum* with altered antibiotic production on selected pathogens in vitro. *Canadian Journal Microbiology* **37:** 659-664.

Gressel J B and Harmann K M (1968) Morphogenesis in *Trichoderma* Action spectrum of photoinduced sporulation. *Planta* **79:** 271-274.

Grigoriev IV, Nordberg H, Shabalov I, Aerts A, Cantor M, Goodstein D, Kou A, Minovitsky S, Nikitin R, Ohm R A, Otillar R, Poliakov A, Ratnere I, Riley R, Smirnova T, Rokhsar D and Dubchak I (2012) The gnome portal of the department of Energy Joint Genome Institute. *Nucleic Acids Res*. **40:** D 26-32.

Gruber S and Seidl-Seiboth V (2012) Self versus no-self: fungal cell wall degradation in *Trichoderma. Microbiology* **158:** 26-34.

Gupta M, Dohroo N P, Gangta V and Shanmugam V (2010) Effect of microbial inoculants on rhizome disease and growth parameters of ginger. *Indian Phytopathology* **63(4):** 438-441.

Gupta V P, Tewari S K, Govindaiah I and Bajpai A K (1999) Ultrastructure of mycoparasitsm of *Trichoderma*, *Gliocladium* and *Laetisaria* species on *Botryodiplodia theobromae. Journal pf Phytopathology* **147:** 19-24.

Gutter Y (1957) Effect of light on sporulation of *Trichoderma viride* Pers. Ex Fries. *Bull Res Counc Israel Sect D Bot* **5:** 273-286.

Haggag W M and Mohamed H A A (2002) Enhancement of antifungal metabolite production from gamma-ray induced mutants of some *Trichoderma* species for control onion white disease. *Plant Pathology Bulletin* **11:** 45-56.

Hanson L E (2002) Reduction of *Verticillium* wilt symptoms in cotton following treatment with Trichoderma virens. *J. Cotton Sci*. **4:** 224-231.

Hanson L E and Howell C R (2002) Biocontrol efficacy and other characteristics of protoplast fusants between *Trichoderma koningii* and *T. virens*. *Mycol. Res*. **106(3):** 321-328.

Harman G E and Bjorjman T (1998) Potential and existing uses of *Trichoderma* and *Gliocladium* for plant disease control and plant growth enhancement. *Enzymes, Biological Control and commercial applications* **2:** 229-265.

Harman G E, Howell C R, Viterbo A, Chet I and Lorito M (2004) *Trichoderma species* – opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol.* **2:** 43-56.

Harman G E (2006) Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology* **96:** 190-194.

Harman G E, Herrera-Estrella A H, Benjamin A and Matteo L (2012) Special issue: *Trichoderma* – from Basic Biology to Biotechnology. *Microbiology* **58*:*** 1-2.

Hassan M M (2014) Influence of protoplast fusion between two *Trichoderma* spp. on extracellular enzymes production and antagonistic activity. *Biotechnology & Biotechnological Equipment* **28(6):** 1014-1023.

Hazra S (2003) *Biocontrol potential and soil biology of some mutant isolates of Trichoderma harzianum Rifai*. Ph.D. Thesis. Bidhan Chandra Krishi Viswavidyalaya.

Henis Y and Papavizas G C (1983) Factors affecting germinability and susceptibility to attack of scerotia of *Sclerotium rolfsii* by *Trichoderma harzianum* in field soil. *Phytopathology* **73:** 1469-1474.

Henis Y, Ghaffar A and Baker R (1987) Integrated control of *Rhizoctonia solani* of radish: Effect of successive plantings, PCNB, and *Trichoderma harzianum* on pathogen and disease. *Phytopathology* **68:** 900-990.

Heraux F M G, Hallet S G, Ragothama K G and Weller S C (2005) Composted chicken manure as a medium for the production and delivery of *Trichoderma virens* for weed control. *HortScience* **40(5):** 1394-1397.

Hermosa M R, Grondona I, Iturriaga E A, Diaz-Mingue J M, Castro C, Monte E and Garcia-Acha I (2000) Molecular characterization and identification of biocontrol isolates of *Trichoderma* spp. *Applied and Environmental Microbiology* **66(5):** 1890-1898.

Hertweck C (2009) The biosynthetic logic of polyketide diversity. *Angew. Chem. Int. Ed.* **48:** 4688-4716.

Hibbett D S (1992) Ribosomal RNA and fungal systematics. *Trans Mycol Soc* **33:** 533-556.

Highley T L and Ricard J (1988) Antagonism of *Trichoderma* spp. and *Gliocladium virens* against wood decay fungi. *Materal und Organismen* **23:** 157-169.

Hjeljod L G, Stensvand A and Tronsmo A (2000) Effect of temperature and nutrient stress on the capacity of commercial *Trichoderma* products to control *Botrytis cinerea* and *Mucor piriformis* in greenhouse strawberries. *Boil. Control* **19(2):** 146-160.

Hoell L A, Klemsdal S S, Vaaje-Koistad G, Hom S J and Eijsink V G H (2005) Overexpressiom and characterization of a novel chitinase from *Trichoderma atroviride* strain PI. *Biochem. Biophys. Acta.* **1748:** 180-190.

Howell C R ad Puckhaber L S (2005) Study of the characteristics of “P” and “Q” strains of *Trichoderma virens* to account for differences in biological control efficacy against cotton seedling diseases. *Biological control* **33(2**).

Howell T A, Ko J., Piccinni G and Marek T (2009) Determination of growth-stage-specific crop coefficients (Kc) of cotton and wheat. *Agricultural Water Management* **96(12):** 1691-1697.

Howell C R, Hanson L E, Stipanivic R D, Puckhaber L S (2000) Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens. Phytopathology* **90:** 248-252.

Howell C R (2002) Cotton seedling pre emergence damping off incited by *Rhizopus oryzae* and *Pythium* spp. and its biological control with *Trichoderma* spp. *Phytopathology* **92:** 177-180.

Howell C R (2003) Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. *Plant dis* **87:** 4-10.

Howell C R and Stipanovic R D (1983) Gliovirin, new antibiotic from *Gliocladium virens* and its role in the biological control of *Pythium ultimum. Canadian Journal of Microbiology* **29:** 321-324.

Hukma R and Pandey R N (2011) Efficacy of bio-control agents and fungicides in the management of wilt of pigeon pea. *Indian Phytopathology* **64(3):** 269-271.

Hutchinson C M (1999) *Trichoderma virens*- Inoculated composed chicken manure for biological weed control. *Biological Control* **16(2):** 217-222.

Ikehata H and Ono T (2011) The mechanisms of UV mutagenesis. *J. Radiat. Res.* **52:** 115-125.

Innocenti G, Roberti R, Montanari M and Zakrisson E (2003) Efficacy of microorganism antagonistic to *Rhizoctonia cerealis* and their cell wall degrading enzymatic activities. *Mycological Research* **107(4):** 421-427.

Itoh Y, Kodama K, Furuya K, Takahashi S, Haneishi T, Takiguchi Y and Aria M (1980) A new sesquiterpene antibiotic, heptelidic acid producing organisms, fermentation, isolation and characterization. *J Antibiot* **33:** 468-473.

Jat J G and Agalave H R (2013) Antagonistic properties of *Trichoderma* species against oilseed-borne fungi. *Sci. Res. Reporter* **3(2):**171-174.

Jensen C E de, Percich J A and Graham P H (2002) Integrated management strategies of bean root rot with *Bacillus subtilis* and *Rhizobium* in Minnesota. *Field Crop Research* **74(2-3):** 107-115.

Kapoor A S (2008) Biocontrol potential of *Trichoderma* spp. Against important soilborne diseases of vegetable crops. *Indian Phytopath.* **61(4):** 492-498.

Katayama A and Matsumur F (1993) Degradation of organochlorine pesticides, particularly endosulfan, by *Trichoderma harzianum. Environ. Toxicol. Chem.* **12:** 1059-1065.

Kaur N P and Mukhopadhyay A N (1992) Integrated control of 'chickpea wilt complex' by Trichoderma and chemical methods in India. *International Journal of Pest Management* **38(4):** 372-375.

Kerry B R (1981) (ed) *Biological Control in Crop Production*. pp 79-90. Totowa N J.

Khan J, Ooka J J, Miller S A, Madden K V and Hoitink H A J (2004) Systemic resistance induced by *Trichoderma harzianum* 382 in cucumber against *Phytophthora* crown rot and leaf blight. *Plant Disease* **88:** 280-286.

Khosla C (2009) Structures and mechanisms of polyketide synthases. *J. Org. Chem.* **74:** 6416-6420.

Kohl J and Schlosser E (1989) Decay of sclerotia of *Botrytis cinerea* by *Trichoderma* spp. at low temperature. *Phytopathology*, **125:** 320-326.

Koide R T, Suomi L, Stevens C M and McCormick L (1998) Interactions between needles of *Pinus resinosa* and ectomycorrhizal fungi. *New Phytologist* **140(3):** 539-547.

Koike N, Hyakumachi M, Kageyama K, Tsuyumu S and Doke N (2001) Induction of systemic resistance in cucumber against several diseases by plant growth-promoting fungi: Lignification and superoxide generation. *European Journal of Plant Pathology* **107(5):** 523-533.

Kotasthane A, Agrawal T, Kushwah R and Rahatkar O V (2015) In-vitro antagonism of *Trichoderma* spp. against *Sclerotium rolfsii* and *Rhizoctonia solani* and their response toward growth of cucumber, bottle gourd and bitter gourd. *European Journal of Plant Pathology* **141(3):** 523-543.

Kredics L, Antal Z, Szekers A, Hatvani L, Manczinger L, Vagvolgyi C and Nagy E (2005) Extracellular protease of *Trichoderma* species. *Acta Microbiol. Immunol. Hung*. **52:** 169-184.

Kudryavtseva K I (1980) *The possibility of using trichodennin in the control of root rot of cucumbers in glass house*. pp 111-119. Sbornik Nauchnykh Trudov Institute, USSR, Lenin Acad. Agric. Sc.

Kuhls K, Lieckfeldt E, Samuels G J, Meyer W C, Kubicek P and Borner T (1997) Revision of *Trichoderma* sp. *longibrachiatum* including related teleomorphs based on analysis of ribosomal DNA internal transcribed spacer regions. *Mycologia* **89:** 442-460.

Kumar V, Sharma D D, Babu A M and Datta R K (1998) SEM studies on the hyphal interactions between a biocontrol agent *Trichoderma harzianum* and a mycopathogen *Fusarium solani* causing root rot disease in mulberry. *Indian Journal of Sericulture* **3:** 17-20.

Latorre B A, Lillo C and Rioja M E (2001) Eficacia de los tratamientos fungicidas para el control de *Botrytis cinerea* de la vid en function de la epoca de aplicacion. *Ciencia e Investigacion Agraria* **28:** 61-66.

Le Doan T, El-Hajii M, Rebuffa S, Rajeswari M R and Bodo B (1986) Fluorescein studies on the interaction of trichozianine a IIIc with model membranes. *Biochemica et Biophisica Acta*. **858:** 1-5.

Levy N O, Meller H Y, Haile Z M, Elad Y, David E, Jurkevitch E and Katan J (2015) Induced resistance to foliar diseases by soil solarization and *Trichoderma harzianum*. *Plant Pathol*. **64:** 365-374.

Lewis J A and Papavizas G C (1983) Production of chlamydospores and conidia by *Trichoderma* spp. in liquid and solid growth media. *Soil Biology and Biochemistry* **15:** 351-357.

Lewis J A (1991) (eds.) The rhizosphere and plant growth. *Agric. Res.* **14:** 279-287.

Lieckfeldt E, Samuels G J, Nirenberg H I and Petrini O (1999) A morphological and molecular perspective of *Trichoderma viride*, is it one or two species. *Applied and Environmental Microbiology* **65:** 2418-2428.

Li N, Alfiky A, Wang W, Islam M, Nourollahi K, Liu X and Kang S (2018) Volatile compound-mediated recognition and inhibition between *Trichoderma* biocontrol agents and *Fusarium oxysporum*. *Front. Microbiol***. 9:** 1-16.

Lindsey D L and Baker R (1967) Effect of certain fungi on dwarf tomatoes grown under gnotobiotic conditions. *Phytopathology* **57:** 1262-1263.

Lin K and Wang A (2001) UV mutagenesis in *Escherichia coli* K-12: cell survival and mutation frequency of the chromosomal genes lacZ, rpoB, ompF, and ampA. *J. Exp. Microbiol. Immunol* **1:** 32-46.

Lin Y R, Lo C T, Li S Y and Peng K C (2012) Involvement of pachybasin and emodin in self-regulation of *Trichoderma harzianum* myco-parasitic coiling. *J Agric Food Chem* **60:** 2123-2128.

Liss S N, Brewer D, Taylor A and Jones G A (1985) Antibiotic activity of an isocyanide metabolite of *Trichoderma hamatum* against rumen bacteria. *Can. J. Microbiol*. **31:** 767-772.

Lo C T, Liao T F and Deng T C (2000) Induction of systemic resistance of cucumber to cucumber green mosaic virus by the root-colonizing *Trichoderma* spp. *Phytopathology* **90:** S47.

Lopez E and Orduz S (2003) *Metarhizium anisopliae* and *Trichoderma viride* for control nests of the fungus-growing ant, *Atta cephalotes. Biological Control* **27(2):** 194-200.

Luckner M (ed) (1990) *Secondary Metabolism in Microorganism, Plants and Animals*. Springer-Verlag, Berlin.

Macauley B J and Griffin D M (1969) Effects of carbon dioxide and oxygen on the activity of some soil fungi. *Transactions of the British Mycological Society* **53(1):** 53-62.

Martin C, Torres H and Henfling J (1985) Chemical control of pink rot of potato (*Phytophthora erythroseptica* PethyB.). *Americal Potato Journal* **62(7):** 355-361.

McIntyre M, Nielsen J, Arnau J, van der Brink H, Hansen K and Madrid S (2004) (ed) Proc 7th *European Conference on Fungal Genetics*. Copenhagen, Denmark.

Meller H Y, Haile M Z, David D, Borenstein M, Shulchani R, Elad Y (2013) Induced systemic resistance against grey mould in tomato (*Solanum lycopersicum*) by benzothiadiazole and *Trichoderma harzianum* T39. *Phytopathology* **104:** 150-157.

Migheli Q, Gonzalez-Candelas L, Dealessi L, Camponogara A and Ramon V D (1998) Transformants of *Trichoderma longibrachiatum* overexpressing the *β*-1, 4-endoglucanase gene egl1 show enhanced biocontrol of *Pythium ultimum* on cucumber. *Phytopathology* **88:** 673-677.

Mohamed H A A and Haggag W M (2010) Mutagenesis and inter-specific protoplast fusion between *Trichoderma koningii* and *Trichoderma reesei* for biocontrol improvement. *American Journal of Scientific and Industrial Research* **1:** 504-515.

Monte E (2001) Understanding *Trichoderma*: between biotechnology and microbial ecology. *International Microbiology* **4:** 1-4.

Moreno L S and Paningbatan R A (1995) Biological control of mango stem end rot caused by *Diplodia natalensis* with *Trichoderma viride. Philippine Phytopathology* **31:** 103-116.

Mortuza H G and Ilag L L (1999) Potential for biocontrol of *Lasiodiplodia theobromae* (Pat.) Griff. and Maubl. in banana fruits by *Trichoderma* species. *Biological Control* **15:** 235-240.

Mukherjee A, Cui Y, Dumenyo C K, Liu Y and Chatterjee A K (1999) *rsm*C of the soft-rotting bacterium *Erwinia caratovora* subsp. *caratovora* negatively controls extracellular enzyme and harpin Ecc production and virulence by modulating levels of regulatory RNA (*rsm*B) and RNA-Binding Protein (RsmA). Journal of Bacteriology **181(19):** 6042-6052.

Mukherjee P K, Buensanteai N, Moran-Diez M E, Druzhinina I S and Kenerley C M (2012) Functional analysis of non-ribosomal peptide synthetases (NRPSs) in *Trichoderma virens* reveals a polyketide synthase (PKS)/NRPS hybrid enzyme involved in induced systemic resistance response in maize. *Microbiology* **158:** 155-165.

Mukherjee P K and Mukhopadhyay A N (1993). Induction of stable mutants of *Gliocladium virens* by gamma radiations. *Indian Phytopathology* **46:**393-397.

Mukherjee P K, Mukhopadhyay A, Sarmah D and Shrestha S (1995) Comparative antagonistic properties of *Gliocladium virens* and *Trichoderma harzianum* on *Sclerotium rolfsii* and *Rhizoctonia solani*: its relevance to understanding the mechanisms of biocontrol. *Journal of Phytopathology* **143:** 275-79.

Mukherjee P K, Horwitz A, Herrera-Estrella A, Schmoll M and Kenerler C M (2013) *Trichoderma* research in the genome era. *Annu. Rev. Phytopathol*. **51:** 105-129.

Naeimi S, Okhovvat S M, Javan-Nikkhah M and Vagvolgvi C (2010) Biological control of Rhizoctonia solani AG1-1A, the causal agent of rice sheath blight with *Trichoderma* strains. *Phytopathologia Mediterranea* **49(3):** 287-300.

Nath V S, Senthil M, Hedge V M, Jeeva M L, Misra R S, Veena S S and Raj M (2013) Molecular evidence supports hypervariability in *Phytophthora colocasiae* associated with leaf blight of taro. *European Journal of Plant Pathology* **136(3):** 483-494.

Nawrocka J and Malolepsza U (2013) Diversity in plant systemic resistance induced by *Trichoderma*. *Biological control* **67(2):** 149-156.

Nodvig C S, Nielsen J B, Kogle M E and Mortensen U H (2015) A CRISPR-cas9 system for genetic engineering of filamentous fungi. *PLoS One* 10.

Oda S, Isshiki K and Ohashi S (2009) Production of 6-pentyl-a-pyrone with *Trichoderma atroviride* and its mutant in a novel extractive liquid-surface immobilization (Ext-LSi) system. *Process Biochem*. **44(6):** 625-630.

Okigbo R N and Oikediugwu F E (2000) Studies on biological control of post-harvest rot in yams (*Dioscorea* spp.) using *Trichoderma viride. Journal of Phytopathology* **148:** 351-355.

Ordentlich A and Chet I (1989) Biological control of soil-borne plant pathogenic fungi by antagonistic *Trichoderma. Israel Agresearch* **3:** 1-2.

Ortiz A and Orduz S (2001) In vitro evaluation of *Trichoderma* and *Gliocladium* antagonism against the symbiotic fungus of the leaf-cutting ant *Atta cepalotes. Mycopathologia* **150(2):** 53-60.

Pahl H L, Krauss B, Schulze-Osthoff K, Dexker T, Traenckner E B, Vogt M, Myers C, Parks T, Warring P, Muhlbacher A, Czernilofsky A P and Baeuele P A (1996) The immunosuppressive fungal metabolite gliotoxin specifically inhibits transcription factor NF-kappaB. *Journal of Experimental Medicine* **183(4):** 1829-40.

Pan S and Das A (2011) Control of cowpea (*Vigna sinensis*) root and collar rot (*Rhizoctonia solani*) with some organic formulations of *Trichoderma harzianum* under field condition. *The J. Plant Prot. Sci*. **3(2):**20-15.

Pandya J R, Sabalpara A N and Chawda S K (2011) *Trichoderma:* a particular weapon for biological control of phytopathogens. *J. Agri. Technol*. **7(5):** 1187-1191.

Papavizas G C (1981) Survival of *Trichoderma hazianum* in soil and in the pea and the bean rhizosphere. *Phytopathology,* **72:** 121-125.

Papavizas G C and Lewis I A (1983) Physiology and biochemical characteristics of stable mutants of *Trichoderma viride* resistant to MBC fungicides. *Phytopathology* **73:** 407-411.

Papavizas G C and Lumsden R D (1980) Biological control of soilborne fungal propagules. *Annual Review of Phytopathology 18(1):* 389-413.

Papavizas G C (1985) *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. *Annual Review of Phytopathology* **23:** 92.

Patel J S, Sarma B K, Singh H B, Upadhyay R S, Kharwar R N and Ahmed M (2016) *Pseudomonas fluorescens* and *Trichoderma asperellum* enhance expression of Gα subunits of the pea heterotrimer G-protein during *Erysiphe pisi* infection. *Frontiers in Plant Science* **6:** 1206.

Pecchia S and Anne J (1989) Fusion of protoplasts from antagonistic *Trichoderma harzianum* strains. *Acta Hortic*. **255:** 303-312.

Pelczar M J Jr, Chan E C and Krieg N R (1993) Control of microorganisms: Principles and physical agents. *Microbiology: concepts and applications* **1:** 200-220.

Perazzoli M, Moretto M, Fontana P, Ferrarini A, Velasco R, Moser C, Delledonne M and Pertot I (2012) Downey mildew resistance induced by *Trichoderma harzianum* T39 in susceptible grapevines partially mimics transcriptional changes of resistant genotypes. *BMC Genomics* **13:** 660.

Perello A, Monaco C, Sisterna M and Dal Bello G (2003) Biocontrol efficacy of *Trichoderma* isolates for Tan Spot of wheat in Argentina. *Crop Protection* **22:** 1099-1106.

Pfeifer G P, You Y H and Besaratinia A (2005) Mutations induced by ultraviolet light. *Mutat. Res. Fundam. Mol. Mech. Mutagen* **571:** 19-31.

Pinto R J, Mapeli N C, Cremon C and Silva E F (2014) Germinacao e crescimento inicial de managaba (*Hancornia speciosa* Gomes) em funcao de preparados homeopaticos Carbo vegetabilis e dias apos o despolpamento para semeadura. *Revista Agrarian* **7(24):** 244-250.

Piotrowski M and Volmer J J (2006) Cyanide metabolism in higher plants: cyanoalanine hydratase is a NIT4 homolog. *Plant. Mol. Biol*. **61:** 111-122.

Potin O, Rafin C and Veignie E (2004) Bioremediation of an aged polycyclic aromatic hydrocarbons (PAHs)-contaminated soil by filamentous fungi isolated from the soil. *International Biodeterioration & Biodegradation* **54(1):** 45-52.

Punja Z K and Utkhede R S (2003) Using fungi and yeast to manage vegetable crop diseases. *Trends in Biotechnology* **21:** 400-407.

Puranen T, Alapuranen M and Vehmaanpera J (2014) Chapter 26-*Trichoderma* enzymes for textile industries. In Biotechnology and biology of *Trichoderma*, pp. 351-362.

Pyke T R and Dietz A (1996) U-21, 963, a new antibiotic I. Discovery and biological activity. *Appl Microbiol* **14:** 506-510.

Raeder U and Broda P (1985) Rapid preparation of DNA from filamentous fungi. *Letters in Applied Microbiology* **1:** 17-20.

Raguchander T, Rajapan K and Samiyappan R (1997) Evaluating methods of application of biocontrol agents in the control of mungbean root rot. *Indian Phytopathology* **50:** 229-234.

Rahman M A, Razvy M A and Alam M F (2013) Antagonistic activities of *Trichoderma* strains against chili anthracnose pathogen. *Int. J. Microbiol. Mycol* **1(1):** 7-22.

Rawal P, Sharma P, Singh N D and Joshi A (2013) Evaluation of fungicides, neem bio-formulations and biocontrol agent for the management of root rot of safed musli caused by *Rhizoctonia solani*. *J. Mycol. Plant Pathol*. **43(30):** 297.

Rebuffat S, El Hajji M, Hennig P, Dvoust D and Bodo B (1989) Isolation, sequene, and conformation of seven trichorzianines B from *Trichoderma harzianum. Int J Pept Protein Res* **34:** 200-210.

Rehman S U, Lawrence R, Kumar E J and Badri Z A (2012) Comparative efficacy of *Trichoderma viride, T. harzianum* and carbendazim against damping-off disease of cauliflower caused by *Rhizoctonia solani* Kuehn. *Journal of Biopesticides* **5(1):** 23.

Reino J L, Guerrero R F, Hernandez-Galan R and Colldo I G (2008) Secondary metabolites from species of the biocontrol agent *Trichoderma. Phytochem. Rev*. **7(1):** 89-123.

Rifai M A (1969) A revision of the genus *Trichoderma*. *Mycol*. **116:** 1-56.

Riker A J and Riker R S (1936) Introduction to research on plant diseases. In: a guide to the principles and practices for studying for various plant diseases problems. University of Winconsin, USA. p.117.

Roy, A (2003*) Biocontrol potential and microhabitat behaviour of some mutant isolates of Gliocladium virens Miller et al*. Ph.D. Thesis. Bidhan Chandra Krishi Viswavidyalaya.

Rudresh D L, Shivaprakash M K and Prasad R D (2005) Potential of *Trichoderma* spp. as biocontrol agents of pathogens involved in wilt complex of chickpea (*Cicer crietinum* L.). J. *Biol. Control* **19:** 157-166.

Saha D K and Pan S (1996) In vitro antagonistic potential of different isolates of *Gliocladium virens* of West Bengal. *J. Nat. Bot. Soc*. **50:** 13-18.

Saha D K and Pan S (1997) Quantitative evaluation of some specific media of *Trichoderma* and *Gliocladium* spp. *J. Mycopathol Res*. **35:** 7-3.

Saifullah and Thomas B J (1996) Studies on the parasitism of *Globodera rostochinensis* by *Trichoderma hazianum* using low temperature scanning electron microscopy. *Afro-Asian Journal of Nematology* **6:** 117-122.

Saksirirat W, Chareerak P and Bunyatrachata W (2009) Induced systemic resistance of biocontrol fungus, *Trichoderma* spp. against bacterial and grey pot in tomatoes. *Asian Journal of Food and Agro-Industry* Special Issue S99-S104.

Sakuno E, Yabe K, Hamasaki T and Nakajima H (2000) A new inhibitor of 5'-hydroxyaverantin dehydrogenase, an enzyme involved in aflatoxin biosynthesis, from *Trichoderma hamatum*. *Journal of Natural Products* **63(12):** 1677-1678.

Salas-Marina M A, Silva-Flores M A, Uresti-Rivera E E, Castro- Longoria E, Herrera-Estrella A and Casas-Flores S (2011) Colonization of *Arabidopsis* roots by *Trichoderma atroviride* promotes growth and enhances systemic disease resistance through jasmonic acid/ethylene and salicylic acid pathways. *Eur. J. Plant. Pathol*. **131:** 15-26.

Salas-Marina M A, Isordia-Jasso M, Islas-Osuna M A, Delgado-Sanchez P, Jimenez-Bremont J F, Rodriguez-Kessler M, Rosales-Saavedra M T, Herrera-Estrella A and Casas-Flores S (2015) The EP11 and Sm1 proteins from *Trichoderma atroviride* and *Trichoderma virens* differentially modulate systemic disease resistance against different life style pathogens in *Solanum lycopersicum. Front. Plant Sci*. **23:** 77.

Saloheimo M, Paloheimo M, Hakola S, Pere J, Swanson B, Nyyssonen E, Bhatia A, Ward M and Swollenin M P (2002) A *Trichoderma reesei* protein with sequence similarity to the plant expansins, exhibits disruption activity on cellulosic materials. *European Journal of Biochemistry* **269:** 4202-4211.

Samolski I, Rincon A M, Pinzon L M, Viterbo A and Monte E (2012) The *qid74* gene from *Trichoderma harzianum* has a role in root architecture and plant biofertilization. *Microbiology* **158:** 129-138.

Samuels G J, Dodd S, Lu B S, Petrini O, Schroers H J and Druzhinina I S (2006) The *Trichoderma koningii* aggregate species. *Stud. Mycol*. **56:** 67-133.

Samuels G J (2006) *Trichoderma*: Systematic, the Sexual State, and Ecology. **96:** 195-206.

Samuels G J (1996) *Trichoderma*: a review of biology and systematic of the genus. *Mycol. Res.* **100:** 923-935.

Samuels G J and Ismaiel A (2009) *Trichoderma evansii* and *T. lieckfeldtiae:* two new *T. hamatum*-like species. *Mycologia* **101:** 142-152.

Sarmah D K and Mukhopadhyay A N (1999) Effect of *Gliocladium virens* on the sclerotia of *Sclerotium rolfsii*. *Indian J. Pl. Path*. **17:** 50-54.

Saraswathy A and Hallberg R (2002) Degradation of yrene by indigenous fungi from a former gasworks site. *FEMS Microbiology Letters* **210(2):** 227-232.

Sarrocco S, Guidi L, Fambrini S, Del'Innocenti E and Vannacci G (2009) Competition for cellulose exploitation between *Rhizoctonia solani* and to *Trichoderma* isolates in the decomposition of wheat straw. *J. Plant Pathol*. **91**: 331-338.

Saxena D, Tewari A K and Dinesh Rai (2014) In vitro antagonistic assessment of *Trichoderma harzianum*, PBT 23 against plant pathogenic fungi. *Journal of Microbiology and Biotechnological Research* **4(3):** 59-65.

Score A J and Palfreyman J W (1994) Biological control of the dry rot fungus *Serpula lacrymans* by *Trichoderma* species: the effects of complex and synthetic media on interaction and hyphal extension rates. *International Biodeterioration and Biodegradation* **33(2):** 115-128.

Seaman A (2003) Efficacy of OMRI-approved products for tomato foliar disease control. *Integrated Pest Management Program Publication* **129:** 164-167.

Seeberg E, Elde L and Bjoras M (1995) The base excision repair pathway. *Trends Biochem. Sci*. **20:** 391-397.

Seidl V, Song L, Lindquist E, Gruber S, Koptchinskiy A, Zeilinger S, Schmoll M, Martinez P, Sun J, Grigoreiv I, Herrera-Estrella A, Baker S E and Kubicek C P (2009) Transcriptomic response of the mycoparasitic fungus *Trichoderma atroviride* to the presence of a fungal prey. *BMC Genomics* **10:** 567.

Selvakumar R, Srivastava K D, Aggarwal R, Singh D V and Dureja P (2000) Studies on development of *Trichoderma viride* mutants and their effect on *Ustilago segetum tritici*. *Indian Pytopath*. **53(2):** 185-189.

Shahid M, Srivastava M, Sharma A, Kumar V, Pandey S and Singh A (2013) Morphological, molecular identification and SSR marker analysis of a potential strain of *Trichoderma*/hypocrea for production of a bioformulation. *Journal of Plant Pathology & Microbiology* **4(10):** 1.

Sharma K, Mishra A K and Misra R S (2009) Morphological, biochemical and molecular characterization of *Trichoderma harzianum* isolates for their efficacy as biocontrol agents. *J. Phytopathol* **157:** 51-56.

Sharma N D, Bandyopadhyay S and Dutta S (2003) Screening of potential *Trichoderma* strains against major root pathogens. *Annals of plant protection sciences* **11(1):** 163-163.

Sharma R, Arunab J and Ramesh C D (2012) A brief review on mechanism of *Trichoderma* Fungus use as biological control agent. *International Journal on Innovations and Bio-Sciences* **2(4):** 200-210.

Sharon E, Bar-Eyal M, Chet I, Herrera-Estrella A, Kleifeld O and Spiegel Y (2001) Biocontrol of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. *Phytopathology* **91:** 687-693.

Shi W B and Feng M G (2006) Field efficacy of application of *Beauveria bassiana* formulation and low rate pyridben for sustainable control of citrus red mite *Panonychus citri* (Acari: Tetranychidae) in orchards. *Biological Control* **39(2):** 210-217.

Shoresh M, Yedidia I and Chet I (2005) Involvement of jasmonic acid/ethylene signalling pathway in the systemic resistance induced in cucumber by *Trichoderma asperellum* T203. *Phytopathology* **95:** 76-84.

Siameto E N, Okoth S, Amugune N O and Chege N C (2010) Antagonism of *Trichoderma harzianum* isolates on soil borne plant pathogenic fungi from Embu District, Kenya. *Journal of Yeast and Fungal Research* **1(3):** 47-54.

Simon A and Sivasithamparam K (1989) Pathogen suppression: a case study in biological suppression of *Gaeumannomyces graminis* var. *tritici* in soil. *Soil Biology and Biochemistry* **21(3):** 331-337.

Singh O V, Agarwal V K and Nene Y L (1973) Seed health studies in soybean raised in the Nainital tarai. *Indian Phytopath* **26:** 260-267.

Singh S B, Zink D L, Doss G A, Polishook J D, Ruby C, Register E, Kelly T M, Bonfiglio C, Williamson J M and Kelly R (2004) Citrafungus A and B, two new fungal metabolite inhibitors of GGTase I with antifungal activity. *Org Lett* **6:** 337-340.

Spiegel Y and Chet I (1998) Evaluation of *Trichoderma* spp. as a biocontrol agent against soilborne fungi and plant-parasitic nematodes in Israel. *Integrated Pest Management* **3(3):** 169-175.

Steyaert J M, Weld R J and Stewart A (2010) Ambient pH intrinsically influences *Trichoderma* conidiation and colony morphology. *Fungal Biol.* **114:** 198–208.

Stipanovic R D and Howell C R (1983) Gliovirin, a new antibiotic from *Gliocladium virens*, and its role in the biological control of *Pythium ultimum. Can J Microbiol* **29:** 321-324.

Suarez-Estrella F, Vargas-Garcia C, Lopez M J, Capel C and Moreno J (2007) Antagonistic activity of bacteria and fungi from horticulture compost against *Fusarium oxysporum* f.sp. *melonis. Crop Protection* **26:** 46-53.

Sumana K and Devaki N S (2012) In vitro evaluations of some bioagents against tobacco wilt pathogen. *J. Biol. Control* **25(1):** 67-73.

Sychev P A and Shaposhnik Yu A (1982) Antagonistic effects of *Trichoderma viride* Fr. In relation to some pathogens of *Cucumis sativum* L. *Mikolog-Fitopathol*. **16:** 154-159.

Szekeres A, Kredics L, Antal Z, Kevei F and Manczinger L (2004) Isolation and characterization of protease overproducing mutants of *Trichoderma harzianum*. *FEMS Microbiology Letters* **233:** 215-222.

Tamimi K M and Hadvan H A (1985) Biological effect of *Neurospora sitophila* and *Trichoderma harzianum* on the growth of a range of Sesamum wilt causing fungi in vitro. *Indian Phytopathology* **38(2):** 292-296.

Tanaka Y, Shiomi K, Kamei K, Sugoh-Hagino M, Enomoto Y, Fang F, Yamaguchi Y, Masuma R, Zhang C G, Zhang X W and Omura S (1998) Antimalarial activity of radicicol, heptelidic acid and other fungal metabolites. *J Antibiot* **51:** 153-160.

Tchameni N S, Ngonkeu M E, Begoude B A D, Nana W L, Fokom R, Owona A D, Mbarga J B, Tchana T, Tondje P R, Etoa F X and Kuate J (2011) Effect of *Trichoderma asperellum* and arbuscular mycorrhizal fungi on cacao growth and resistance against black pod disease. *Crop Prot.* **32:** 1321-1327.

Trivedi R K S (2010) *Strategies to control Alternaria radicina in carrot seed production*. Ph.D. Thesis. Lincoln University, Seed Res. Centre, Bio-Protection Res. Centre, Lincoln University Canterbury, New Zealand.

Tronsmo A and Dennis C (1987) Effect of temperature on antagonistic properties of *Trichoderma* species. *Transactions of the British Mycological Society* **71(3):** 469-474.

Tucker E J B, Bruce A and Staines H J (1997) Application of modified international wood preservation chemical testing systems standards for assessment of biocontrol treatments. *International Biodeterioration and Biodegradation* **39(2-3):** 189-197.

Tu J C (1980) *Gliocladium virens*, a destructive mycoparasite of *Sclerotinia sclerotium*. *Phytopathology* **70(7):** 670-674.

Tu J C and Vaartaja O (1981) The effect of the hyperparasite (*Gliocladium virens*) on *Rhizoctonia solani* and on *Rhizoctonia* root rot of white beans. *Canadian Journal of Botany* **59:** 22-27.

Turner W B and Aldridge D C (1983) *Fungal metabolites II*. Academic Press, London.

Utkhede R and Koch C (2004) Biological treatments to control bacterial canker of greenhouse tomatoes. *Biocontrol* **49:** 305-313.

Vargas-Garcia M C, Lopez M J, Suarez F and Moreno J (2005) Laboratory study of inocula production for composting processes. *Bioresource Technology* **96:** 797-803.

Verma M, Brar S K, Tyagi R D, Valero J R and Surampalli R Y (2005) Wastewater sludge as a potential raw material for antagonistic fungus (*Trichoderma* sp.): role of pre-treatment and solids concentration. *Water research* **39(5):** 3587-3596.

Vestberg M, Kukkonen S, Saari K, Parikka P, Huttunen J, Tainio L, Devos N, Weekers F, Kevers C, Thonart P, Lemoine M-C, Cordier C, Alabouvette C and Gianinazzi S (2004) Improving the growth and health of micro propagated strawberry. *Applied Soil Ecology* **27(3):** 243-258.

Vey A, Hoagland R E and Butt T M (2001) (eds.) *Fungi as biocontrol agents: progress, problems and potential*. pp 311-346. CABI, Bristol, UK.

Villena, G K and Guiterezz-Correa M (2006) Production of cellulase by *Aspergillus niger* biofilms developed on polyester cloth. *Letters in Applied Microbiology* **43(3):** 262-268.

Vinale F, Marra R, Scala F, Ghisalberti E L, Lorito M and Sivasithamparam K (2006) Major secondary metabolites produced by two commercial *Trichoderma* strains active against different phytopathogens. *Lett. Appl. Microbiol*. **43:** 143-148.

Viterbo A, Harel M and Chet I (2004) Isolation of two aspartyl proteases from *Trichoderma asperellum* expressed during colonization of cucumber roots. *FEMS Microbiol. Lett.* **238:** 151-158.

Wagh S, Ingle S T, Dandale S and Mane S S (2015) Improvement in biocontrol ability of *Trichoderma viride* through gamma irradiation. *Trends in Biosciences* **8(20):** 5622-5626.

Walnuj A A and Jhon P. (2013) Role of mutation in *Trichoderma*. *Rashtriya Krishi***.** **8(2):**68.

Whipps J M (2001) Microbial interactions and biocontrol in the rhizosphere. *J. Exp Bot* **52:** 487-511.

Whipps J M and Lumsden R D (2001) (eds.) *Fungal Biocontrol Agents: Progress, Problems and Potential*. pp 9-22. CABI Publishing, Wallingford.

Weist A, Grzegorski D, Xu B W, Goulard C, Rebuffat S, Ebbole D J, Bodo B and Kenerly C (2002) Identification of peptaibols from *Trichoderma virens* and cloning of a peptaibol synthetase. *J. Biol. Chem.* **277:** 20862-68.

Wilhite S E, Lumsden R D and Straney D C (1994) Mutational analysis of gliotoxin production by the biocontrol fungus *Gliocladium virens* in relation to suppression of *Pythium* damping-off. *Phytopathology* **84:** 816-821.

Windham G L, Windham M T and Williams W P (1989) Effects of *Trichoderma* spp. on maize growth and *Meloidogyne arenaria* reproduction. *Plant Diseases* **73:** 493-494.

Wright J M (1956) The production of antibiotics in soil III. Production of gliotoxin in wheat straw buried in soil. *Annu. Appl. Boil*. 44: 461-466.

Wu T, Sharda J N and Koide R T (2003) Exploring interactions between saprotrophic microbes and ectomycorrhizal fungi using a protein-tannin complex as a N source by red pine (*Pinus resinosa*). *New Phytologist* **159(1):** 131-139.

Wu W S, Liu S D, Chang Y C and Tschen J (1986) Hyperparasitic relationships between antagonists and *Rhizoctonia solani*. *Plant Protection Bullentin* **28:** 91-100, Taiwan.

Yamano T, Hemmi S, Yamamoto I and Tsubaki K (1970) Trichoviridin, a New Antibiotic. Japan, Tokyo Koho, JP Patent 45015435.

Yasui A, Kanno S and Takao M (2003) DNA damage and repair. *Nihon Ronen Igakkal zasshi Japanese J. Geriatr* **58:** 557-580.

Yedidia I, Benhamou N and Chet I (1999) Induction of defence responses in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum. Applied and Environmental Microbiology* **65:** 1061-1070.

Yedidia I, Benhamou N, Kapulnik Y and Chet I (2000) Induction and accumulation of PR proteins activity during early stages of root colonization by the mycoparasite *Trichoderma harzianum* strain T-203. *Plant Physiol. Biochem*. **38:** 863-873.

Yoshioka Y, Ichikawa H, Naznin H A, Kogure A and Hyakumachi M (2012) Systemic resistance induced in *Arabidopsis thaliana* by *Trichoderma asperellum* SKT-1, a microbial pesticide of seed-borne diseases of rice. *Pest Manage. Sci.* **68:** 60-66.

Zaldivar M, Steiner J, Musalem M and Contreras I (1987) Isolation of a Cellulase Hyperproducer *Trichoderma pseudokoningii* Mutant. *Microbiologia* **3(1):** 33-44.

Zeilinger S, Galhaup C, Payer K, Woo S L, Fekete C, Lorito M and Kubicek C P (1999) Chitinase gene expression during mycoparasitic interaction of *Trichoderma harzianum* with its host. *Fungal Genet. Biol*. **26:** 131-140.

Zeilinger S, Reithner B, Scala V, Peissl I, Lorito M and Mach R L (2005) Signal transduction by Tga3, a novel G protein alpha submit of *Trichoderma atroviride. Applied and Environmental Microbiology* **71:** 1591-1597.

Zhang F, Yuan J, Yang X, Cui Y, Chen L, Ran W and Shen Q (2013) Putative *Trichoderma harzianum* mutant promotes cucumber growth by enhanced production of indole acetic acid and plant colonization. *Plant Soil* **368:** 433-444.

urkund

Document Information

Analyzed document	Tulipa De Thessis.pdf (D79657925)
Submitted	9/22/2020 5:07:00 PM
Submitted by	Kalyan Kanti Das
Submitter email	daskk3@gmail.com
Similarity	1%
Analysis address	daskk3.ubkv@analysis.urkund.com

Sources included in the report

W	URL: https://www.intechopen.com/online-first/biological-efficacy-of-trichoderma-spp-and ... Fetched: 9/22/2020 5:07:00 PM	1
W	URL: https://www.researchgate.net/publication/314946122_Trichoderma_A_Potential_Fungal_ ... Fetched: 9/22/2020 5:07:00 PM	3
W	URL: https://www.researchgate.net/publication/303632190_Trichoderma_Trichoderma_as_Bioc ... Fetched: 10/14/2019 12:32:19 PM	2
W	URL: https://docplayer.net/89597218-Proceedings-of-the-27-th-training.html Fetched: 9/22/2020 5:07:00 PM	1
W	URL: https://worldwidescience.org/topicpages/t/trichoderma+asperellum+strain.html Fetched: 2/17/2020 6:21:14 PM	1

Ayonkay 22/09/2020.
Signature of the Chairman,
Advisory Committee

Tulipa De
Signature of the Student

VITA

Name of the student	: Tulipa De
Father's name	: Lakshman Chandra De
Mother's name	: Soma De
Nationality	: Indian
Date of birth	: 3rd December 1996
Permanent home address	: Vill + P.O. Kushadwip, Dist-Bankura, West Bengal, Pin - 722207

EDUCATIONAL QUALIFICATION

Bachelor's degree

Name of the University	: UTTAR BANGA KRISHI VISWAVIDYALAYA
Year of award	: 2018
OGPA	: 8.73

Master's degree

OGPA	: 9.09
Year of award	: 2020
Title of Master's Thesis	: Antagonistic Potential of mutants of *Trichoderma* species
Awards	: Best Poster of the session IV in Eastern Zonal Conference (IPS) and National Symposium on Mitigating Biotic Stresses in Agriculture for 21st Century: Changing Market Paradigm held in Uttar Banga Krishi Viswavidyalaya on Nov 5-6, 2019
Scholarships	: UBKV merit Scholarship